RENEWABLE CHEMICALS PRODUCTION FROM AQUATIC WEED: *PISTIA STRATIOTES*

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in
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by

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CANDIDATE'S DECLARATION

I, Sweeti hereby certify that the work which is being presented in the thesis entitled **"Renewable chemicals production from aquatic weed: *Pistia stratiotes***" in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from December, 2018 to June, 2024 under the supervision of Prof. Jai Gopal Sharma, Department of Biotechnology, Delhi Technological University, Delhi and Co Supervision of Dr. Rashmi Kataria, School of Bioscience and Technology, Vellore Institute of Technology, Vellore 632014, Tamil Nadu, India.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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This is to certify that the student has incorporated all the corrections suggested by the examiners in the thesis and statement made by the candidate is correct to the best of our knowledge.

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Certified that **Sweeti** (2k18/PHDBT/504) has carried out their search work presented in this thesis entitled **"Renewable chemicals production from aquatic weed: *Pistia stratiotes*"** for the award of **Doctor of Philosophy** from Department of Biotechnology, Delhi Technological University, Delhi, under our supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or anybody else from this or any other University/Institution.

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Renewable chemicals production from aquatic weed: *Pistia stratiotes*

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ABSTRACT

A renewable chemical that can replace fossil fuels is bioethanol. *Pistia stratiotes*, an aquatic weed, was used as the substrate for the synthesis of ethanol in the current investigation. Initially, chemical composition was analyzed and shows this weed contains ash content (18.36%), total solid content (92.85 %), moisture content (7.15%), cellulose content (25.90%), hemicellulose content (18.44%), lignin content (25.25%), starch content (0.6 %), protein content (21.38 %). This composition shows that it contains a high amount of cellulose and hemicellulose which can be utilized as a carbon source for ethanol production after pretreatment. Reducing structural recalcitrance and enhancing hydrolysis efficiency are crucial factors for increasing fermentable sugars and the production of valuable products. Response surface methodology was employed to optimize acidic pretreatment and alkaline pretreatment. In the alkaline pretreatment, cellulose content was exposed and this was further followed by enzymatic hydrolysis, aiming to enhance the saccharification of *Pistia stratiotes*. On the other hand in the acidic pretreatment direct hydrolysis was done and reduced sugar was directly followed by the fermentation process. Alkaline concentration, time and temperature (0.5 - 3 % NaOH, 30 - 60 minutes, 60 - 120 °C) were taken as independent variables in the optimization of alkaline pretreatment. The substrate comprises lignin, hemicellulose, and cellulose. The NaOH concentration during pretreatment significantly influenced the delignification process, resulting in an increased cellulose content. The highest cellulose content was achieved with 2.47 % NaOH at 120 ºC for 60 minutes, leading to enhanced cell porosity and facilitating greater enzyme saccharification accessibility. Under these optimized conditions, the sample exhibited 51.66 % cellulose content. Enzymatic hydrolysis of this cellulose was performed with a commercially available cellulase enzyme. It resulted in 31.06 g/L of reduced sugar liberation after 72 hours. In the Optimization of acidic pretreatment conditions, three independent variables including acid concentration (0.15 - 3.1 % H_2SO_4 concentration), time (12.96 - 97.04 minutes) and temperature (93.18 - 126.82 °C) with responses like sugar and cellulose concentration. Subsequently, the optimized condition was subjected to fermentation for the production of ethanol using *Saccharomyces cerevisiae* and *Pichia stipites* strains. The physiochemical characteristics of the cellulose obtained after pretreatment were analysed using SEM, FTIR, and TGA. The maximum amount of sugar is produced under optimal conditions (2.5% acid concentration) at 120°C for 15 minutes. For the

fermentation stain two microbial strains were procured by NCIM, Pune, these are *Saccharomyces cerevisiae* and *Pichia stipitis*. Initial sugar was taken as 10g/l in the hydrolysate, which is consumed by the strains with maximum sugar conversion rate (0.24 g/l/h). The quantification of the ethanol was done by gas chromatography. Maximum sugar consumption *by S. Cerevisiae* and *P. stipites* was 85.9 % and 87.9 %, respectively. Thus, we present the optimized acidic pretreatment conditions for maximum production of sugar, and thereby, the maximum yield of ethanol from *Pistia stratiotes* using *S. Cerevisiae* and *P. stipitis*. This study demonstrates that acidic and alkaline pretreatment of *Pistia stratiotes* significantly increased its reduced sugar content and cellulose content, leading to a higher sugar yield during enzymatic hydrolysis and maximum ethanol was produced by the *P. stipitis* strains because it can convert both pentose and hexose sugar into the ethanol under anaerobic conditions.

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Sweeti

Dedicated

To

My Father

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List of Abbreviations and Symbols

AIIMS	All India Institute of Medical Sciences
AuNPs	Gold nanoparticles
C. barbata	*Chloris barbata*
CCD	Central composite design
C5	Five-carbon
C6	Six-carbon
CO	Carbon monoxide
CO_2	Carbon dioxide
FPU	Filter paper unit
FTIR	Fourier transform infrared spectroscopy
H_2SO_4	Sulphuric acid
GC-FID	Gas Chromatography- Flame Ionization Detector
NREL	National renewable energy laboratory
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxy butyrate
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
%	Percentage
cm	Centimeter
gm	Gram
h	Hour
HCL	Hydrochloric acid
H_2O_2	Hydrogen peroxide
kV	Kilo volt
kGy	Kilo Grays
LCBs	Lignocellulosic Biomass
MPa	Mega pascal
Mg	Milligram
MHz	Megahertz
min	Minutes
mL	milliliter
NaOH	Sodium Hydroxide

ODW	Oven dried weight
OD	Optical density
RSM	Response Surface Methodology
S. cerevisiae	Saccharomyces cerevisiae
P. stipitis	*Pichia stipitis*
P .stratiotes	*Pistia stratiotes*
SSF	Simultaneous saccharification and fermentation
SHF	Separated hydrolysis and fermentation
°C	Degree Celsius
IU/ml	International units per millilitres
U/g	Units/gram
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight

CHAPTER – 1

INTRODUCTION

CHAPTER (1)

INTRODUCTION

1.1 Background

In the contemporary economy, fossil fuels are the main source of energy. In 2011, the United States derived 36% of its energy from petroleum, 26% from natural gas, and 20% from coal (Sathre, 2014). The current energy situation involves using fossil fuels excessively to meet demand, which raises the price and greenhouse gases. The Intergovernmental Panel on Climate Change (IPCC) released a report citing greenhouse gas emissions from anthropological activity as the cause of global warming. Because of these activities and the use of fuel derived from petroleum in 2005, the dominant greenhouse gas in the atmosphere, CO2, rose by 280 parts per million to 379 parts per million. Hydrothermal power, geothermal power, coal, natural gas, and nuclear energy are the sources of energy used on a global scale. Of the total energy used worldwide, more than 460 quadrillion comes from petroleum (170 quadrillion). Approximately 50% of petroleum is utilized for transportation (Galbe and Zacchi, 2002). Overall 80 % of energy is produced in the world by fossil fuel burning (Escobar et al., 2009). Bioconversion, the process of converting biomass into biofuels, is thought to be a cutting-edge way to lower carbon emissions and pollution in the environment (Saravanan et al., 2022). A sustainable method of lowering environmental pollution and the consumption of crude oil is to use biomass for the production of ethanol. Typically, biomass that contains sucrose, starch, and lignocellulosic material is utilized as feedstock to produce ethanol. The leaders in biofuel production are the United States (US) and Brazil. In 2016, the United States produced approximately 57.7 billion tons of ethanol from corn. Brazil has the capacity to produce approximately 27.6 billion litres of bioethanol from molasses in a single year (Liu et al., 2019).

First, second and third-generation bioethanol production depends upon the biomass used in it. In the first generation, the feedstock used for the production is sugars or by-products of sugar crops and starchy crops. The production of ethanol by these substrates is easy because of the presence of simple sugars in edible substances as compared to lignocellulosic biomass. The main disadvantage of the first-generation feedstock is that it is competitive with the food material. In the second generation, the substrate used for the production is lignocellulosic

biomass. It is commonly a waste material that contains a high amount of carbohydrates, non-competitive to edible material with, low cost and high availability. Environment pollution can be reduced by the use of it, which can reduce greenhouse gas emissions as compared to the first-generation feedstock used (Alonso et al., 2010). The lignocellulosic biomass is divided into different categories as mentioned in Fig 1.1, the sources of the lignocellulosic biomass included energy crops and their residues, grasses aquatic weeds, and forest residues with industrial and municipal waste. Forest residues included softwood and hardwoods with forest waste. Agricultural waste (straw, husk and cereals) (Zabed et al., 2016a).

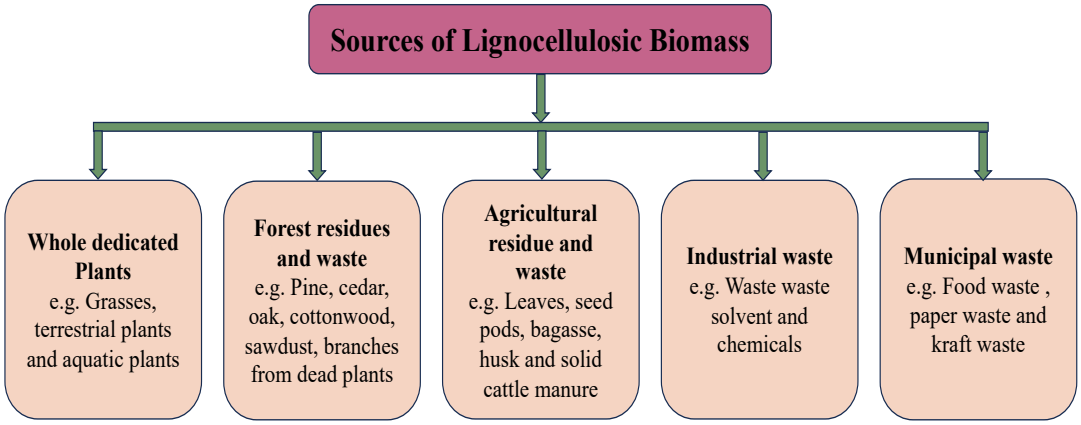


Fig. 1.1 Different lignocellulosic sources for the production of bioethanol

Bioethanol production by LCBs on an industrial scale is very challenging because of some limitations like complex structure (Fig. 1.2) and some technical barriers which count as hurdles in the production as compared to the simple sugar used as substrate. The limitations are energy-consuming pretreatment with the formation of toxic compounds in the hydrolysate. A high number of conversion steps with different compositions of different types of lignocellulosic biomass. They contain both the sugar pentose and hexose sugar and the availability of microbes that can convert both sugars into ethanol after fermentation. Many scientists are working to resolve these barriers in recent years. The initial step of selection of raw material, the selection of naturally specific microbes with the development of genetically modified microbes, development of new strategies for the production of ethanol with low cost production (Zabed et al., 2016.).

The process of producing third-generation bioethanol involves using algal biomass as a feedstock. The freshwater bodies contained this kind of feedstock. Liquid biofuels are produced

using this. Proteins, lipids, and carbohydrates can be found in the freshwater macroalgae of India, such as *Cladophora, Enteromorpha, Hydrodictyon, Microspora, Mougeotia, Oedogonium, Rhizoclonium, Spirogyra, Tribonema, Ulothrix, Vaucheria, and Zygnema* (Kumar et al., 2018).

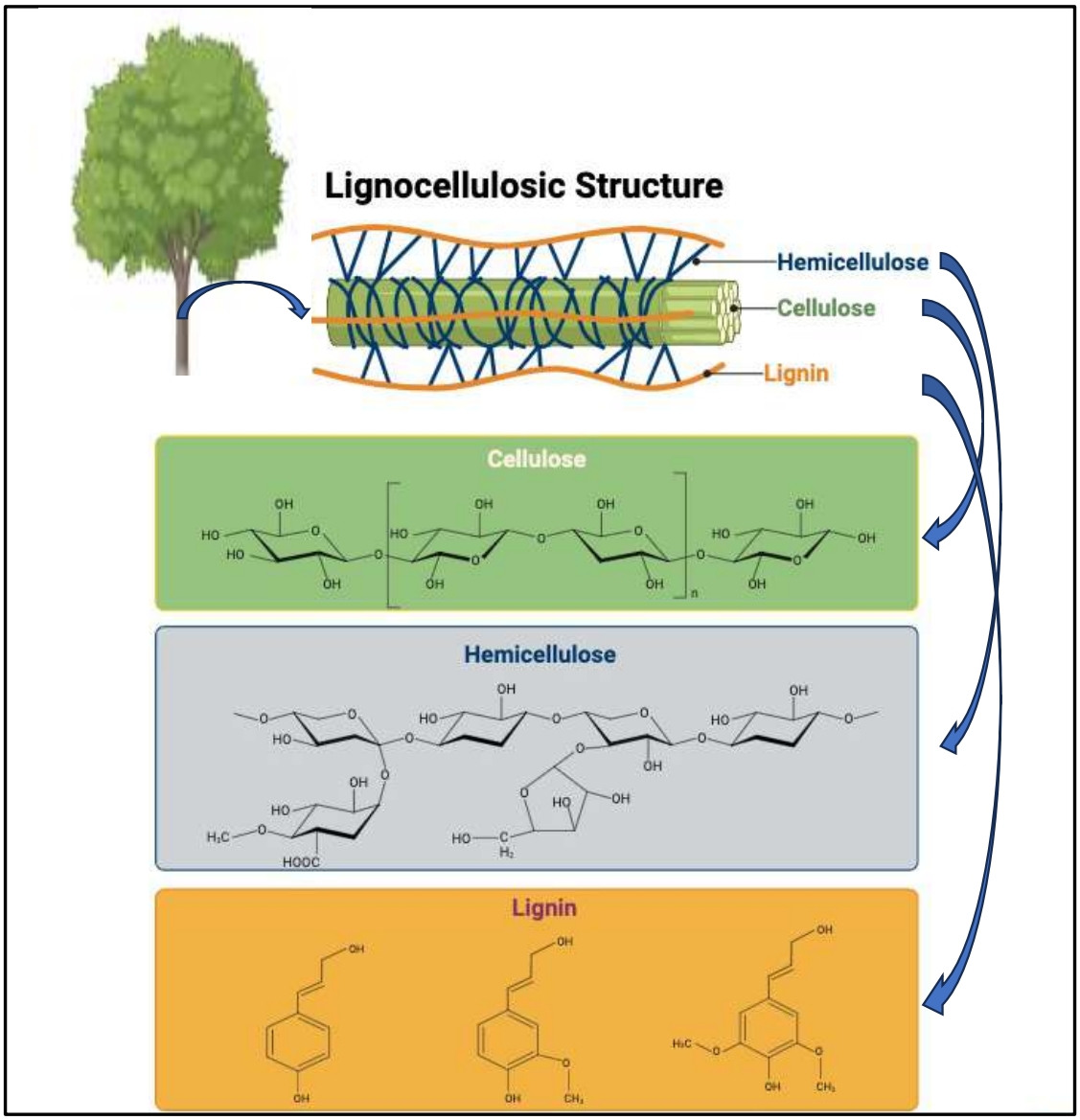


Fig. 1.2 Lignocellulosic biomass structure

Lignocellulosic biomass states to herbal biomass which can be divided into four parts hardwood, softwood, agricultural waste and grasses on the terrestrial and aquatic levels. This is obtained from different types of sources like crop waste, forest residue, agricultural waste, municipal solid waste and aquatic weeds (Kumar et al., 2009)(Sahoo et al., 2022). In the lignocellulosic biomass, mostly agricultural and energy crops are mainly used. Corn crops and their residue like corn cob, stem and husk are utilized for ethanol production. Bagasse obtained from sugarcane is another agricultural waste that is used as a substrate in biofuel production.

Bioethanol production is challenging due to high enzyme cost and technological barriers, such as biomass recalcitrance, diligent pretreatment and inhibitory product formed during pretreatment, which decreases the fermentation efficiency. The polysaccharides, cellulose and hemicellulose, present in lignocellulosic biomass constitute 60% of its total mass and are reluctant to break it into simple sugar naturally (Wyman and Yang, 2017). The complex structure of plant cell wall prohibits the degradation of plant biomass into simple sugar. Different steps such as retreatment, hydrolysis and fermentation are required to convert the plant biomass into valuable products (Kataria et al., 2018a).

In recent decades, A lot of research has been conducted on the optimization of processes for maximizing the production of valuable products from lignocellulosic biomass. Animal dung, agricultural waste, and organic waste are examples of lignocellulosic biomass, which is pretreated before being transformed into biogas. Due to the complex material's pretreatment degradation into simple sugars, the results were incredibly effective. Various pretreatment methods are used for aquatic weeds, but 1% NaOH (alkali) pretreatment is used for *Pistia stratiotes*, which increases biogas production (Sinbuathong, 2019). Effective pretreatment methods are required to increase the accessibility of substrate to the enzymes by changing the complex lignocellulosic structure into a simpler form (Baadhe et al., 2014). The development of systematic pretreatment methods resolves the initial obstacle in cellulosic ethanol production (Mikulski and Kłosowski, 2023). Many pretreatment methods, viz. physical, mechanical and chemical (acidic and alkaline etc.) pretreatment, have been applied to different types of lignocellulosic materials. The main motive of these pretreatment methods is to solubilize the cell wall components (cellulose, hemicellulose and lignin). Acidic pretreatment is much more effective compared to other pretreatment methods as it does not require hydrolysis of the sample to solubilization of sugar reducing the energy consumption for the process (Phwan et al., 2019). H_2SO_4 is commonly used in pretreatment due to its effectiveness in the removal of

hemicellulose and lignin (Dahunsi, 2019). During acidic pretreatment, lignocellulosic biomass is heated by an autoclave with a range of 100 °C-120 °C, during this procedure some toxic compounds were generated, which can be minimized by adding the different chemicals (Hendriks and Zeeman, 2009).

Aquatic weeds present in freshwater bodies cause complications in irrigation and aquaculture projects. These weeds grow rapidly in the presence of proper nutrients and restrict the penetration of light on the lower surface of water bodies, which impairs the growth of aquatic biota. So it has become a topic of research to use them as a renewable carbon source for the production of valuable products in a sustainable manner (Gusain and Suthar, 2017). The main advantages of these aquatic weeds are 1) an economical carbon source, 2) higher CO_2 diminution impact as compared to the terrestrial plants, and 3) low lignin content as compared to the terrestrial plants (John et al., 2011). Extensive studies are conducted for the production of bioethanol from aquatic weeds due to these advantages.

Pretreatment optimization gives a good direction in the field of bioethanol production. Besides pretreatment, other barriers in biofuel processing are the commercialization of lignocellulosic biorefinery, feedstock organization, and ample water consumption for the cultivation of weeds (Kaur et al., 2019). The aquatic weed *Pistia stratiotes* is composed of high carbohydrate content and can grow rapidly in the water. Therefore, it was selected as a substrate for ethanol production in our study. It was established in the earlier research on *Pistia stratiotes* that this weed's dry biomass produces the best results following pretreatment. Following acid treatment, alkaline treatment was administered (Whangchai et al., 2021). In our study, we have performed the optimization of acidic pretreatment on this weed to get the maximum sugar, which was not done previously by any researcher. The parameters used for acidic pretreatment can be optimized with the help of response surface methodology (RSM) design by Minitab software. The main objective of this model is to find out the main response in a particular area of interest, optimize the response, achieve the specific condition using a minimum number of experiments, and observe the interaction between the parameters (Gunst et al., 1996). A combination of mathematical and statistical methods in RSM is used for making the model formation by which optimization of factors is done. The RSM method takes quantitative data from different experiments to estimate the regression model for optimization of responses (dependent variable) affected by process variables (independent variables) (Hinkelmann, 2012). Central composite design (CCD) is a fractional factorial design consisting of 2^n factorial

runs with 2*n* axial runs and centre runs that decide the experimental error. Independent variables determine the number of runs in the model. If the number of variables increases, the number of runs also increases in the replicates of the model. RSM method for optimization consists of three steps. The first step is designing of experiment statistically, the second step is to calculate the coefficients in the model, and the last determine the response and examine the competency of the model within the design of the experiment (Mahalik et al., 2010).

The sugar obtained from the optimized conditions was used for ethanol production. *Saccharomyces cerevisiae* and *Pichia stipites* both strains were used for production. *Pichia stipites* can use both C5 (e.g. xylose) and C6 (e.g. glucose) carbon sources present in the acid hydrolysate. This research makes a genuine contribution to the advancement of the technology used in the production of ethanol and may help in the development of an economical and sustainable approach for the production of ethanol by acidic pretreatment.

1.2 Conversion Technologies involved in bioethanol production

1.2.1. Pretreatment

Pretreatment is an essential step in the liberation of sugars from plant-based biomass. Lignocellulosic biomass is mainly composed of complex polymers including, cellulose, hemicellulose, and lignin. Lignin is the main obstacle to hydrolysis of carbohydrate complex; it is tightly bound with cellulose and hemicellulose and restricts the saccharification process. After the pretreatment step, lignin is removed (Fig. 1.3), and the free cellulose or hemicellulose can be enzymatically hydrolyzed into monomer sugars, these sugars are further used by microbes for ethanol production. Depending on the biomass composition, different types of pretreatment methods are used in the process of production of ethanol from different types of biomasses. The Various classes of conventional pretreatment methods include Physical, chemical, physicochemical, and biological and many emerging technologies are also there like microwave irradiation, microwave-assisted solvolysis, microwave-assisted pyrolysis, ultrasound, a combination of microwave and ultrasound, gamma ray, electron beam irradiation, pulsed electric field, high hydrostatic pressure and high pressure homogenization (Fig. 1.4) (Hassan et al., 2018).

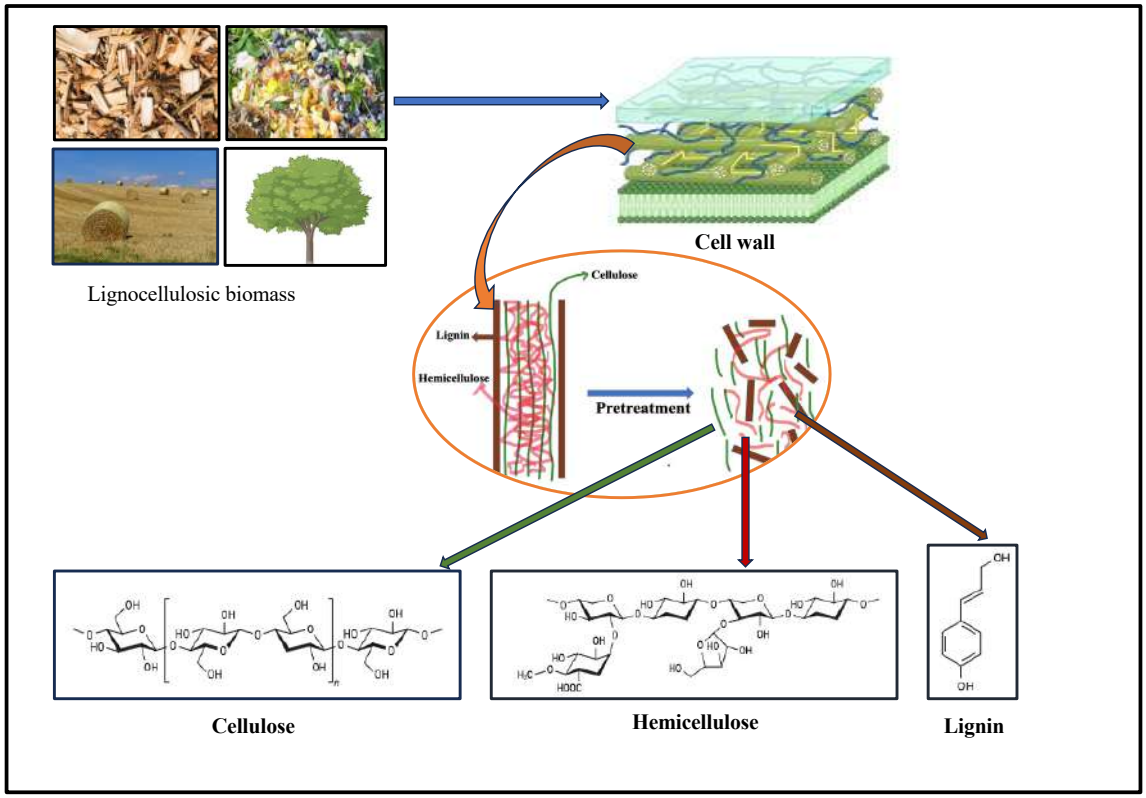


Fig. 1.3 Effect of pretreatment on lignocellulosic biomass

1.2.1.1 Physical pretreatment

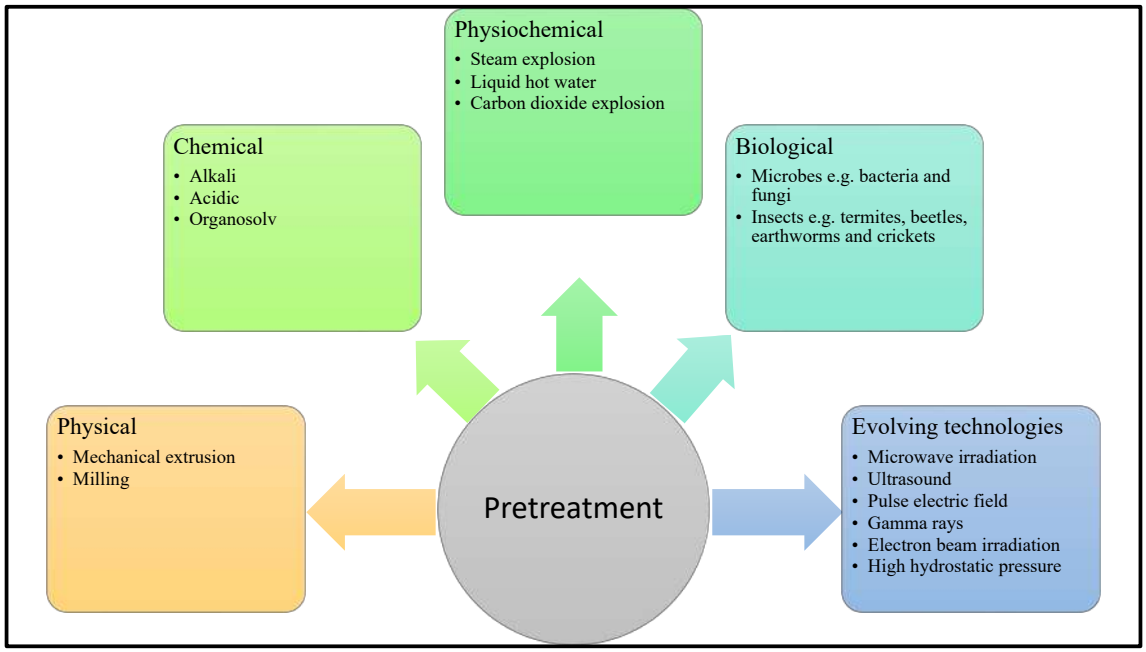


Fig. 1.4 Classification of various pretreatment

Mechanical extrusion is a technique by which reduction in particle size of the organic waste is performed, which increases the surface area of the substrates and efficiently hydrolyzes (Hjorth et al.,2011) It is a promising process of treatment, high pressure is applied to the substrate, and the liquid fraction is formed after this pretreatment (Cesaro et al., 2021). Extruders are of two types, 1) single-screw extruders and 2) twin-screw extruders. A single-screw extruder is made up of a single solid screw while a twin-screw extruder is made up of two screws that are connected to the motor, hopper, and temperature regulators (Duque et al., 2017).

Milling is a process in which the particle size of the substrate is decreased by the grinding instrument. The milling instrument is made up of an electromotor (1.1kW) and a grinder to decrease the particle size (Q. Liu et al., 2016). Generally, this pretreatment is performed with a combination of other pretreatment methods. The grinder of electromotor grinds the substrate and revolves at a particular rpm. The substrate should be chemical-free during this process. After this pretreatment, centrifuge the slurry for further enzymatic hydrolysis (Zhang et al., 2021). Water in the substrate affects this process, a low moisture

content in the substrate speeds up the milling process. A fast grinding and high glucose content form when there is no water content during the milling process (Gu et al., 2018).

1.2.1.2 Chemical pretreatment

Alkali and acidic pretreatments are efficient pretreatment methods for the accessibility of enzymes and the breakdown of complex material in plant cells into simple sugars for further use in bioethanol production. Different acids, such as sulphuric acid, hydrochloric acid, acetic acid, boric acids, etc. are used for acid pretreatment. Here, mostly hemicellulosic sugars are recovered. However, for alkali pretreatment sodium hydroxide, ammonia, and calcium hydroxide are used, which result in the solubilization of lignin components. These chemicals are used with a combination of temperature and various time duration and concentrations, depending upon the biomass type. As compared to the acidic pretreatment alkali pretreatment is most beneficial for enzymatic hydrolysis (Murciano Martínez et al., 2015).

Organosolv pretreatment is used to extract lignin from the lignocellulosic biomass. Many organic solvents are used with a combination of acid or alkali in this pretreatment. Ethanol, methanol, acetone, butanol, and diethylene glycol are used as a solvent. Glycerol and methanol are commonly used for efficient sugar liberation (Joy and Krishnan, 2022). The organosolv pretreatment method is also used to obtain the highly purified lignin from the lignocellulosic biomass. About 97% of pure lignin was recovered by using ethanol as a solvent (Parot et al., 2022).

1.2.1.3 Physiochemical Pretreatment

The steam explosion pretreatment process is performed at high temperature and high pressure on the biomass. High pressure is generated by a steam generator in the steam explosion equipment. The biomass is treated with this steam for a short duration of time, which can be from a few seconds to several minutes and the pressure is released to atmospheric pressure instantly. The sudden change in pressure causes disruption of the substrate by the release of sugars and lignin disruption. This pretreatment could be performed alone or in combination with another pretreatment. In a study, sequential pretreatment is performed, after steam explosion pretreatment, acidic pretreatment with 1.5 % sulphuric acid is done. Optimized conditions for banana pretreatment were found to be 219.31 ºC temperature with 10 minutes

of residence time for PHB production (Mabazza et al., 2020). Acid-catalyzed reaction during steam explosion gives higher xylose recovery which is further used for pentose utilizing microbe for the production of value-added chemicals (Cavalaglio et al., 2021).

Liquid hot water pretreatment reduces the recalcitrance property of lignocellulosic material. It is also called hydrothermal pretreatment and this pretreatment does not release any toxic materials, hence enhancing enzymatic activity for hydrolysis of cellulose (Martín-Lara et al., 2020). This treatment is beneficial for the fermentation process because, in the fermentation step, microbes utilize sugars free from any toxic material. However, the sugar yield is lower in comparison to other pretreatment processes (Zhuang et al., 2016).

Carbon di-oxide explosion is a technique in which biomass is placed in a reactor that contains pressurized carbon dioxide. Anaerobic digestion is an essential step in the waste management process and the pretreatment process for sustainable valuable chemical production (Ampese et al., 2022). Carbon dioxide works as a solvent in this process and it mixes with the substrate in the reactor with high pressure which the breakdown of hemicellulose and cellulose into simple forms of sugars (Das et al., 2021). Benefits of this pretreatment include; low cost, the release of a low amount of hazardous molecules and this process needs low temperature (Agbor et al., 2011).

1.2.1.4 Biological pretreatment

Chemical pretreatments release toxic bioproducts in the hydrolysate which become hazardous to the environment as well as fermenting bacteria. Biological pretreatment is an alternative to chemical pretreatment. Bacteria and fungi are the main microbes involved in this process. During biological pretreatment, some controlled factors like pH, inoculum, temperature, moisture content, and time duration are used to optimize the process. The breakdown of lignin is the first step for rapid enzymatic saccharification for the production of biodegradable products (Naik et al., 2021). Microbes such as *Cellulomonas fimi, Paenibacillus compinasensis*, *Zymomonas mobilis, Azospirillum lipoferum*, *Pseudomonas*, *Rahnella* are used as cellulolytic. Some fungus strains such as *Byssochlamys nivea* can grow on the chemical compounds and decompose them (Zanellati et al., 2021). Other than microbes, some insect species like termites, beetles, earthworms, and crickets carry the ability of enzymatic

degradation of lignin. Various enzymes present in the guts of these organisms are effective for this enzymatic degradation process (J. Sun et al.,2014).

1.2.2 Evolving technologies for the pretreatment

There are many evolving techniques like microwave irradiation, microwave-assisted solvolysis, microwave-assisted pyrolysis, ultrasound, a combination of microwave and ultrasound, gamma ray, electron beam irradiation, pulsed electric field, high hydrostatic pressure and high pressure homogenization (Hassan et al., 2018).

1.2.2.1 Microwave irradiation

Electromagnetic radiation including in the microwave with wavelength from 1mm to 1m. These waves, which selectively transfer energy to different molecules, are nonionizing and fall within the electromagnetic spectrum, which spans from 300 to 300000 MHz (Huang et al., 2016). The first evidence of the effectiveness of microwave radiation in promoting hydrolysis, esterification, and alkylation was presented by researchers in the 1980s, marking the advent of the microwave (Gedye et al., 1986). Researchers have discovered that this kind of pretreatment works well for lignocellulosic biomass. Microwave pretreatment is done for the breakdown of the lignin. Heat is provided to the sample to disrupt the lignin. The range of heat may be 60-140 ºC with different time ranges (Yan et al., 2021). This technique is majorly used for lignin extraction, however, the sugar content can decrease under harsh operating conditions (Sun et al., 2021). This is a potential technique for highly purified lignin extraction (Zhong et al., 2022). The maximum recovery of sugar by this process obtained is 0.512g/g with corn cob under the optimized conditions which also enhanced the productivity of bioethanol (Ocreto et al., 2021). It can be applied in two ways: 1) microwave assisted solvolysis at a low temperature <200 °C, which breaks down the biomass into simpler bioproducts, and 2) microwave assisted lignin pyrolysis at a high temperature >400 °C, which breaks down the biomass into bio-gas and bio-oil. The primary benefit of this kind of treatment over traditional heating is that (a) it produces results quickly and efficiently. (b) offer consistent and targeted heating (c) be energy-efficient and simple to use (d) have little distortion and side product formation (e) remove more acetyl groups from the hemicellulose

1.2.2.2 Ultrasound

Over 97 years ago, the impact of ultrasound treatment was estimated on cellular biomass (Wood and Loomis, 1927). Ultrasonic pretreatment is performed by an ultrasonic bath reactor with different frequencies. Single, dual, and multiple frequencies are used in the generator to perform the pretreatment. Samples in the tube are placed 4 cm deep in this water bath, with different frequencies such as 20,40, and 60 kHz. Different pretreatment time settings are used with fixed temperatures (Yan et al., 2021). The combination of alkaline in ultrasonic pretreatment could increase the saccharification of the lignocellulosic biomass and a sugar recovery of 90% is obtained (Saratale et al., 2020).

1.2.2.3 Pulse electric field

Pulse electric field is another pretreatment technology, in which a high electric field is applied on the substrate for milli-seconds at a short pulse. Pulse electric field pretreatment gives better performance for enzymatic accessibility of the substrate (Kovačić et al., 2021). Lipid extraction is essential for industrially important chemical synthesis. Wastewater *Chlorella pyrenoidosa* (microalgae) could be a good carbon source for biodiesel and other metabolites including PHA production. For the delignification step pulse, the electric field is an efficient pretreatment method (Han et al., 2019). Lignocellulosic biomass is treated with a short burst of a high-intensity electric field for a fraction of second and after that the polymers of the cell wall breakdown into monomers of sugar for further use in the industry (Haldar and Purkait, 2021).

1.2.2.4 Gamma rays

The radioisotopes cobalt-60 and cesium-137, which are investigated for their potential to treat lignocellulosic biomass, release gamma rays. These ionizing radiations readily enter the cell structure, aiding in the disintegration of cellulose and altering the composition of lignin. The free radicals that decay quickly after the radiation stops facilitated the process's effect (Dai et al., 2017). The effects of radiation on microcrystalline cellulose in comparison to alternative pretreatments, such as ionic liquid, acid aqueous ionic liquid, 1% HCL, and 1% H2SO4. The most effective radiation dose, which was higher than other tested pretreatments and yielded the same result as the ionic liquid pretreatment, was 891 kGy (Liu et al., 2015). Numerous studies

have been conducted to demonstrate how radiation affects lignocellulosic biomass and how it can improve enzymatic hydrolysis (Y. Liu et al., 2016)(Liu et al., 2017).

1.2.2.5 Electron beam irradiation

Electron beam radiation is produced by linear accelerators. It can break down the lignin, hemicellulose, and cellulose that make up the cell wall's crystallinity. Following this pretreatment, free radicals with a decrease in the degree of polymerization and decalcification of cell wall are formed (Grabowski, 2015). This treatment can improve the fermentation and hydrolysis of the rice straw that has been soaked in water. During the treatment process, lignocellulosic water-soaked electron beam irradiation does not produce any inhibitory compounds. Scanning microscopy images demonstrate that lignocellulosic biomass undergoes surface changes (Bak, 2014). The FTIR spectra show that structural changes occur when the microcrystalline cellulose is exposed to an electron beam. Lower transmittance was detected at O-H in these spectra, indicating that the maximum amount of cellulose could be recovered during pretreatment (Zhang et al., 2018).

1.2.2.6 High hydrostatic pressure

The food industry uses high hydrostatic pressure technology, which uses pressures between 100 and 600 MPa, to pasteurize food without compromising its quality. This method is mostly applied to the storage of food in the United States, Japan, and Europe. The disadvantage of this is the relatively high cost of the equipment used. The basic principles of this technique are as follows: 1) equal pressure is applied to all parts of the substances without taking into account the size and shape of the biomass. 2) Put pressure on the biomass's volume to decrease (Eisenmenger and Reyes-De-Corcuera, 2009). During the enzymatic hydrolysis, the engineering of the enzymatic reaction was influenced by the high hydrostatic pressure. By changing the pressure and the time affects the rate of hydrolysis. The change in the pressure (300-400 MPa) and time (15-45 min) increases the 10-15-fold hydrolysis rate after pretreatment. The optimal pressure in the sugarcane baggies was found 250 MPa which gives a significant increase in the sugar concentration after pretreatment (Castañón-Rodríguez et al., 2013).

1.3 Detoxification

Detoxification is an essential step after the chemical pretreatment, especially acid pretreatment. Many toxic compounds are generated due to the reaction between substrate and acid. Different types of material are used for the detoxification of hydrolysates like activated carbon, membrane Nanofiltration, and calcium carbonate. Charcoal is activated by mixing with NaOH and then this activated charcoal is added to the hydrolysate slowly for 1 hour. After that, it is removed from hydrolysate by vacuum filtration. GE-Sepa CF cross-flow module with pressure pump used for the membrane filtration. Complete removal of toxic material is done by this filtration unit (Tavares et al., 2022). Calcium carbonate is another chemical that is used during the neutralization of hydrolysate after the acidic pretreatment which can remove the toxic materials from the hydrolysate (Ahmed et al., 2019). Ca $(OH)_2$ is used as a chemical for detoxification. Optimal over liming gives the best result by reduction of furans, and phenolic compounds by 51.9% and 41.6% (Martinez et al., 2001).

1.4 Hydrolysis

Hydrolysis is the main step between pretreatment and fermentation, It enhances the accessibility of sugars for the microbes and enzymes for the production of bioethanol. Chemical hydrolysis and enzymatic hydrolysis are mainly performed during the production of valuable products. Enzymes like cellulase and hemicellulase are generally used for carbohydrate breakdown into simple forms of monomers. The substrate is mixed with the sodium acetate buffer, enzyme and incubated at 50 ^{0}C for a different duration of up to 3-4 days. The detection of monomeric sugars is performed by HPLC (Van Thuoc et al., 2021). A successful pretreatment can eliminate lignin and hemicelluloses from lignocellulosic feedstocks, at least in part, improving the availability of cellulose to cellulases. That being said, it is not cost-effective to remove all lignin to get purely cellulose materials for enzymatic hydrolysis because this requires a lot of chemicals and extreme conditions. Thus, it is imperative to address the issue of lignin's impact on cellulose's enzymatic hydrolysis, which has been extensively researched in recent decades (Cai et al., 2023). Hydrolysis is also done by dilute acid; it can be sulphuric acid or hydrochloric acid. Toxic chemical generation after pretreatment is the main disadvantage of acid hydrolysis. For the removal of these toxic materials, another detoxification step is to be necessary for further steps (Kucera et al., 2017).

1.5 Fermentation

To produce ethanol, a combination of pretreatment, enzymatic saccharification and fermentation are required (Fig. 1.5). In bioprocess engineering, three primary types of fermentation processes are included. Three methods exist for bioprocessing: consolidated bioprocessing, simultaneous saccharification and fermentation (SSF), and separated hydrolysis and fermentation (SHF). These various fermentations were chosen based on the biomass that was chosen for the procedure (Tomás-Pejó et al., 2008).

1.5.1 Separate hydrolysis and fermentation

The SHF process has been used historically. Hydrolysis and fermentation were carried out independently in this procedure. This procedure is advantageous since it was carried out at the ideal pH and temperature. 45°C to 50°C is the ideal temperature range for hydrolytic enzymes, and 30°C to 37°C is the ideal range for fermenting microorganisms. An additional benefit of enzymatic hydrolysis is the elimination of insoluble solid matter from the hydrolysate In addition to the benefits, there are certain drawbacks, such as the buildup of free sugar, which can impede the enzymatic hydrolysis process and lower the hydrolysis yield (Olsson et al., 2004). In addition to the benefits, there are certain drawbacks, such as the buildup of free sugar, which can impede the enzymatic hydrolysis process and lower the hydrolysis yield (Andrić et al., 2010).

This particular set of fermentation proved successful in the lignocellulosic biomass. Both the semi-simultaneous saccharification and fermentation process (SSSF) and the SHF with steam explosion pretreatment were applied when using substrate from the cardoon fields. The experiment was conducted in 5-liter bioreactors, and the results show that the SSSF produced more ethanol which was 13.64g/100g of biomass than the SHF, which produced 13.17g/ 100 g of biomass. Throughout the process, the severity conditions in the SSSF method were also lessened (Cotana et al., 2015).

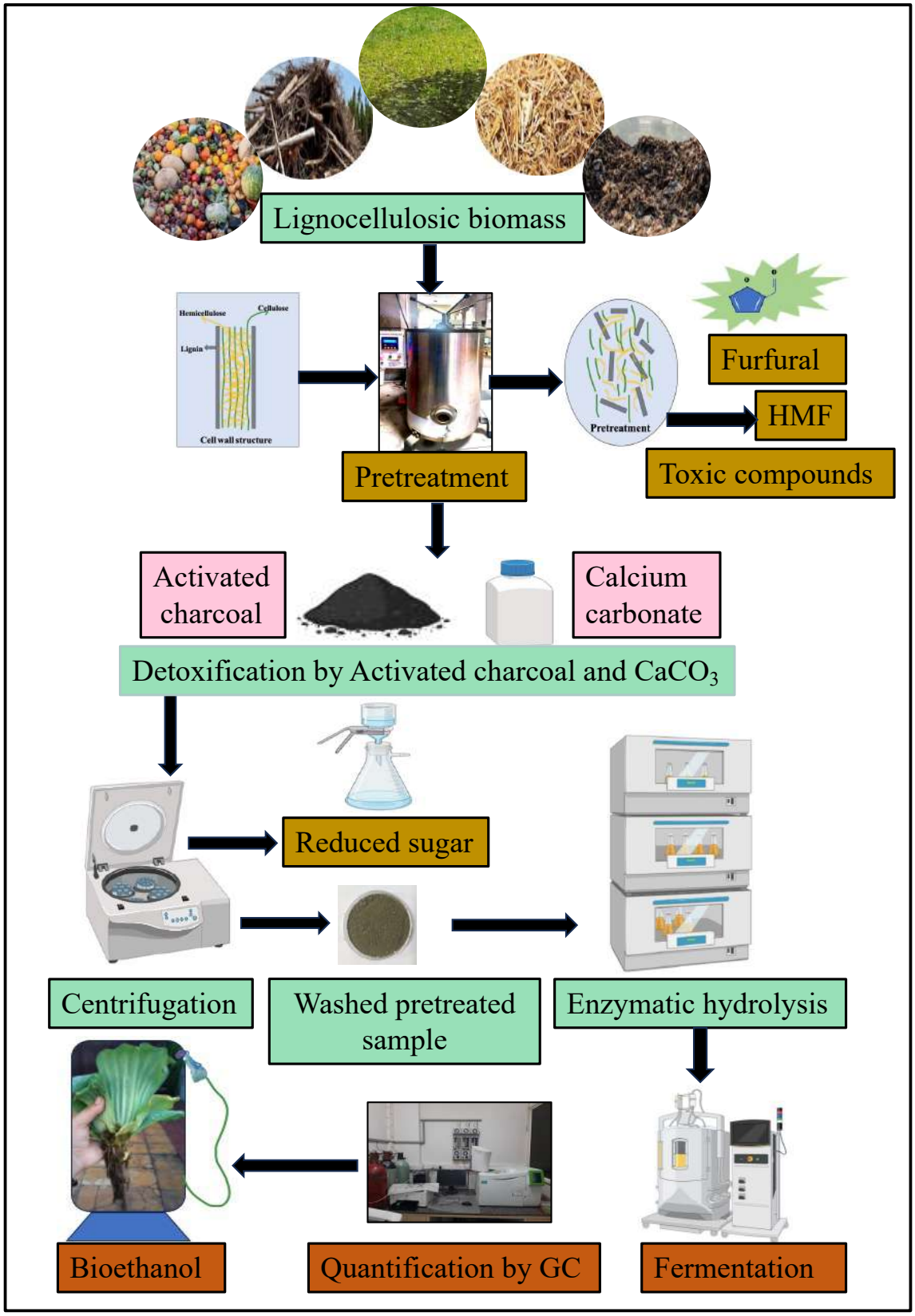


Fig. 1.5 Flow diagram of lignocellulosic biomass to ethanol

1.5.2 Simultaneous saccharification and fermentation (SSF)

Enzymatic hydrolysis and fermentation are done in the same vessel. The main advantage of this method is a reduction in the cost with reduction in the end product inhibition at the time of the enzymatic hydrolysis process. The beneficial outcomes during this process are the higher enzymatic hydrolysis yield, less reaction time and low risk of contamination by which sugar is converted into ethanol as soon as possible. Both fermentation and enzymatic hydrolysis take place in the same vessel. This method has primary benefits are cost effective, and decreased end product inhibition during the enzymatic hydrolysis process. The advantages of this process include a faster rate of enzymatic hydrolysis yield, a shorter reaction time, and a lower risk of contamination, all of which help to quickly convert sugar into ethanol (Alfani et al., 2000). A compatible pH and temperature are needed for this process to facilitate fermentation and hydrolysis. When the temperature is at 40 °C, which lowers the likelihood of contamination and lowers the cost of cooling, thermotolerant microbes are needed. However, this circumstance decreased the fermenting microbes' tolerance to the inhibitory substances (Abdel-Banat et al., 2010). The considering process between SHF and SSF depends on the biomass composition, biomass loading, pretreatment method, saccharifying enzymes and fermenting microbes used in the fermentation process.

1.5.3 Consolidated bioprocessing (CBP)

It consists of integrating the fermentation process, enzymatic hydrolysis, enzyme production and fermentation into a bioprocessing system. Because it lowers operating costs, this procedure is economical (Lynd et al., 2005; Kumar et al., 2016). The CBP process involves a large number of cellulolytic and non-cellulolytic microbes. Most bacteria that are studied are Clostridium spp. By adhering to the cell surface, these anaerobic bacteria can break down cellulose and produce sugars (Shao et al., 2011; Jin et al., 2012). Microorganisms that facilitate CPB should be able to solubilize biomass and generate a high yield of the product on an industrial scale. Because these characteristics of microbes are not present in nature, genetic engineering is necessary to create these kinds of microbes. Three groups of microorganisms can be used to diversify native strategies: fungi, bacteria that form cellulosomes, and bacteria that can produce and excrete enzymes. The organisms that were discovered to be capable of CBP were producing a lesser amount of ethanol at that time. Thus, co-cultivating various microorganisms (a

consortium) is a potential strategy to speed up biomass conversion and boost the yield of ethanol (Olson et al., 2012). Some bacteria have extracellular enzymatic systems known as cellulosomes. In the CBP process, Thermoanaerobacterium saccharolyticum and E. coli are two examples of engineered bacteria (Bokinsky et al., 2011; Chung et al., 2014).

1.6 Bioethanol

It is a first-generation biofuel made through the fermentation and enzymatic hydrolysis processes. It is simple to blend with gasoline, which helps lessen reliance on fossil fuels. The Indian national policy for biofuels 2018 provides a suggestive goal of attaining 20% ethanol blending with petrol by 2030 (Kothari et al., 2020). Because ethanol contains more oxygen, the blending stability increased and the emission of harmful compounds decreased. The benefits of blending gasoline and ethanol include reduced hydrocarbon emissions, refined octane number, and increased combustion efficiency and CO emissions. Due to its polar properties, ethanol exhibits complete miscibility with water and a strong affinity for it (Kunwer et al., 2022). Due to the high octane number of ethanol, its blending is possible so that a separate engine is not required for vehicles because the same engine works for ethanol after blending. The annual data for ethanol production worldwide is shown in Table 1.1 and Fig. 1.6 (“RFA Releases 2023 Ethanol Industry Outlook and Pocket Guide: ‘Ready. Set. Go!,’” 2023).

Table 1.1 Ethanol production worldwide in million gallons

Country	Year (2019)	Year (2020)	Year (2021)	Year (2022)	Ethanol production worldwide (%)
United States	15,778	13,941	15,016	15,400	55 %
Brazil	8,860	8,100	7,320	7,420	27 %
European Union	1,350	1,280	1,350	1,330	5 %
China	1,010	930	870	1,090	3 %
India	460	540	850	920	3 %
Canada	497	429	434	460	2 %
Thailand	430	390	360	380	1 %

Argentina	290	210	270	290	1 %
Rest of World	655	650	820	870	3 %
Total	29,330	26,470	27,290	28,160	

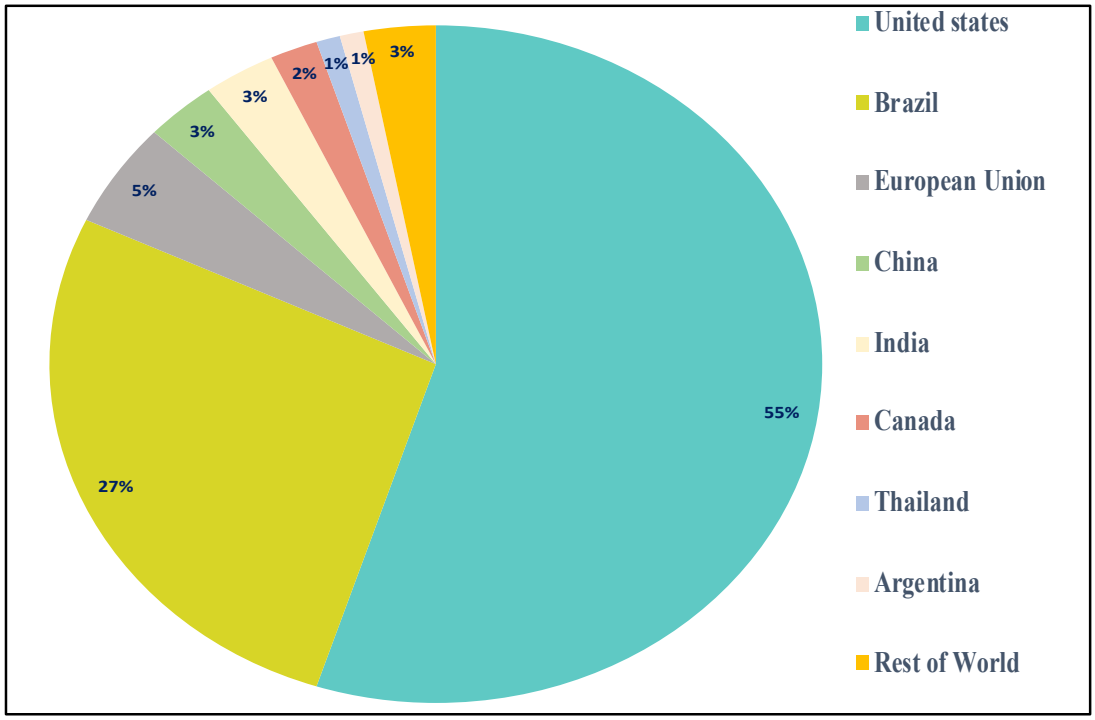


Fig. 1.6 Ethanol production worldwide in 2022

Biofuel production by renewable feedstock shows the perfect balance between the total amount of carbon dioxide released and the consumption of carbon dioxide by plants during photosynthesis.

1.7 Review literature

In the present scenario, energy demands are fulfilled by fossil fuels, which are rapidly depleted with the release of an excess number of green house gases like CO_2 and CO during combustion. To overcome the problem for future perspective, search for fossil fuel alternatives, such as biobutanol, bioethanol and biodiesel, that work sustainably to clean the environment with fulfil the energy demand (Karunanithy and Muthukumarappan, 2011). The need for energy has grown significantly in recent years, placing additional strain on the use of fossil fuels, a resource that is running out quickly. To meet the demands of the world's expanding population, significant production and resource extraction will continue to accelerate due to the rising consumption of natural resources, particularly the non-renewable ones, which power most countries' economies today (Gu, 2020). Renewable and sustainable energy resources gained focus as an alternative to fossil fuels due to their lesser impact on the environment. Biomass from renewable feedstock attains interest in the research and development field due to its composition containing high amounts of carbohydrates, proteins, and lipids and cost effectiveness (Sudhakar et al., 2021). Ecofriendly substrate obtained from plants is considered a renewable energy source with carbon-free emissions (Shrivastava and Sharma, 2023). Many scientists are employed worldwide in the field of waste-based renewable energy production By using greener and more innovative methods, renewable energy sources are displacing fossil fuels. Biofuel is a form of renewable energy obtained from sources such as agricultural waste, food waste, algae and municipal waste (Wannapokin et al., 2018). One likely way to achieve the Sustainable Development Goals (SDGs) is through the production of biofuels from lignocellulosic biomass (LCBs) (Nazari et al., 2021). LCBs contain secondary cell wall enriched with sugars that are feasible, sustainable and economically directed to the production of valuable products such as biofuels, polymers and biochemicals (Culaba et al., 2022).

At the industrial level bioethanol has been produced by corn, wheat, sugar beet and sugarcane are first-generation biomass. However, these feedstocks may have an unfortunate effect on the farmland, causing conflict in food and fuel crop generation. Lignocellulosic biomass falls under the second-generation feedstock, such as certain invasive plants, weeds and other lignocellulosic biomass from terrestrial and aquatic ecosystems. This material could be used as a carbon source in an economic way for energy production. US (United States of America), Russia, China, Germany, Brazil and India have taken action to generate bioenergy products from agriculture and industrial waste (Yadav et al., 2021). Currently United States

and Brazil are front runners in bioethanol production using corn as a substrate, thereby creating the deficiency of food crops (Tse et al., 2021). Both on land and in water, a variety of invasive species can be used as a substrate for the long-term, sustainable production of valuable products. These lignocellulosic invasive weed species serve as an alternative to food crops in the same capacity. A lot of work has gone into using terrestrial lignocellulosic substrates to produce reduced sugars that microbes can use to produce biofuels and biogas (Moerman, 1996). The inability of lignocellulosic biomass to grow quickly due to a lack of agricultural resources and land presents a barrier to the industrial production of bioenergy (Chen et al., 2015).

Aquatic biomass could be utilised as a substrate for the synthesis of biofuel to address the aforementioned issue. The advantage of aquatic weed over terrestrial lignocellulosic biomass is its lower lignin content and faster growth rate. Because of their components, a variety of aquatic plants have the potential to produce bioethanol. Aquatic weeds present in freshwater bodies cause complications in irrigation and aquaculture projects. These weeds grow rapidly in the presence of proper nutrients and restrict the penetration of light on the lower surface of water bodies, which impairs the growth of aquatic biota. These weeds have unique biochemical composition and adaptability in adverse conditions with efficient utilization. The composition of different aquatic weeds has been described in Table 1.2. So it has become a topic of research to use these aquatic weeds as a renewable carbon source for the production of valuable products in a sustainable manner (Gusain and Suthar, 2017; Kaur et al., 2018). The main advantages of these aquatic weeds are 1) they are an economical carbon source, 2) they have a higher CO_2 diminution impact as compared to the terrestrial plants, and 3) they possess lower lignin content as compared to the terrestrial plants (John et al., 2011). Aquatic weeds come in three different varieties: 1) emerging 2) floating and 3) submerged weeds. *Phragmites australis*, *Typha lotifolia*, *Cyperus difformis* are included in emergent weeds; *Azolla pinnata*, *Lemna minor*, *Pistia stratiotes Eichhornia crassipes* and *Salvinia molesta*, are free floating weeds; submerged rooted weeds include *Hydrilla verticillate* and *Utricularua flexuosa*, *Vallisneria spiralis, Najas minor*, and *Potmogeton natans.* Extensive studies have been conducted on the production of bioethanol from aquatic weeds due to these advantages (Alam et al., 2021a).

Table 1.2 Composition of different aquatic weeds

Biomass	Ash content (%)	Total solids (%)	Moisture content (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Starch (%)	Reference
Water hyacinth	1.5	---	---	24.5	34.1	8.6	---	(Gaurav et al., 2020)
Azolla filiculoides	7.3	---	11	21.8	13.5	10.3	6.05	(Miranda et al., 2016)
L. minor	18.7	---	3.5	---	---	---	---	(Gusain and Suthar, 2017)
P. stratiotes	22.7	---	5	---	---	---	---	(Gusain and Suthar, 2017)
Eichhornia sp.	24.9	---	24.9	---	---	---	---	(Gusain and Suthar, 2017)
Eichhornia crassipes	---	---	---	35.84	19.42	13.27	---	(Manivannan and Narendhirakannan, 2015)
Typha sp.	---	---	---	36.7	16.9	12.5	---	(Froese et al., 2020)
Eichhornia crassipes	13.3	88.5	11.5	35	24.8	7.72	---	(Kaur et al., 2019)
Azolla microphylla	19.7	90.8	9.2	27.4	15.67	10.13	---	(Kaur et al., 2019)
Lemna minor	7.2	87.95	12.05	28.8	22.3	4.1	7.2	(Kaur et al., 2019)
Water hyacinth	---	---	---	18.2–18.4	48.7–49.2	3.5–3.55	---	(Zabed et al., 2016a)
Pistia stratiotes	23.8	91.25	---	---	---	---	---	(Pantawong R., Chuanchai A., Thipbunrat P.,

								Unpaprom Y., 2015)
Water hyacinth	---	---	---	19.7	27.1	---	4.1	(Mishima et al., 2008a)
Pistia stratiotes	---	---	---	16.5	17.3	---	6.4	(Mishima et al., 2008a)
Azolla sp.	15.89	---	94.70	---	---	---	---	(SAJAD Rabani, 2019)
Water primrose	28.37	90.0	7.28	---	---	---	---	(THU THI NONG et al., 2021)
Hydrilla verticillata	18.32	---	90.20	---	---	---	---	(SAJAD Rabani, 2019)
Ipomoea aquatica leaf	7.28	---	80.0	---	---	---	---	(Ali and Kaviraj, 2018)
Pistia stratiotes	24.91	90.27	9.73	19.78	25.38	34.85	---	(Sutaryo et al., 2022)
Pistia stratiotes	18.36	92.85	7.15	25.29	18.44	16.73	0.6	This study

Bioethanol production is challenging due to high enzyme cost and technological barriers, such as biomass recalcitrance, diligent pretreatment and inhibitory product formed during pretreatment, which decreases the fermentation efficiency. The three primary constituents of lignocellulosic waste are cellulose, hemicellulose, and lignin. The polysaccharides, cellulose and hemicellulose, present in lignocellulosic biomass constitute 60% of its total mass and are reluctant to break it into simple sugar naturally (Wyman and Yang, 2017). The complex structure of plant cell wall prohibits the degradation of plant biomass into simple sugar. Different steps such as pretreatment, hydrolysis and fermentation are required to convert the plant biomass into valuable products (Kataria et al., 2018a). In recent decades, lots of research has been conducted on the optimization of processes for maximizing the production of valuable products from lignocellulosic biomass. Animal dung, agricultural waste, and organic waste are examples of lignocellulosic biomass, which is pretreated before being transformed into biogas. Due to the complex material's pretreatment degradation into simple sugars, the results were

incredibly effective (Olatunji et al., 2021). The most abundant material in the environment is cellulose. To produce bioethanol, the complex plant component must first be broken down into simple sugar. The cellulose separation is obtained from the hemicellulose and lignin through pretreatment. The most important stage in the entire ethanol production process is the pretreatment. Effective pretreatment can be used to avoid the need for reduced biomass particle size, inhibit the formation of furfural compounds that hinder the growth of fermentative microbes, and lower costs and energy requirements (Mosier et al., 2005). In addition to these standards, other factors include the choice of pretreatment technique that yields high carbohydrate recovery rates, high cellulose digestibility for enzyme accessibility, and the requirement that sugars be detoxified and released into the liquid fraction (Galbe and Zacchi, 2012).

Pistia stratiotes (water lettuce) is a monocotyledonous weed found in fresh water. It can obstruct the water channel due to its dense growth connected with the root nodules. This weed's primary drawback is that it can deteriorate the quality of the water, which is why it's considered an invasive weed. When used as a substrate for the production of bioethanol, this weed's drawback can be turned into a useful resource for financial gain (Jayanth, 2000). Early in the 1990s, *Pistia stratiotes* was discovered to be a valuable carbon source for the creation of products like biogas. In addition to the weed's anaerobic digestion, which yields biogas, continuous fermentation also forms an alternative by producing bioethanol (Yang et al., 2022). Harvesting the aquatic weeds is the first stage in turning them into biofuels. Compared to alternative strategies, mechanically more efficient methods can be used to remove these weeds (Alam et al., 2021b). The aquatic weed *Pistia stratiotes* is composed of high carbohydrate content and can grow rapidly in the water. Therefore, it was selected as a substrate for ethanol production in our study. It was established in the earlier research on *Pistia stratiotes* that this weed's dry biomass produces the best results following pretreatment. Following acid treatment, alkaline treatment was administered (Whangchai et al., 2021). Various pretreatment methods are used for aquatic weeds, but 1% NaOH (alkali) pretreatment is used for *Pistia stratiotes*, which increases biogas production (Sinbuathong, 2019). Effective pretreatment methods are required to increase the accessibility of substrate to the enzymes by changing the complex lignocellulosic structure into a simpler form (Baadhe et al., 2014). The development of systematic pretreatment methods resolves the initial obstacle in cellulosic ethanol production (Mikulski and Kłosowski, 2023). Many pretreatment methods, viz. physical, mechanical and chemical (acidic and alkaline etc.) pretreatment, have been applied to different types of

lignocellulosic materials. The main motive of these pretreatment methods is to solubilize the cell wall components (cellulose, hemicellulose and lignin).

Pretreatment optimization gives a good direction in the field of bioethanol production. Besides pretreatment, other barriers in biofuel processing are the commercialization of lignocellulosic biorefinery, feedstock organization, and ample water consumption for the cultivation of weeds (Kaur et al., 2019). The parameter used for pretreatment can be optimized with the help of response surface methodology (RSM), a statistical tool designed by Minitab software. RSM methodology involves combining the results of each experiment to optimise multiple variables under various experimental conditions (Ali et al., 2015; Manmai et al., 2020). It is a statistical tool that draws on scientific techniques such as analytical and classical methods, producing responses that are influenced by a variety of variables and that ultimately relate to optimisation. Numerous lignocellulosic biomass, including rice straw, napier grass, switchgrass, and corncob, are optimised using RSM (Başar et al., 2021; Gundupalli et al., 2022; Selvakumar et al., 2022). The most popular and frequently utilised RSM design is the central composite design (CCD). Enzymatic hydrolysis requires cellulase enzyme for conversion of cellulose into reduced sugar. The source of carbon has the lowest concentration limit at which the balance between newly produced and dead cells keeps the number of living cells relatively constant (Gao and Gu, 2021).

Lignocellulosic substrates are highly recalcitrant as a consequence of lignin along with cellulose and hemicellulose in the cell wall of plants. For the saccharifying enzymes to access the substrate, this is a significant barrier. Pretreatment is necessary to get rid of lignin and hemicellulose and make cellulose more accessible to hydrolysing enzymes to improve the saccharification from lignocellulosic biomass (Fillat et al., 2017). Pretreatment raises the biomass porosity by eliminating lignin and hemicellulose (Asghar et al., 2015). Pretreatment can be categorized as biological (fungi) like white-rot fungus (Saha et al., 2016), physical (milling, extrusion, microwave heating), chemical (alkali, acidic, ionic, and with organic solvents) and physiological (CO_2 explosion and wet oxidation) (Aguilar-Reynosa et al., 2017). However, none of these meet the requirements in terms of inherent advantages and drawbacks. Thus, to improve the effectiveness of the conversion process, a valuable pretreatment procedure is evaluative. When fungi are co-cultivated during a biological pretreatment, their combined growth can sometimes inhibit the growth of the individual fungi, but it also has a synergistic effect on lignin degradation and the expression of lignolytic enzymes (Meehnian et

al., 2017). When various pretreatment techniques were employed to optimise reduced sugar content in sugarcane bagasse, it was utilised as a substrate for the production of bioethanol. In essence, pretreatment with hydrothermal, organosolv, alkali, and alkaline peroxide was carried out. When the sample was treated with 5% alkaline peroxide, the least amount of energy was used and the least amount of waste was produced. With the least amount of waste generated (38.9 kg waste/kg bioethanol), the energy efficiency of bioethanol production was 162 kg bioethanol/kWh. The production of bioethanol is enhanced by the simultaneous saccharification and fermentation of 5% w/w H_2O_2 and NaOH at 25 °C for 24 hours of incubation (0.101 kg bioethanol/kg biomass) (Raina et al., 2024).

Alkaline pretreatment is a more intently studied technique and has many advantages, such as effective lignin solubilization in contrast to other pretreatment methods. Alkaline pretreatment is used for delignification with minimal loss of reduced sugar and unescorted by inhibitory compound formation (Alvira et al., 2010). Saphonication and solvation are the causes of swelling in alkaline pretreatment. The lignin and hemicellulose eater bonds break down as a result of the saponification reaction. The surface area of cellulose increases as the polymerization and crystallinity decrease as a result of the swelling of the biomass. Alkaline concentration, reaction temperature, and reaction time are some of the parameters that need to be optimised to get the most out of the alkaline pretreatment (Kim and Han, 2012). Alkali pretreatment of *Salix viminalis L*., the effects of granulation, catalyst concentration (NaOH), temperature and pretreatment duration were examined. Experiments were conducted, using the Box-Behnken design for four different factors. Utilising cellulolytic enzymes immobilised on diatomaceous earth, the pretreated substrate was saccharified. A statistical model for the ultimate conditions of alkali pretreatment estimation is put forth based on the results obtained. If saccharification efficiency and cost analysis are taken into account, the optimum conditions for an alkaline pretreatment are: granular of 0.75 mm with 7 % NaOH concentration for 6 hours, at 65 °C (Łukajtis et al., 2018). A research investigates the impact of alkali pretreatment on lignocellulosic substrate (paddy straw and sugarcane bagasse), at different concentrations of NaOH (2% - 10%), as well as how this pretreatment affects the structure of the biomass and the subsequent production of bioethanol. In comparison to untreated samples, samples treated with 2% NaOH pretreatment showed a notable reduction in lignin and an increase in reducing sugar yields. The reduced sugar found from paddy straw was 8.37 g/L and 7.64 g/L from sugarcane bagasse (Tharunkumar et al., 2024).

The cellulose content of *C. barbata*, a terrestrial lignocellulosic weed, was exposed to a mildly alkaline treatment with the aid of an autoclave. This cellulose was accessible to cellulolytic enzymes, which increased the efficiency of saccharification. When the sample was treated with 2% NaOH for 60 minutes at 110 °C, optimal condition was observed. Following that, there was a five-fold increase in sugar content over the raw sample, indicating that this weed may be a possible source of bioethanol (Obeng et al., 2019). Aquatic weed *L. flava* was utilised to produce bioethanol following an alkaline pretreatment with varying bases, such as 0%, 1%, 2% CaO, and 2% NaOH, to delignify biomass. The pretreatment's results show that 1% CaO significantly increases reduced sugar, which is then used in the fermentation process to produce bioethanol. Following this pretreatment, 50.81 g/L of total sugar and 28.88% reducing sugar were formed, and after 24 hours of fermentation, the yield of ethanol was 6.31±0.72 g/L. Because of the safe and economical treatment, it is appropriate for this procedure (Mejica et al., 2022). *Ludwigia hyssopifolia* (water primrose) is a potential source of biogas production. To increase its productivity, an alkali pretreatment optimisation study was conducted using 10% of total solids and alkali concentrations of 0,1,2, and 4% w/v of NaOH solution. the optimal condition was identified at 2% NaOH concentration and a biogas yield of 8072 ml was formed which was 88.4% higher than without the treated sample. Once this ideal pretreatment was applied, the energy efficiency was computed, yielding satisfactory results (Nong et al., 2022). The combination of both alkali pretreatment and ensilage was applied to the water lettuce for the production of reduced sugar. The biomass was treated with NaOH to release lactic acid during storage. Even though the alkali pretreated sample did not increase the water soluble carbohydrate. The reduced sugar conversion ratio was increased with increased the loss of carbohydrates during the neutralization of the alkali treated sample. More research was required to improve the procedure and decrease the loss of carbohydrates (Chen et al., 2015)

Acidic pretreatment is much more effective compared to other pretreatment methods as it does not require hydrolysis of sample to solubilization of sugar reducing the energy consumption for the process (Phwan et al., 2019). H_2SO_4 is commonly used in pretreatment due to its effectiveness in the removal of hemicellulose and lignin (Dahunsi, 2019). During acidic pretreatment, lignocellulosic biomass is heated by an autoclave with a range of 100 °C - 120 °C, during this procedure some toxic compounds were generated, which can be

minimized by adding the different chemicals (Hendriks and Zeeman, 2009). After a diluted acidic and hot water pretreatment, bamboo and sugar cane bagasse were taken as lignocellulosic biomass for the production of valuable products. RSM performed the optimisation for this comparative study, and the results were extremely pleasing. The temperature range of 80°C to 150°C, the biomass loading of 5–10 wt.%, and the treatment duration of 10–20 minutes were considered the independent variables. For the acidic pretreatment, the acid concentration was kept at 0.5% w/v. When the sugarcane bagasse and bamboo sample were treated with acidic pretreatment and hot water pretreatment, the maximum total reduced sugar (23.49 g/L, 26.50 g/L) was obtained; the conditions were 100 °C, biomass loading of 5%, and treatment time of 20 min (Timung et al., 2015). During the pretreatment step, a bacteria was added to the acidic pretreatment condition, resulting in a higher digestibility of the sample. The rice straw sample's lignin droplets that formed following the acidic pretreatment are acted upon by *Cupriavidus basilensis B-8* bacteria. The sample's surface becomes rough and porous as a result of the bacteria removing these droplets, making it easier for the cellulolytic enzymes to hydrolyse the material. Thus, there was a 35-70% increase in rice straw's digestibility. This bacteria enhanced physiochemical procedure provides the pretreatment with a new direction (Ahmed et al., 2019). After receiving an acidic pretreatment, the biomass feedstock made from water hyacinth has produced bioethanol with satisfactory results. The Yamuna River in Delhi, India served as the sample collection site. Five distinct locations were selected to collect the samples and were optimized to have less sugar. After the sample was treated with 3% sulphuric acid for 90 minutes at 121°C, the optimal condition was reached. This ideal setting helped to release the lignin-hemicellulose bond and extract the most reduced sugar possible from the material (Chauhan et al., 2020). At the moment, a study comparing the pretreatment methods of sulphuric acid and biphasic, which contain pentanol/ *p*- toluenesulfonic acid, is being conducted on the pretreatment of the pinewood biorefinery. The conclusion was that, in comparison to the traditional acidic methods, the biphasic pretreatment caused less environmental damage. This biphasic pretreatment has a 13.05 per cent lower environmental impact because it uses less energy, produces more bioethanol during the process, and produces more xylonic acid (Khounani et al., 2024). To remove lignin and hemicellulose, wheat straw is physically ground and then chemically pretreated.

Following pretreatment, cellulose is created and then treated with a thermophilic cellulase enzyme that is derived from filamentous fungi Aspergillus fumigatus and manure-detachable *Rhizomucor pusillus*. *Aspergillus fumigatus* with an exonuclease activity of 147.11 U/mg, an endonuclease activity of 515.9 U/mg, and a total cellulase activity of 453.78 U/mg wheat straw. The substrate's cellulose is exposed by the pretreatment with 88.91 %. Hydrolysate after hydrolysis with cellulase (obtained for the *Aspergillus fumigatus* and *Rhizomucor pusillus)* gives 21.88 g/L and 24.02 g/L ethanol after fermented by Saccharomyces cerevisiae (Valamonfared et al., 2023). *Aspergillus niger* and *Aspergillus flavus* produced the cellulase enzyme, which was used to extract raw sugar from lignocellulosic biomass. In comparison to the free enzyme, the immobilized enzyme's maximum temperature stability (between 20 and 70 °C) was measured. The free enzyme's carboxymethyl cellulase (CMCase) activity peaked at pH 4 (1.26 IU/ml); the immobilized enzyme's activity peaked at pH 5 (2.09 IU/ml). Both *A. niger* and *A. flavus* strains exhibit good CMCase activity when using coffee pulp as a substrate (7.62 IU/ml and 6.47 IU). When used as a nitrogen source, ammonium sulphate exhibits the highest level of CMCase activity. In contrast to free *S. cerevisiae*, the immobilized strain exhibits the highest rate of ethanol production as fermentation duration increases, reaching a maximum of 71.39 mg/ml for *A. niger* and 11.73 mg/ml for *A. flavus* after 72 hours. (Alabdalall et al., 2023).

Agricultural residues such as corn stover, rice husk, and wheat straw a common substrates for ethanol production after providing the pretreatment. Rice straw makes an excellent substrate for the synthesis of bioethanol. *Zymomonas mobilis*, facultative anaerobic bacteria, and *Pecoramyces sp.* F1, a fungus used to produce ethanol, concurrently ferment to ethanol using the same substrate. After the fourth day, the maximum production was determined to be 0.32 g ethanol/g sugar (Y. Li et al., 2022). Because it is inexpensive and simple to experiment with, the biochemical pretreatment applied to the corn stover is an advantageous pretreatment method for preventing the production of toxic compounds through chemical pretreatment. In comparison to the untreated biomass, the lignocellulosic biomass (corn stover) increased in density four times when treated with acidic chemicals. There was a strong resistance to microbial contamination in this dense bed. This kind of pretreatment's primary benefit was its ease of storage, packing, and transportation. Compared to conventional acidic treatment, dense lignocellulosic biomass treated at 121 °C in an autoclave has higher enzyme digestibility and fermentability. After pretreatment, 68.1 g/L of ethanol was produced without any detoxification

of the biomass (Yuan et al., 2022). The biomass growth rate increases the substrate's efficiency in producing bioethanol. The next generation of the substrate is made up of aquatic weeds that can grow in wastewater because they reproduce more quickly, have higher cellulose and hemicellulose contents, and require less lignin. Because they can grow in wastewater streams, this biomass has the potential to be beneficial for phytoremediation and bioenergy production, which can lessen the need for fertilized freshwater bodies and land (Kaur et al., 2018).

Numerous aquatic weeds exist, including *Lemna minor*, *Azolla microphylla*, *Eichhornia crassipes*, and *Pistia stratiotes*. They are suitable for producing bioenergy by performing different sets of experiments. Four distinct treatments were applied to *Lemna minor*, *Azolla microphylla*, and *Eichhornia crassipes*. (1) Anaerobic digestion and hydrothermal treatment, followed by fermentation (2) Anaerobic digestion and fermentation followed by thermochemical treatment; (3) Hydrothermal treatment and fermentation followed by anaerobic digestion; and (4) sequential hydrothermal and anaerobic digestion. In the first condition, hemicellulose is removed by 68.5-73.5% while cellulose content is increased by 41.2–54.5%. The ethanol yield was higher under this condition (0.170–0.231 g/g biomass) than under the other three (Kaur et al., 2019). The efficacy of aquatic weeds *L. minor, L. gibba, Eichhornia sp.* and *P. stratiotes* for ethanol production was estimated. the starch was used for the production of ethanol by these weeds. The outcome of ethanol from Aquatic weeds *Alternanthera philoxeroides* and *Brachiaria mutica* were employed in the production of bioethanol. The weeds were first pretreated with an alkali (NaOH), and then the cellulase enzyme hydrolyzed them. AuNPs were used to immobilize the cellulase enzyme, and calcium alginate was also used to immobilize yeast cells. Next, fermentation and saccharification were carried out concurrently (Gusain and Suthar, 2017) .

The immobilized enzymes exhibit the best results regarding the hydrolysis and the immobilized yeast cells when both conditions are applied for fermentation: the free and immobilized yeast for fermentation, and the immobilised enzymes and immobilized cellulase enzyme for hydrolysis. After scarification by cellulase immobilised on the AuNPs, ethanol production was successfully formed, with 45.09 and 50.1% from the alkali-pretreated sample of *A. philoxeroides* and *B. mutica* substrate (Aarti et al., 2022). The primary issue facing the ecological niche that negatively affects aquatic and terrestrial life systems is environmental pollution. Fossil fuel consumption is the primary source of this pollution. The majority of environmental contaminants are hydrophobic, so they dissolve slowly in the water, which

restricts microorganisms from utilizing them as carbon sources (Gu, 2016). The fermentation process of water hyacinth was statistically optimised using Taguchi orthogonal array design. Simultaneous saccharification and fermentation process was used. E. coli BL21 (DE3) was used to produce recombinant cellulase (GH5) and hemicellulase (GH43) for the hydrolysis process. For the fermentation process, the strains *Saccharomyces cerevisiae* and *Candida shehatae* were primarily utilised. Six distinct factors were used in the optimisation process: pH, temperature, inoculum volumes of *S. cerevisiae* and *C. shehatae*, GH43 hemicelluase, and GH5 cellulase. Ethanol was detected at 1.84 g/L under optimal conditions (Das et al., 2016).

1.8 Research gap

Aquatic weed is a valuable substrate for the production of valuable products like bioethanol, bioplastic and lactic acid. Many advantages of aquatic biomass as compared to terrestrial biomass give weight to this type of substrate, like rapid growth rate, minimal land requirements, and ideal composition. Apart from this, many points need to be addressed by the scientific community like challenges of harvesting biomass, inefficient pretreatment, hydrolysis and fermentation processes for the production of biofuel at an industrial level. The process cost is another issue in this field. For higher production of biofuels, hybrid pretreatment methods and optimization processes pave the way to this aim. Less research at present on aquatic weed as compared to terrestrial weed is also a limitation at the scientific level. In previous studies, optimization of pretreatments is not yet reported on *Pistia stratiotes.* There is a need for the identification of advanced biotechnologies to produce bioethanol from aquatic weeds worldwide.

1.9 Objectives of the work

The present study demonstrates the production of ethanol from the low commercial-valued weed *Pistia stratiotes*. During the fermentation process, this invasive weed is pretreated with acid and alkali, then reduced sugar is extracted by enzyme hydrolysis and consumed by S. cerevisiae, which converts hexose sugars to ethanol, and *Pichia stipitis*, which converts hexose and pentose sugars through fermentation to ethanol. The aforementioned benefit led to the following objectives being set out to assess how well aquatic weed *Pistia stratiotes* produce bioethanol.

- **The major objectives of the present research investigation include**

1) Composition of biomass and optimization of alkali and acidic pretreatment conditions
2) Enzymatic hydrolysis of biomass
3) Microbial fermentation for bioethanol production

CHAPTER - 2

MATERIAL AND METHODS

CHAPTER (2)

MATERIAL AND METHODS

2.1 Collection and preparation of biomass

Pistia stratiotes were collected from a pond located at Hauz Rani City Forest Delhi, India (28.5159^0 N, 77.2111^0E) shown in Fig. 2.1. The average Plant size was 42.6 cm (Fig. 2.2), after the collection of a sample, it was washed with tap water 4 times and then air dried in shadow at room temperature for 80 h. The air-dried sample was put in the oven for 3 hrs. at 40 °C and further ground using an electric grinder. Grounded samples were sieved with particle size 425 µm and stored in an air-tight jar for further use at room temperature shown in Fig. 2.3 (Awoyale and Lokhat, 2021).

In our study, we have performed the optimization of acidic pretreatment on this weed to get the maximum sugar, which was not done previously by any researcher. The main objective of this model is to find out the main response in a particular area of interest, optimize the response, achieve the specific condition using a minimum number of experiments, and observe the interaction between the parameters (Gunst et al., 1996).

Fig. 2.1 Site of sample collection

Fig. 2.2 Average height in plant biomass

Fig. 2.3 Collection and preparation of sample

2.2 Reagents

All the reagents used in this study were of analytical gradient grade. Sulphuric acid and sodium hydroxide used for the acidic and alkali pretreatment were purchased from Fisher Scientific, India. For the ethanol analysis, HPLC grade ethanol, purchased from Sigma Aldrich, USA, was used in GC analysis.

2.3 Composition analysis of the biomass

The composition of biomass is essential to determine its capability for bioethanol production. Physicochemical analysis was performed on the raw sample through proximate analysis. The determination of the composition of biomass gives the exact component present in the biomass. Whenever the composition is identified then further experiments could be planned according to the component present in the biomass. We have determined ash, total solids, moisture, cellulose, hemicellulose, lignin, protein and starch content in our weed.

2.3.1 Determination of ash, total solids and moisture content

All of these composition were done by the NREL protocols. The ash content of oven dried sample was estimated by the muffle furnace. In a pre-heated crucible 1 g sample was burned at 575 ºC for 6 h. Then the crucible was put into a desiccator until it cooled down and weight was recorded (Fig. 2.4) (Sluiter et al., 2008)(Singh et al., 2017). The moisture content and total solid content were estimated by oven-dry procedure. The crucible was preheated at 105 °C for 4 h and its weight was recorded. 1g sample was put in the crucible at 105 °C for 4 h and then the crucible was transferred to the desiccator for cooling and record the weight after heating. Put the crucible again in a hot air oven for reheating until a stable weight is found (Singh et al., 2017). The estimation of all the compositions shown in Eqn. 2.1, 2.2 and 2.3.

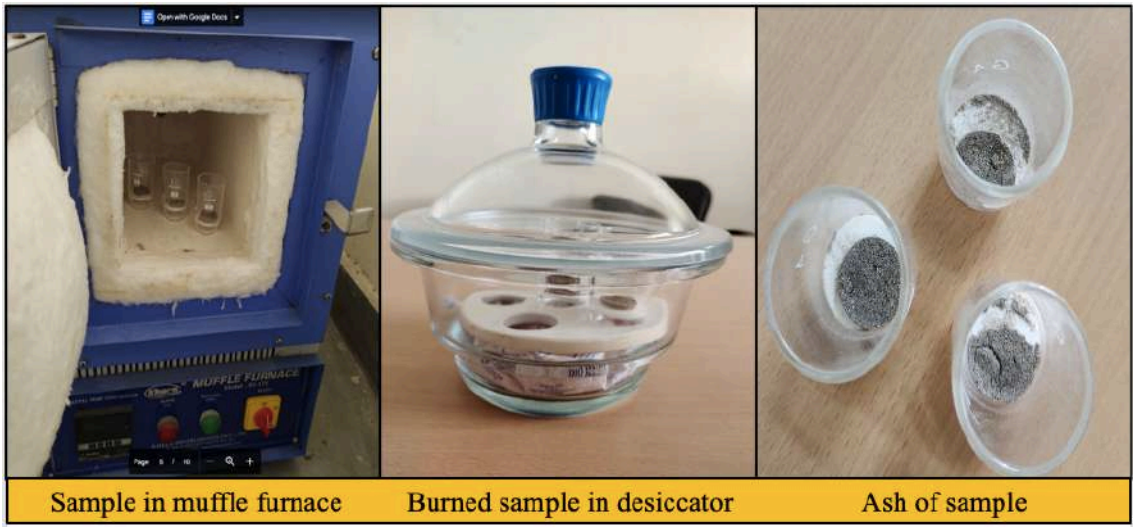


Fig. 2.4 Determination of ash content in the sample

$$\%\ Ash = \frac{Weight\ (crucible\ plus\ ash\ ) - Weight\ (crucible)}{ODW\ (sample)} x\ 100 \qquad (Eq.\ 2.1)$$

$$ODW = \frac{Weight\ (air\ dry\ sample\ ) x\ \%\ Total\ solids}{100} \qquad (Eq.\ 2.1.1)$$

$$Total\ solids\ (\%) = \frac{(weight_{dry\ pan+dry\ sample}) - (weight_{dry\ pan})}{Sample\ initially\ used} \times 100 \qquad (Eq.\ 2.2)$$

$$Total\ moisture\ (\%) = 100 - total\ solids \qquad (Eq.\ 2.3)$$

2.3.2 Determination of cellulose content

Cellulose content was estimated by the Up-degraff method (Bauer and Ibáñez, 2014).. Initially, for washing 70 mg sample was incubated in 1.5 ml of 70 % ethanol at 70 ºC for 1 hour and

then removed out ethanol by an aspirator twice. After that 1 ml of acetone was added for 2 minutes and then aspirated out and put the sample in a fume hood for 3-4 hours, keep the sample in the oven at 37 ºC overnight, resulting in what is known as alcohol-insoluble residue (AIR). This sample was stored at room temperature for further use. This AIR was then subjected to a 30-minute treatment at 100 ºC in a water bath using 3 ml Acetic nitric reagent (80% glacial acetic acid: 10 % nitric acid: 20 % Milli-Q). Following this treatment, the sample underwent rinsing with ethanol and water until cellulose became apparent. After that 4 ml acetone was added to the sample and evaporated in a fume hood. The resulting residue was subsequently placed in an oven overnight at 37 ºC. Subsequently, 1 ml of 67 % H_2SO_4 was added to the sample, and the mixture was placed in a shaker for thorough mixing. After the cellulose was effectively mixed with the acidic solution then glucose standards were made from 100mg/ml stock solution. Anthrone reagent was prepared by mixing 0.3 % anthrone in H_2SO_4. For testing 500 µl sample and blank take out in the Eppendorf and add 1 ml anthrone reagent in each, now boil each for 5 min and take the OD at 620 nm. The anthrone test was employed to estimate the amount of glucose present in the cellulose (Kumar and Turner, 2015).

2.3.3 Determination of hemicellulose

Estimation of the hemicellulose content was done by fibre analysis method (Wolfrum et al., 2009). The difference between Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) gives the total hemicellulose in a given sample (Eqn. 2.4). All the chemicals used in this experiment were procured from Fisher Scientific Ltd. A neutral detergent solution was prepared by EDTA, Sodium lauryl sulphate, sodium borate decahydrate, disodium hydrogen orthophosphate(anhydrous) and ethoxy ethanol whereas cetyl trimethyl bromide (c-tab) and acetone with 1 N H_2SO_4 was used for the preparation for acid detergent solution. 1 g sample was treated with 100 mL Neutral detergent solution with the addition of 2 mL decahydronaphthalene and 0.5 g sodium sulfite in a refluxing flask for 1 h. The sample was then transferred to the pre-heated crucible. The sample was filtered and washed two times with Milli-Q and acetone consecutively. Then the sample was transferred to the hot air oven at 100 °C for 8 h and then put in a desiccator, recording the weight after heating. On the other hand, for the determination of ADF, 1 g sample was treated with an acid detergent solution in a refluxing flask for 1 h and the same procedure was followed as for NDF (Holtzapple, 2003).

$$Hemicellulose\ (\%) = NDF - ADF \qquad (Eq.\ 2.4)$$

2.3.4 Determination of lignin content

This was performed by the NREL protocols. Before the estimation of the lignin, the extraction of the sample was performed using Milli-Q in a 1:20 ratio (Zhou et al., 2017). 300 mg extracted sample with 72% H_2SO_4 in a pressure tube was put in the water bath at 30 °C for 1 h with periodic stirring. 4% dilution was done using Milli-Q and the tube was autoclaved for 1 h shown in Fig. 2.5. The preheated and labelled crucible was weighted, and the sample was filtered by a filtration unit in the crucible and then washed twice. After that, the sample was dried at 105 °C for 4 h, cooled in a desiccator, and Acid Insoluble Residue (AIR) was calculated. Later, the crucible was transferred to a muffle furnace at 575 °C for 24 hours to calculate the % of ash in the filter (Xia et al., 2022). The lignin content (%) was calculated using AIR (%) and ash in the filter (%). Acid-soluble lignin was calculated by measuring absorbance at 280 nm (Nomanbhay et al., 2013). All the calculations were performed using the formulas mentioned below (Eqn. 2.5, 2.6 and 2.7)

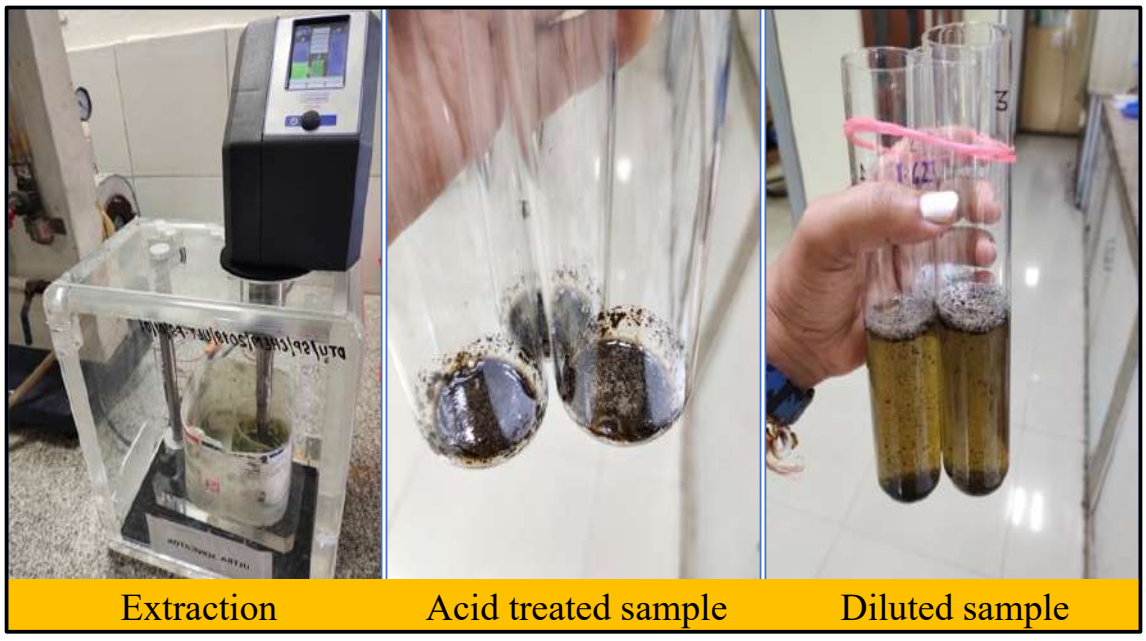


Fig. 2.5 Steps included in the estimation of lignin from the sample

$$\%\ Acid\ insoluble\ Lignin = \%\ AIR - \%\ Ash\ in\ filter \qquad (Eq.\ 2.5)$$

$$\% \, AIR \; = \frac{(Crucible \; plus \; AIR \; weight - Crucible \; weight)}{Sample \; loading \; in \; Crucible} \times 100 \qquad (Eq. 2.5.1)$$

$$\% \, Ash \; in \; filter = \frac{(Crucible \; plus \; ash \; weight - Crucible \; weight)}{Sample \; weight} \times 100 \qquad (Eq. 2.5.2)$$

$$\% \, Acid \; soluble \; lignin = \frac{UV_{absorbance \, (280nm)} \times Voulme \; of \; filtrate \times dilution}{Absorptivity \times dry \; weight \times pathlength} \qquad (Eq. 2.6)$$

$$\% \, Lignin = \% \, Acid \; insoluble \; lignin + \% \, Acid \; soluble \; lignin \qquad (Eq. 2.7)$$

2.3.5 Determination of starch

Starch was estimated by anthrone reagent. Chemicals used in this composition were anthrone reagent which was prepared in 95% sulphuric acid, 52% perchloric acid and 80% ethanol. The initial step of this was done by washing the sample with 80% ethanol repeatedly till the colour of the residue was removed. Then the residue was dried in a hot air oven, mixed with perchloric acid, and incubated for 20 min. The tubes were centrifuged at 10000 rpm for 10 minutes, and the supernatant was taken out. The glucose standard was prepared using 1 mg/mL stock solution. Anthrone is added in the supernatant of sample and glucose standard, and heated at boiling temperature. After heating, the reading was taken on a spectrophotometer at 630 nm for colour detection and starch concentration was calculated (Zorić et al., 2019).

2.3.6 Determination of proteins

Protein estimation was performed by the kjeldahl method with N factor 6.25. Three steps are included for the estimation of nitrogen these are digestion, distillation and titration. For digestion, 1 g sample was mixed with 7 g K_2SO_4, 0.8 g $CuSO_4$ x $5H_2O$ and 12 ml H_2SO_4. All

the chemicals are procured by Fisher Scientific. The sample was poured into the digestion tube with a blank where the sample was not present in other chemicals. Digestion was done at 420^0C for 1 hour and then cooled for 2 hours for further step which is distillation. The distillation unit is made up of four subunits which are alkali, receiver, distilled water and wastage tank. Bromocresol green and methyl red dyes are used as indicators in the boric acid solution. Distillation was performed automatically by the Foss instrument for protein analyser. Dilution of the digested sample with 80 ml H_2O and then add 50 ml of 40 % NaOH solution in it. The receiver flask contains 30 ml of receiver solution. The distillation process released the ammonia from the sample. The whole process takes only five minutes. After distillation titration was done by 0.1 N HCL for nitrogen estimation in the sample shown in Fig. 2.6 (Appenroth et al., 2017). Formula used for nitrogen and protein estimation shown in Eqn. 2.8 and 2.9.

$$\% N = \frac{(T - B)\ X\ N\ X\ 14.007\ X\ 100}{WEIGHT\ sample\ (mg)} \qquad (Eq. 2.8)$$

T= Sample titration

B= Blank titration

N= Normality of titrant

$$\% \text{ Protein} = N \text{ X } F \qquad (Eq.\ 2.9)$$

F= 6.25 for cereals (AOAC 945.18 B)

Fig. 2.6 Steps included in the estimation of the protein in the sample

2.4 Biomass characterization

The characterization of the sample was done by Fourier-transform infrared (FTIR) spectroscopy, TGA (Thermo-galvanometric analysis), and scanning electron microscopy (SEM). The FTIR analysis and TGA were done at the Delhi Technological University, Delhi (India), while SEM was carried out at AIIMS, Delhi (India). The details for this analysis are mentioned below.

2.4.1 Fourier-transform infrared (FTIR) spectroscopy

The changes in functional groups after providing the acidic and alkali pretreatment were analysed by FTIR spectroscopy (PerkinElmer 400 FTIR/FTIR) control with a frequency range of 4000 cm^{-1} – 400 cm^{-1} with the control sample. The pellet preparation was done with potassium bromide (KBr) and scanning was performed at 4 cm^{-1} resolution (Kataria et al., 2018b). 10 mg sample was used and the ratio of KBr and sample was 100:1.

2.4.2 Thermogravimetric analysis (TGA)

The TGA of raw and pre-treated samples were performed by PerkinElmer TGA 4000. 10 mg samples were heated from room temperature to 600 °C at a heating range of 10 °C min^{-1} with 10 mL/min nitrogen flow rate (Kataria et al., 2018b)(Umesh et al., 2022a).

2.4.3 Scanning electron microscopy (SEM)

The changes in the morphology of the pretreated sample were investigated with SEM (Model: EV018 Zeiss, Germany) with 5 kV voltage at 10,000 magnifications. The breakdown of the cell wall was identified after SEM analysis (Umesh et al., 2022a).

2.5 Statistical analysis and model fitting in RSM design

A combination of mathematical and statistical methods in RSM is used for making the model formation by which optimization of factors is done. The RSM method takes quantitative data from different experiments to estimate the regression model for optimization of responses (dependent variable) affected by process variables (independent variables) (Hinkelmann, 2012). Central composite design (CCD) is a fractional factorial design consisting of 2^n factorial runs with $2n$ axial runs and centre runs that decide the experimental error. Independent variables determine the number of runs in the model. If the number of variables increases, the number of runs also increases in the replicates of the model. RSM method for optimization consists of three steps. The first step is designing of experiment statistically, the second step is to calculate the coefficients in the model, and the last determine the response and examine the competency of the model within the design of the experiment (Mahalik et al., 2010).

The statistical tool known as Analysis of variance (ANOVA) was employed for divination of the statistical factors required in the assessment of the RSM model between the independent variables and response of parameters. Utilising the software Design Expert, regression analysis of trial data was employed to generate the Contour plot, Pareto chart, and optimised condition. The influence of independent variables on the cellulose concentration is shown by the contour plot and pareto chart. The outcome of the model predicted statistically significant values by the F test and p-value. If the value was less than 0.05, then the model was stated as statistically significant with a lack of fit test that is insignificant in the same model. Independent factors significantly impacting the response were determined by p test value less than 0.05 with a confidence level above 95 %. The significance of the model was accessed by R^2 predicted and R^2 adjusted value (Ramaraj and Unpaprom, 2019).

In this study, three independent variables (acid concentration (X_1, vol.%), time (X_2, min) and temperature (X_3, °C) with two dependent variables (reduced sugar (mg/mL) and cellulose (%)) as response were chosen for experiment design. These three independent variables along

with their respective ranges were found to be critical parameters for maximum sugar production. It is expected in the design that the independent variable is managed by experiments with minor errors. The main aim of this model was to optimize the response variables (Y). This statistical model gives the approximate correlation between independent variables and dependent variables (responses) (Gunaraj and Murugan, 1999). Experiments were run in random order for negligible error in the model. To determine the ideal condition divided into linear, quadratic, and interactive components, the effects of independent variables on the dependent variable (response) were estimated using a polynomial equation (Eqn. 2.10).

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + e \qquad (Eq. 2.10)$$

where Y is the variable of Response; β_o constant coefficient; β_i the linear constant; β_{ii} the quadratic coefficients; β_{ij} the interaction effect of coefficients; X_i and X_j the coded values used for variable parameters; and e is the random error (Dahunsi et al., 2019).

2.5.1 Optimization of acidic Pretreatment using RSM

Acidic pretreatment was performed with sulphuric acid. The experiment design was done to optimize the conditions and determine the maximum reduced sugar liberation that was further used in ethanol production. The biomass sample was mixed with acid with a 10% biomass loading. Pretreatment was done in an autoclave and neutralization of the sample was done by the $CaCO_3$ for detoxification. After pretreatment, the estimation of reduced sugar is done by the DNSA method and cellulose is determined by the up-degraff method. Experiment designing for pretreatment was done by the Central Composition Design (CCD) and the Response Surface Methodology (RSM) approach was used to analyse the data (Awoyale and Lokhat, 2021). Three variables, including Temperature (93.18 °C, 100 °C, 110 °C, 120 °C and 126.82 °C), Time (12.96, 30, 55, 80 and 97.04 min) and Acid concentration (0.15%, 0.75%, 1.63%, 2.5% and 3.1%) were selected to find out the optimized condition. Reducing sugar and cellulose was recorded as a response for this design. A total of 20 runs in design with three levels (-1, 0, +1) with a 1.68 alpha value were performed. Parameters are mentioned with alpha value in Table 2.1.

Table 2.1 Independent variables with experimental levels in the RSM model for Acidic pretreatment

Factors	Symbols	Units	- 1.68	-1	0	+1	+1.68
Acid Concentration	A	% (w/v)	0.15	0.75	1.63	2.5	3.1
Time	B	Minutes	12.96	30	55	80	97.04
Temperature	C	°C	93.18	100	110	120	126.82

After the pretreatment, the detoxification of liquid residue was done by calcium carbonate ($CaCO_3$) powder. Toxic compounds may be retained in the hydrolysate, which can inhibit the microorganism's growth. After pretreatment in an autoclave, the residue was centrifuged, and the liquid hydrolysate was separated from the solid fraction. This liquid fraction was detoxified by adding calcium carbonate to liquid hydrolysate and pH was monitored by the amount of calcium carbonate addition shown in Fig. 2.7 (Ahmed et al., 2019).

2.5.2 Detoxification of hydrolysate

Dry biodetoxification was done by CaCO3 powder because during this biodetoxification phenolic compounds (furfural, HMF) were not retained in the hydrolysate which are toxic. Detoxification of hydrolysate was done after autoclaving it then the hydrolysate and separate the solid and liquid fractions found after centrifugation. This liquid fraction was neutralized by calcium carbonate by mixing it with liquid hydrolysate and then removed by centrifugation at 1000rpm for 5 min. On the other hand, neutralization was also done by the aqueous alkali solution but this detoxification was not done only neutralization was done (Ahmed et al., 2019).

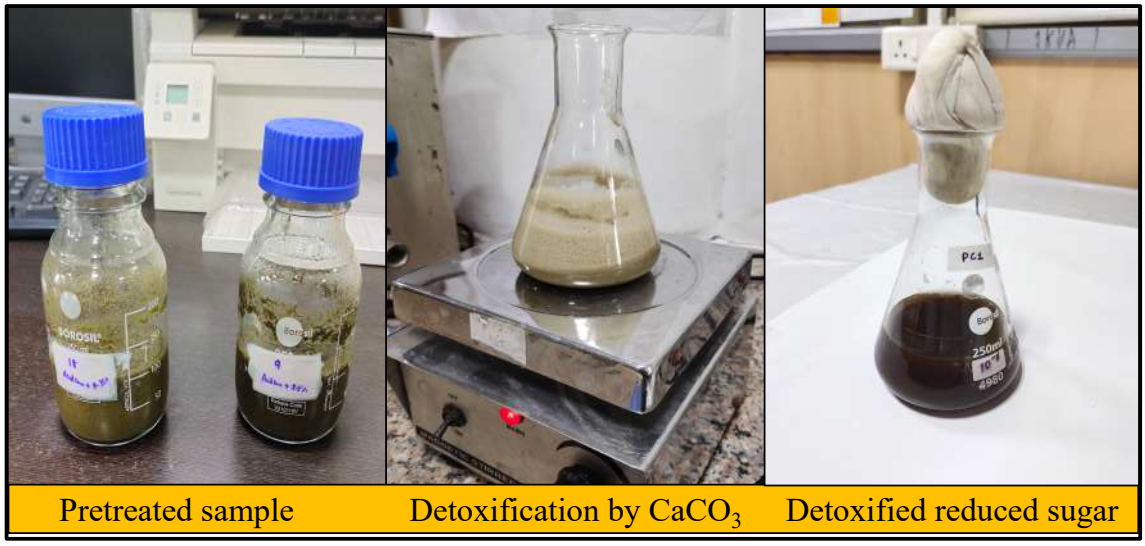


Fig. 2.7 Pretreated sample and detoxification of pretreated sample with $CaCO_3$

2.5.3 Optimization of alkali pretreatment using RSM

RSM is a multivariant statistical tool that provides a new approach for determining the ideal pretreatment state. Design Expert software assisted in the development of a central composite design, which was used to determine the effects of various independent variables on cellulose extraction.

RSM was responsible for determining the results. The variables in this model were represented by codes, which were represented by the numbers -1,0, and 1. The neighbouring distance from the value of the central point determines how variables are coded. The experiment's independent variables, such as the temperature, time, and NaOH concentration, were transformed into code variables Xi (Eqn. 2.11)

$$Xi = 2\left(\frac{OV - V}{\Delta}\right) \qquad (Eq.\,2.11)$$

Equation 2 comprises the following terms:

Δ (difference between largest and smallest values (range)), OV (original variable), and V (average of the largest and smallest values of variables or mid value). Three independent variables with lower and higher levels are mentioned in Table 2.2.

Table 2.2 Independent variables with experimental levels in the RSM model in Alkali pretreatment

Symbols	Independent variables	Unit	Relation of codes with original independent variables		
			-1	**0**	**1**
A	NaOH concentration	ºC	0.5	1.75	3
B	Reaction Time	min	30	45	60
C	Reaction Temperature	% w/v	60	90	120

Three variables, including, NaOH concentration (0.5, 1.75, and 3 %), Time (30, 45, and 60 min) and Temperature (60, 90, and 120 ºC) were selected to find out the optimized condition. Cellulose was recorded as a response to this design. A total of 20 runs in design with three levels (-1, 0, +1) were performed.

2.6 Enzymatic hydrolysis

After pretreatment, the sample was washed until the neutral pH and then dried for further use. 10 ml of citrate buffer (0.05M, pH 4.8) was added to 150 ml test tubes containing 0.5 g of both pretreated and untreated samples for enzymatic hydrolysis. The commercially available cellulase enzyme (40000 U/g) was sourced from IndiaMart, and the Filter Paper Unit (FPU) was calculated following NREL protocols. The cellulose loading was adjusted to 25, 50, and 75 FPU/g of dry biomass.

The hydrolysis was carried out in an incubator shaker (New Brunswick Innova 44 series, Germany) with a rotation speed of 150 rpm for 96 hours at 50 ºC. Samples were withdrawn at 24, 46, 72, and 96 hours. The reducing sugar was quantified using the DNSA method as described by Liu et al. (2021). After enzymatic hydrolysis, the sugar yield was determined using Eqn. 2.12, and each experiment was conducted in triplicate.

$$Sugar\ yield\ (\%) = \frac{0.9\ X\ reducing\ sugar\ \left(\frac{g}{L}\right) X\ volume\ (L)}{solid\ cellulose\ fibre\ wt.(g)}\ X\ 100 \qquad (Eq.2.12)$$

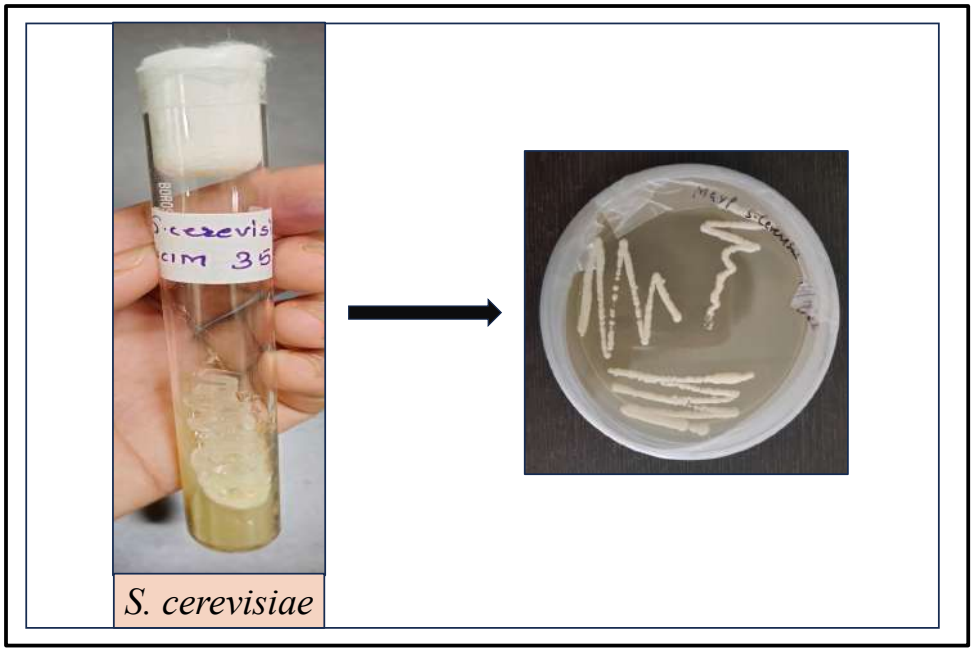
2.6.1 Total reducing sugar analysis

Total reducing sugars in the liquid hydrolysate were determined by the 3,5-Dinitrosalicylic acid (DNSA) method (Miller, 1959). DNSA reagent was prepared and stored in an amber bottle for further use. Glucose stock solution was prepared for standard curve determination. This DNSA reagent was added to the standards as well as a sugar sample, boiled for 15 min, and cooled down at room temperature. The sample's optical density (OD) was measured at 540 nm for the estimation of reducing sugar with the help of a standard curve (Miller, 1959). The total reducing sugar of raw and pre-treated samples was quantified.

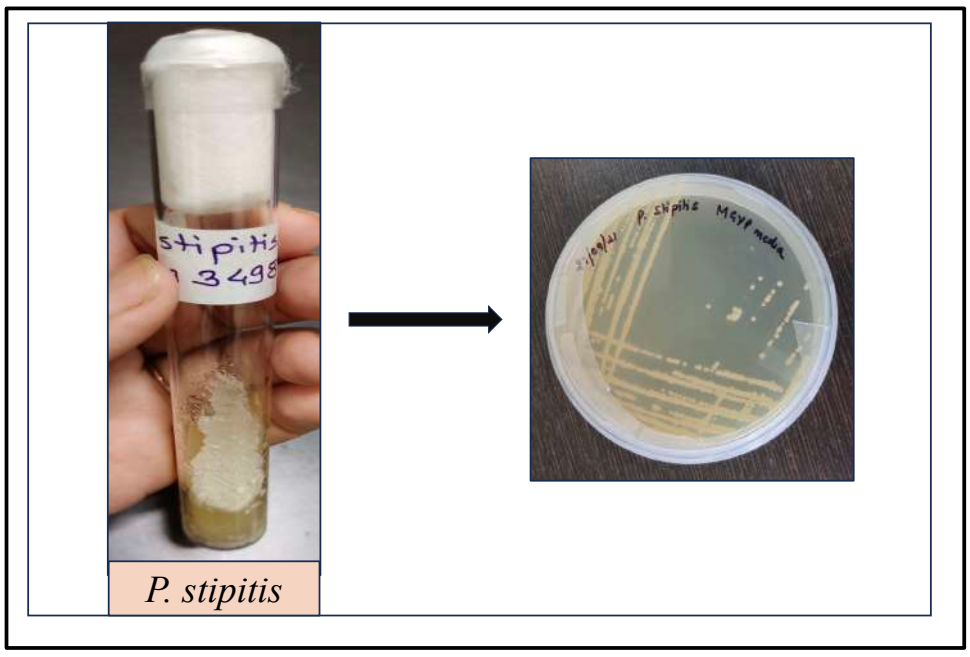
2.7 Fermentative process

2.7.1 Microorganisms and culture revive

Saccharomyces cerevisiae (ATCC 834) (NCIM 3594) and *Pichia Stipitis* (NCIM 3498) for ethanol production were procured from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune (India) in the slanted form (active culture) at 4^{o} C. Both of the strains were maintained in MGYP media as shown in Fig. 2.8. For reviving the stains they were transferred to the petri plate from the active slant by the prescribed media. For 100 ml of media, 0.3 g malt extract,1 g glucose, 0.3 yeast extract, and 0.5 g peptone in 100 ml milli Q with 2 g agar were taken by adjusting the pH 6.4- 6.8. This media was autoclaved before pouring media into the petri plates for streaking the culture. After streaking the plates were inoculated at 28 °C for 24 h and stored the plate at 4 °C for further use.



(a)



(b)

Fig. 2.8 Microbial strains procured from NCIM, Pune and revived on petri plate (a) *S. cerevisiae* (b) *P. stipitis*

2.7.2 Growth curve of *Saccharomyces cerevisiae* and *Pichia stipites*

The growth of these two microbes in the MGYP broth was determined by a shake flask assay. The first step is to prepare the seed culture in the same media. Inoculate the fresh culture from the petri plate in the MGYP broth keep on a shaker at 150 rpm for 12 hours and inoculate it in a secondary culture for growth and sugar analysis.

2.7.3 Inoculum Preparation

S. cerevisiae and P. stipitis were grown in the MGYP media (10g/L glucose, 3g/L malt extract, 3g/L yeast extract and 5 g/L peptone) (Ben Bader et al., 2022). Seed culture was prepared in the conical flask with 50 ml of media inoculated with a loopful of cells and incubated in an orbital shaker at 150 rpm at 30 °C. The mid-exponential phase culture was used to inoculate of culture media with an optical density of around 0.8, which was measured at 600 nm in a UV-VIS spectrophotometer (Gonçalves et al., 2016). The fermentative media was inoculated with 5% (v/v) of seed culture for ethanol production.

2.7.4 Hydrolysate filtration after pretreatment :

Following the acidic pretreatment, calcium carbonate is used to neutralise the hydrolysate to detoxify the sample and estimate its sugar content. This sample was filtered with a syringe filter or vacuum filter to remove any excess solid material before being used for fermentation with the addition of microbes. Following a vacuum filter, the material was processed into a pure hydrolysate (Fig. 2.9), which contained sugar and was ready for S. cerevisiae and *P. stipitis* to use to convert it to ethanol.

Fig. 2.9 Pretreated sample prepared for fermentation after vacuum filtration

2.7.5 Fermentation for ethanol production

Fermentation was performed in a 250 mL shake flask with 150 mL working volume containing synthetic media (malt extract 0.3%, yeast extract 0.3%, peptone 0.5% and glucose 1%) and hydrolysate detoxified by $CaCO_3$ in a triplet. For fermentation, 5% (v/v) of the seed culture was inoculated in the detoxified hydrolysate. All the experiments were performed for 56 h. Sample collection was done periodically for determination of cell growth, sugar consumption and ethanol production. The ethanol was separated by Gas chromatography (Clarus 580 $_{PerkinElmer}$, USA) equipped with a ZB-wax column (60 m X 0.32 mm internal diameter 0.25 um; Phenomenex, UK). The oven temperature was set in between 150 °C and 180 °C. Ethanol data was captured from programmed software (Total Chrome Workstation Ver 6.3) that was pre-installed. The ethanol estimation in the sample was done by comparing it with the standard of ethanol (EMSURE ACS, Sigma Aldrich, USA). All samples are performed in triplicates and the concentration was mentioned in the g/L (Goswami et al., 2022).

2.7.6 Calculation of ethanol-related kinetic parameters

The kinetic parameters related to ethanol yield were analysed based on the aforementioned reports (Eqn. 2.13, 2.14 and 2.15) (Pereira et al., 2015)(Pooja et al., 2018)(Mithra et al., 2018).

$$Ethanol\ Yield(\mathrm{YE}) = \frac{Ethanol\ concentration\left(\frac{g}{L}\right) in\ fermentation\ media\ (Ef)x1}{Sugar\ consumed\ (\frac{g}{L})} \qquad (Eq.2.13)$$

$$Volumetric\ Ethanol\ Productivity\left(\frac{\frac{g}{L}}{h}\right) = \frac{Ehanol\ concentation\left(\frac{g}{L}\right) in\ fermented\ broth}{Fermentation\ time\ (h)} \qquad (Eq.2.14)$$

$$Fermentation\ efficiency(\%) = \frac{Ethanol\ yield(YE)X100}{Theoretical\ ethanol\ yield} \qquad (Eq.2.15)$$

CHAPTER - 3

RESULT AND DISCUSSION

CHAPTER (3)

RESULT AND DISCUSSION

3.1 Composition analysis of Raw sample

In the *P. stratiotes*, the carbohydrate concentration is high so that it can be used as a carbon source for ethanol production (Table 3.1). The amount of cellulose is observed as 25.29% with a low level of lignin (16.73%) in comparison to another study by Sutario et al. The lignin composition is 34.85% (Sutaryo et al., 2022). In the present study, 18.44% hemicellulose was observed while Mishima et al. reported 17.3% (Mishima et al., 2008a). This variation in compositions could be due to cultivation conditions, age, and location. Approximately 60% of biomass was composed of carbohydrates in this *P. stratiotes*. *P. stipitis* utilizes both pentose and hexose sugar from the hydrolysate by which maximum production of ethanol may be achieved. Considering the convenience and utilization, this plant was taken as a carbon source for bioethanol production.

Table 3.1 Composition analysis of *Pistia stratiotes*

Pistia stratiotes		
S. No.	Biochemical composition	(%)
1	Ash content	18.36 ± 1.31
2	Total solid	92.85 ± 0.11
3	Moisture content	7.15 ± 0.11
4	Cellulose	25.90 ± 0.017
5	Hemicellulose	18.44 ± 0.71
6	Lignin	25.25 ± 1.15
7	Starch	0.6 ± 0.03

3.2 Optimization of alkali pretreatment by RSM and validation

After pre-treating *P. stratiotes* with alkali (NaOH), which had an impact on the raw material's depolymerization, cellulose was the final product, with lignin removed. The reduced sugar obtained from the hydrolysis of cellulose can be used to further produce ethanol. The best conditions were achieved by optimising biomass using RSM, which resulted in 2.47 (w/v) NaOH at 120 ºC for 60 min after autoclaving. The optimal condition gives the result of 51.66 % cellulose. The result of the alkali pretreatment under various conditions is cellulose, which is shown in Table 3.2. Cellulose was estimated by the up-degraff method with the help of anthrone reagents shown in Fig. 3.1.

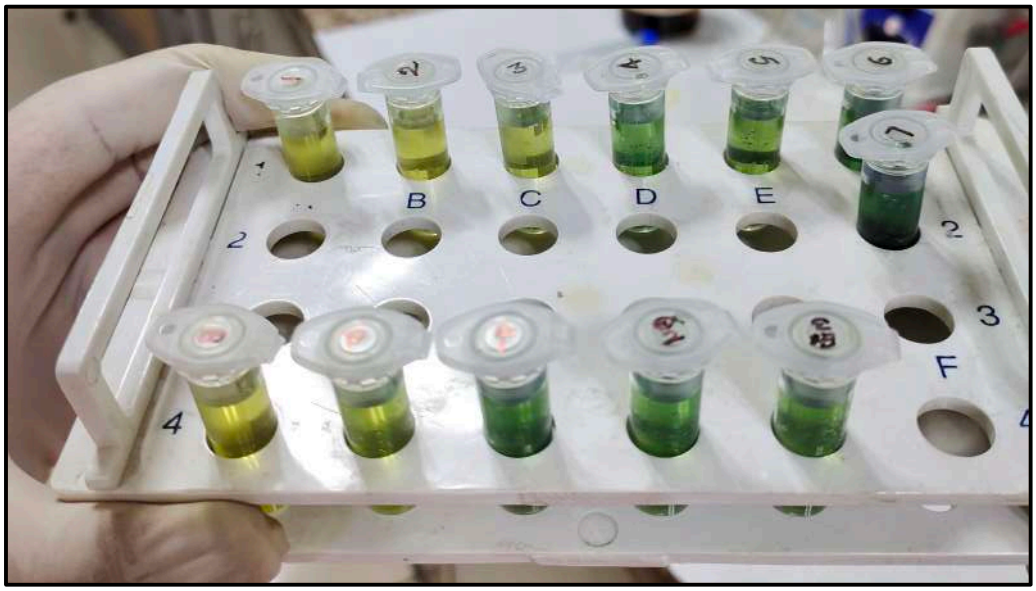


Fig. 3.1 Cellulose estimation by anthrone reagent

Table 3.2 Experiment list with different levels of independent variables (alkali conc., time and temperature) with response (cellulose)

Experiment no.	Alkali concentration	Time	Temperature	cellulose (%)
1	1.75	45	90	37.3 ± 2.61
2	1.75	45	120	41.5 ± 3.51

3	1.75	45	90	36.92 ± 2.44
4	3	60	120	50.88 ± 1.40
5	0.5	30	60	27.38 ± 0.93
6	1.75	45	90	35.01 ± 2.95
7	3	30	60	36.31 ± 3.24
8	0.5	60	60	27.64 ± 2.61
9	0.5	30	120	21.17 ± 1.04
10	1.75	45	90	34.39 ± 2.1
11	1.75	45	60	34.2 ± 1.38
12	1.75	45	90	35.27 ± 0.72
13	3	60	60	27 ± 3.83
14	3	45	90	32.57 ± 1.91
15	3	30	120	41.12 ± 2.98
16	1.75	45	90	34.08 ± 2.51
17	1.75	60	90	40.83 ± 3.25
18	1.75	30	90	34.67 ± 2.34
19	0.5	60	120	42.28 ± 3.41
20	0.5	45	90	29.15 ± 2.39

The factors that affect the expected changes in the mean response when the factors change from a lower to a higher level, also establish the coded coefficients. The alkali concentration shows a major positive impact so this parameter has an effect on increased response (cellulose concentration) proceeding from 0.5 % to 3 %. If the alkali concentration is higher, than the cellulose concentration will be high. However other factors like time, temperature, alkali conc.*alkali conc. and alkali conc.*time, shows the negative sign which successively impacts reducing the response.

3.2.1 Analysis of Variance (ANOVA)

Table (B) illustrates an ANOVA statistical model for cellulose estimation from CCD. ANOVA and the lack of fit test are used to analyse the model's fitness. When the experimental data fits the model, significant regression and non-significant lack of fit are displayed. This model's statistical advantage was evaluated and attributed to the interactions between the model's factors and p-value. Table 3.3 shows that the model's p-value for cellulose concentration is 0.000, which is less than 0.050 and suggests that the response to the model is substantial and meaningful. The model is considered highly significant when the p-value is less than 0.001. A p-value greater than 0.1 indicates that the model is not significant. However, because of the large f value (53.79), the model result has a significant impact on the response. This f-value is probably going to demonstrate the significance of the model. There is only a 0.01 % chance in the statistical model that a large f value could arise (Pashaei et al., 2020).

Table 3.3 Analysis of Variance (ANOVA) results and statistical parameters of the model quadratic correlation versus alkaline conc., reaction time and reaction temperature

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	9	818.693	90.966	53.79	0.000
Model	9	79.187	26.396	15.61	0.000
Linear	3	44.593	44.593	26.37	**0.000**
Alkali conc.	1	15.012	15.012	8.88	**0.014**
Time	1	30.042	30.042	17.77	**0.002**
Temp.	1	40.584	13.528	8.00	**0.005**
Square	3	38.954	38.954	23.04	**0.001**
Alkali conc.*Alkali conc.	1	3.007	3.007	1.78	0.212
Time*Time	1	3.576	3.576	2.11	0.177
Temp.*Temp.	1	305.185	101.728	60.16	**0.000**
2-Way Interaction	3	54.702	54.702	32.35	**0.000**

Alkali conc.*Time	1	51.288	51.288	30.33	**0.000**
Alkali conc.*Temp.	1	199.195	199.195	117.80	**0.000**
Time*Temp.	1	16.910	1.691		
Error	10	8.084	1.617	0.92	0.537
Lack-of-Fit	5	8.826	1.765		
Pure Error	5	835.603			
Total	19				

3.2.2 Model summary

The adjusted R^2 of 96.15 % and the predicted R^2 value of 92.48 % agree rationally, indicating a difference of less than 4 %. This result demonstrates that this model can accurately represent the percentage of cellulose extraction from *P. stratiotes* and can be shown as coded variables, as shown in the regression equation Eqn. 3.1.

$$\textit{Cellulose} = 64.90 + 12.18\ \text{Alkali conc.} - 0.986\ \text{Time} - 0.697\ \text{Temp.} - 2.409\ \text{Alkali conc.*Alkali conc.} + 0.00465\ \text{Time*Time} + 0.001267\ \text{Temp.*Temp.} - 0.1395\ \text{Alkali conc.*Time} + 0.0675\ \text{Alkali conc.*Temp.} + 0.01109\ \text{Time*Temp.} \quad (Eq.\ 3.1)$$

3.2.3 Contour plot

The contour plot describes the impact of different variables on the response change in cellulose content depending on the temperature and time). The effects of temperature and time on cellulose are interpreted in Fig. 3.2. A notable increase in cellulose is seen as a result of the temperature rising over time. A rise in cellulose content of more than 50% was noted after 55–60 minutes at 110–120 ºC. Conversely, after pretreatment, a higher amount of cellulose content is obtained by raising the temperature and alkali concentration. The maximum cellulose content, or >50%, is achieved at 120 ºC and >2.5 % alkali content, as Fig. 3.3 illustrates.

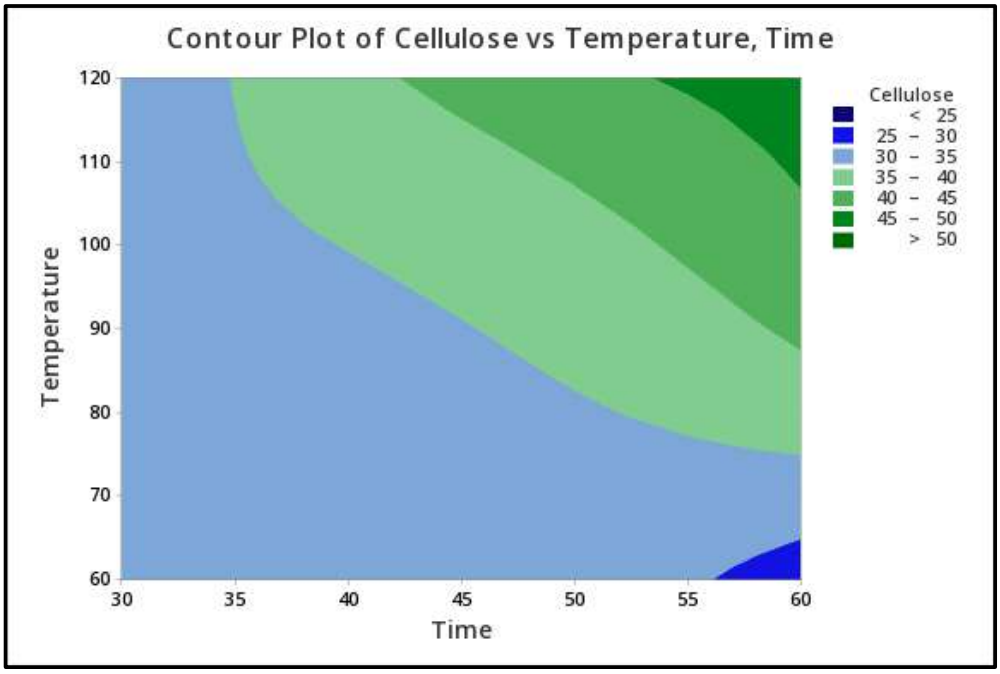


Fig. 3.2 Contour plot shows the effect of temp. and time on the cellulose content. An increase in cellulose content was noticed when temperature increased with time

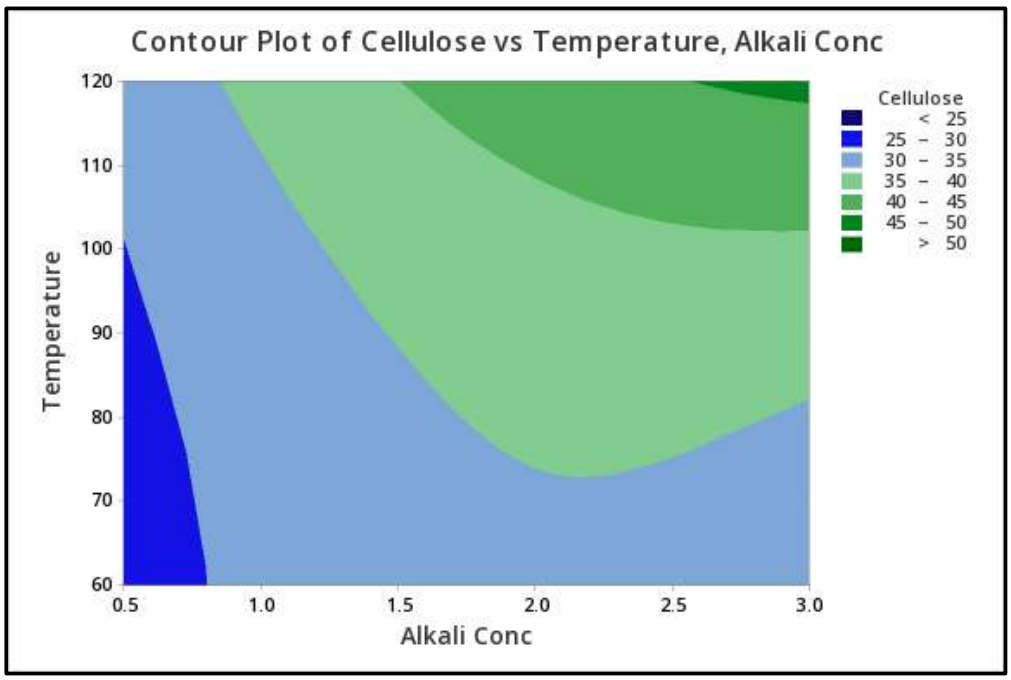


Fig. 3.3 Contour plot demonstrates the effect of alkali conc. and temperature on cellulose content. With the increase in the alkali conc. with temperature, the cellulose content also rises

3.2.4 Pareto chart

The Pareto chart was used to clearly illustrate the significance and magnitude of the impact of independent variables on response. The complete values of the regulated effects, arranged from highest effect to lowest effect, are shown in the pareto chart. The chart's reference line indicates statistical significance for the effect. The statistical significance is shown by the bar that crosses the reference line. The cellulose pareto chart is shown in Fig. 3.4. This chart indicates that the parameters are statistically significant because of time * temperature (BC), alkali conc.* time (AB), alkali conc.* temp. (AC), alkali conc. (A), alkali conc.* alkali conc. (AA), temp. (C), and time (B) cross the reference line.

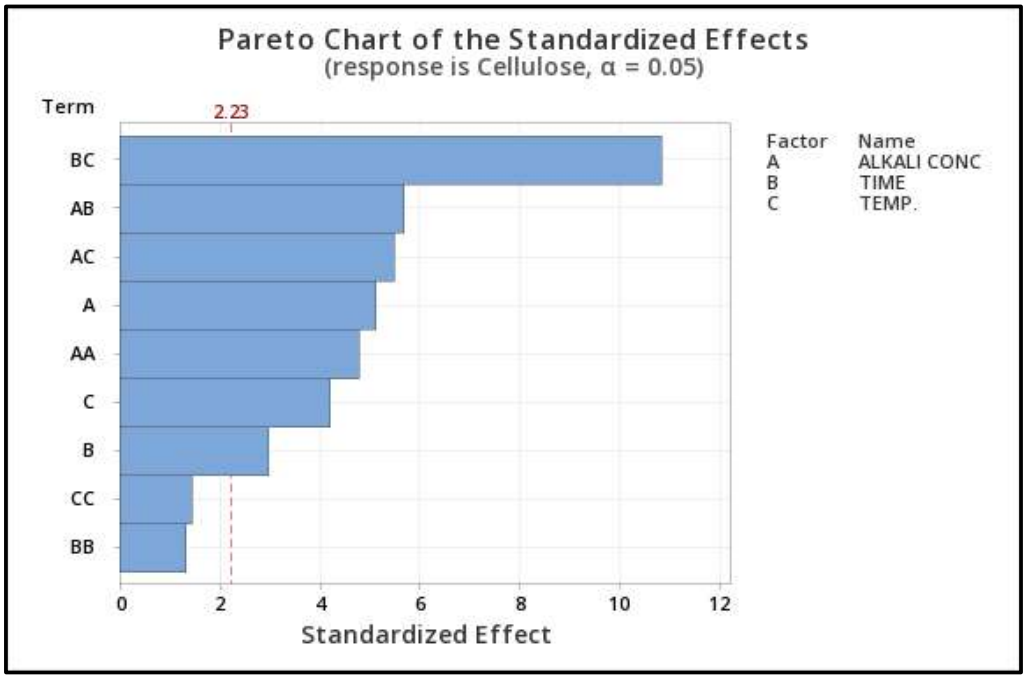


Fig. 3.4 Pareto chart of standard effects of independent variables on response. This chart illustrates that the factors time *temp., alkali conc.*time, alkali conc.*temp, alkali conc., alkali conc.*alkali conc., temp., and time are statistically significant

3.2.5 Response outcome after multiple response prediction

RSM was used to determine the response (cellulose content) outcome, and it fit 51.66 % of the cellulose content with a standard error of 1.06. With a prediction interval ranging from 47.92 to 55.39 %, the confidence interval fell between the range of 49.92 to 54.01 % cellulose content.. After statistical analysis of the optimisation model, the target cellulose content was 50.87 %, and the objective was to maximise the cellulose outcome with a lower cellulose content of 21.17 %. The ideal condition was discovered at 2.4 % alkali concentration with 60 min at 120 ºC temperature after multiple responses. Post the optimization experiment the cellulose fit was 51.65 % with 1 % composite desirability.

3.2.6 Optimization plot

Optimization of alkaline pretreatment was performed, optimized condition considered at 2.47 % NaOH, 120 ºC with 60 min. This optimized condition was performed separately to check the reliability. After performing the optimized condition, 51.67 % cellulose was found in the hydrolysate which shows the reliability of the model. A model created by Minitab software in which independent variables are adjoined to estimate the target response. Combined desirability ranges from 0-1 are helpful for the calculation of optimization. Separate desirability for the optimization experiment is shown in Fig. 3. Cellulose has an independent desirability outcome of 1.0 as a predicted outcome of 51.65 %. The separate desirability of 1.0 is an excellent score which shows that response is immediate to their absolute setting. The optimization of response (cellulose) gives an ideal desirability score as cellulose was an absolute setting that is included in the justifiable range. The optimized condition established after the RSM model is represented in Fig. 3.5. The sample colour and texture changed after performing all the pretreated conditions as shown in Fig. 3.6

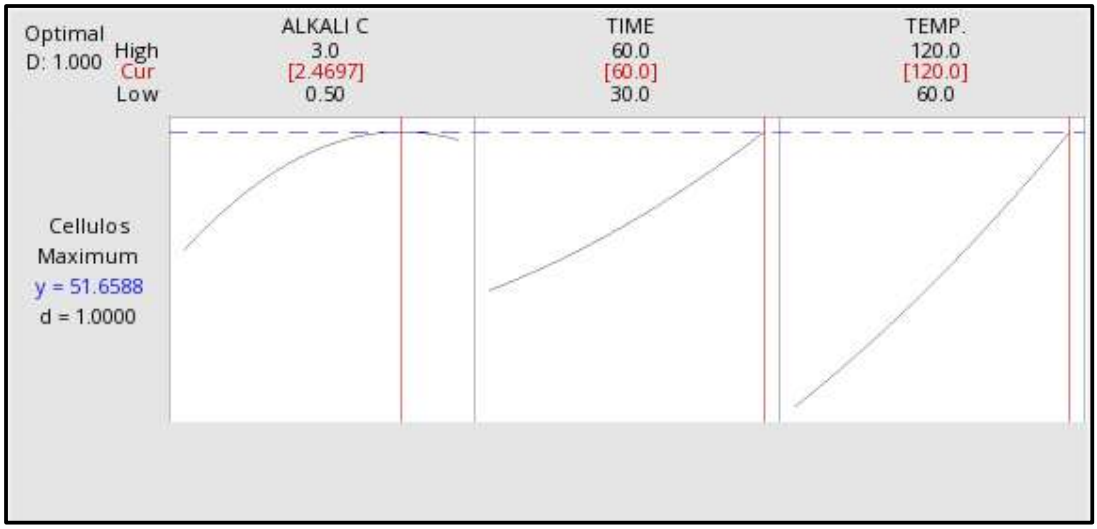


Fig. 3.5 Optimized condition demonstrates when the cellulose substrate is treated with high conc. of alkali (2.47 %) at 120 ºC for 60 min then the maximum cellulose content was obtained with optimal density 1 which is statistically significant.

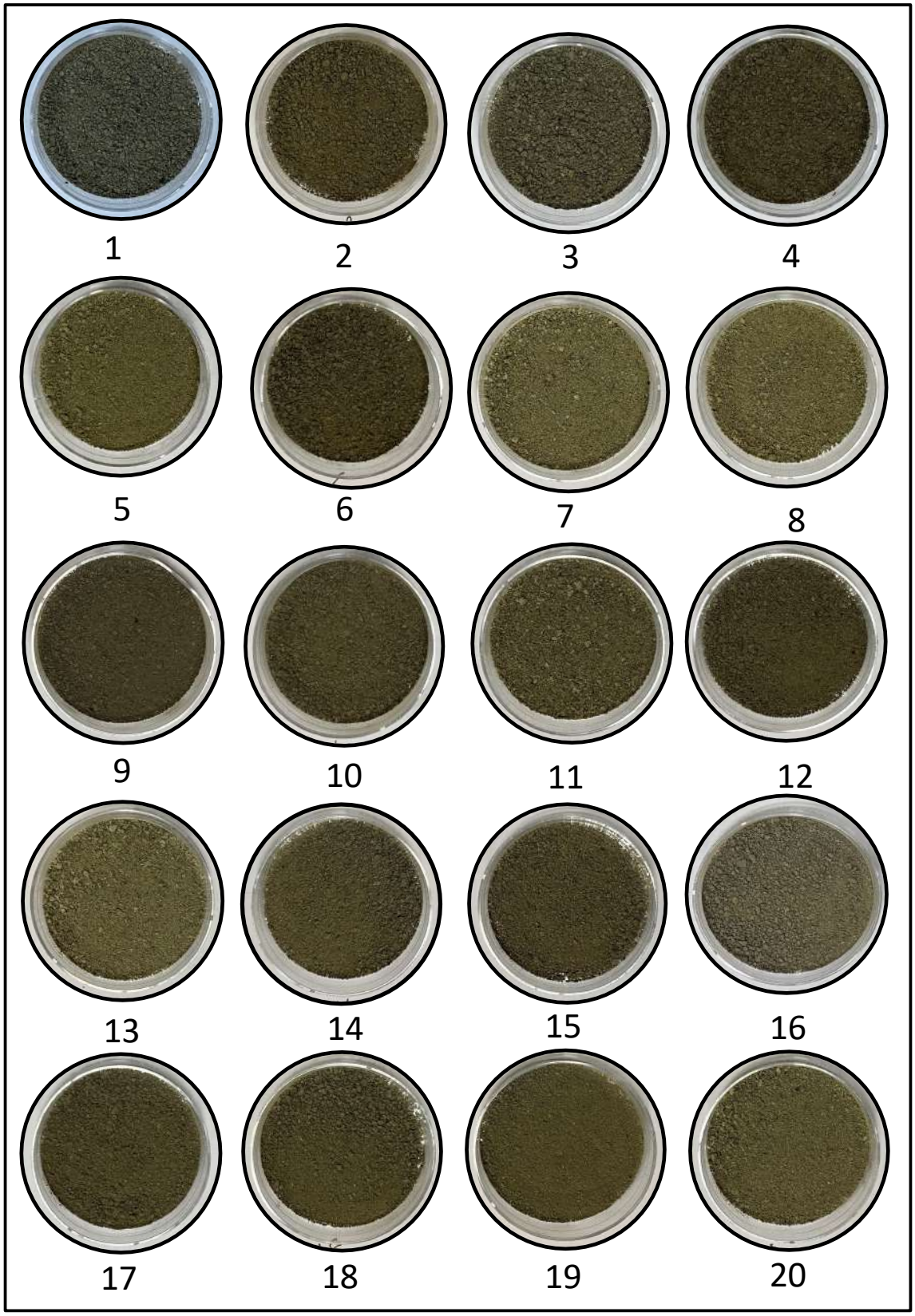


Fig. 3.6 Alkaline pre-treated samples after RSM conditions were performed

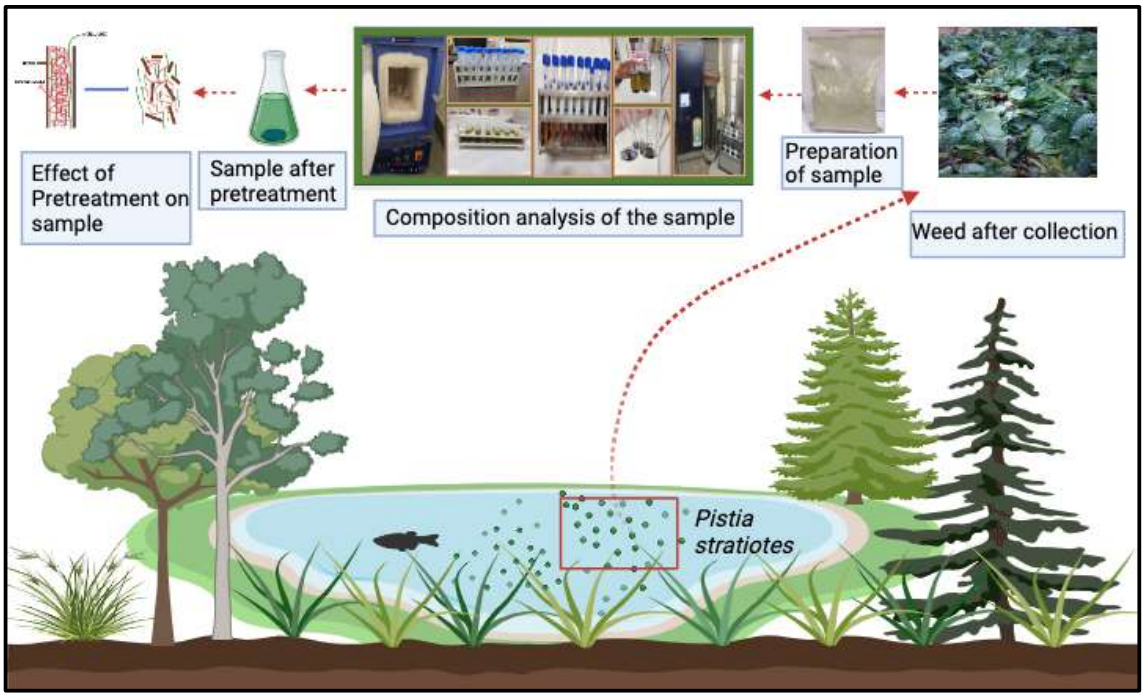
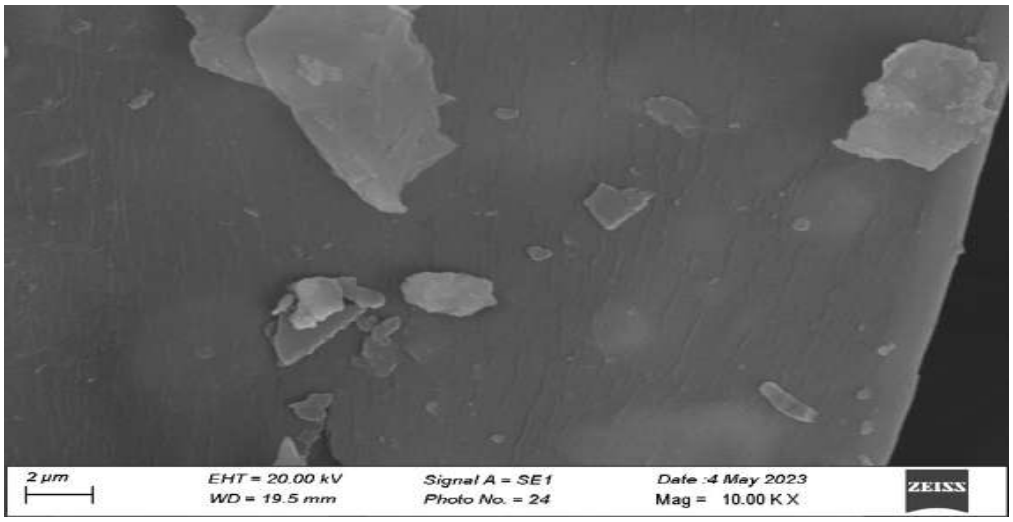


Fig. 3.7 Overall process of collection, preparation, composition analysis and pretreatment of the sample

3.3 Improvisation of the raw and alkali-treated sample

3.3.1 Phonological modulation in raw material (SEM analysis)

The sample's initially smooth surface transforms into a rough texture under the influence of elevated temperatures and alkaline conditions, causing the breakdown of cellulose and hemicellulose bonds upon lignin removal. This alteration increases the surface area of the biomass, enhancing its accessibility to enzymes, as demonstrated by Kataria et al. (2017). Post-alkaline treatment, noticeable structural changes in the untreated sample are evident, as depicted in Fig. 3.8. Comparative analysis with the untreated raw material reveals a more pronounced disruption in the sample following alkaline pretreatment. In Figure (b), the most significant disruption is observed after alkaline pretreatment. Notably, severe conditions (3 per cent NaOH concentration) also exhibit substantial disruptions, as illustrated in Fig. (d) In contrast, mild pretreatment conditions (0.5 % NaOH concentration) are shown in Fig. (c).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WD = 19.5 mm
Signal A = SE1
Photo No. = 24
Date :4 May 2023
Mag = 10.00 K X
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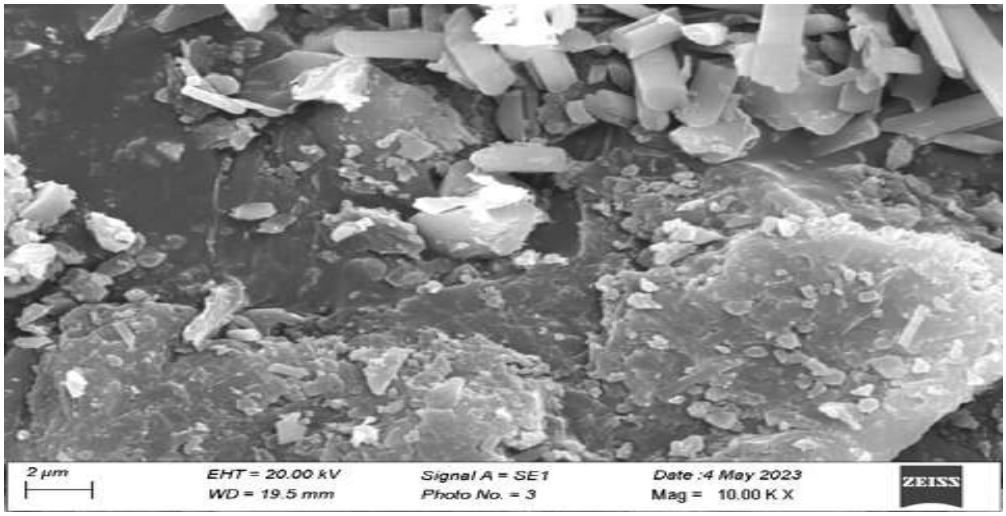
(a)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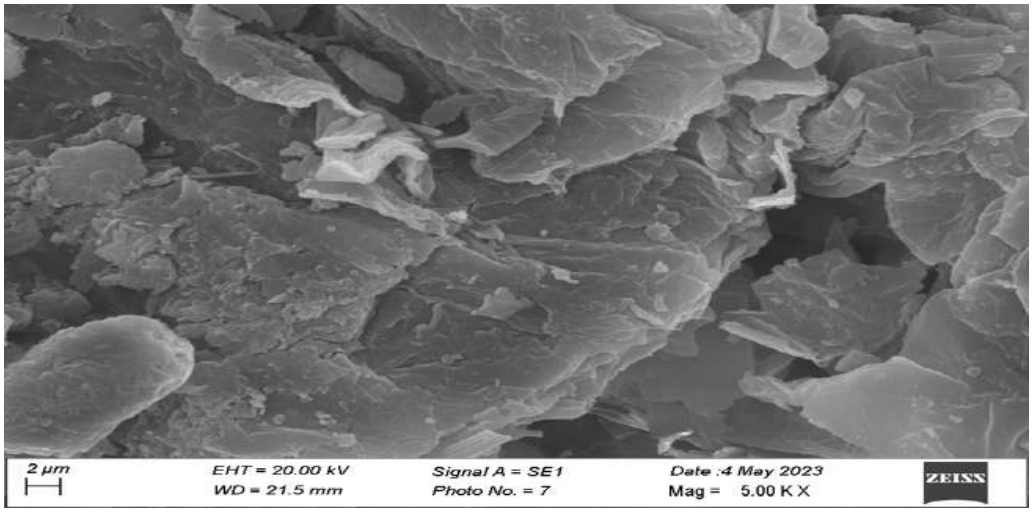
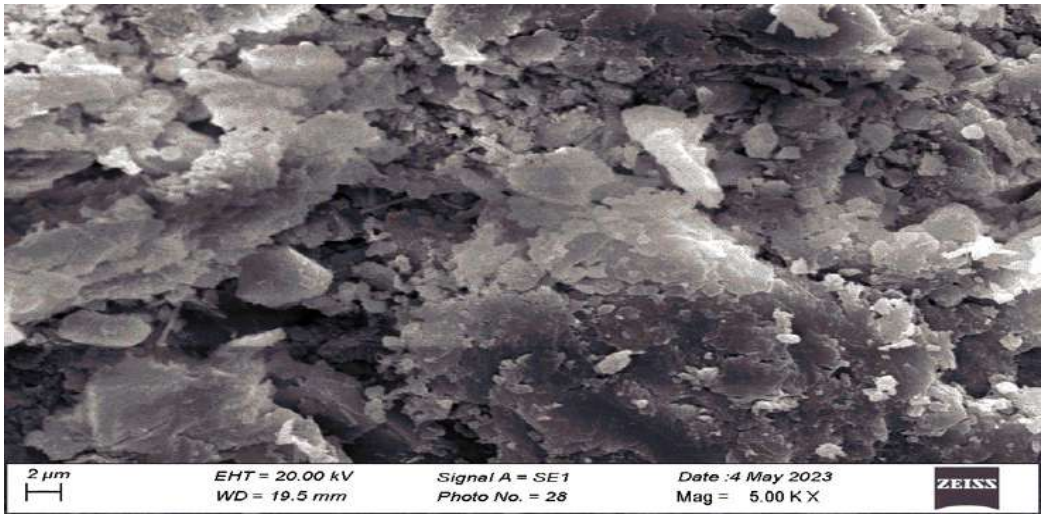
(b)



(c)



(d)

Fig. 3.8 **(a)** SEM analysis for untreated (Raw), **(b)** Optimized condition (2.47 % NaOH conc.,60 min and 120 ºC). **(c)** Mild condition (0.5 % NaOH conc.,30 min and 60 ºC), and **(d)** harsh (3 % NaOH Conc., 60 min. and 120 ºC). The optimized condition shows maximum disruption due to the removal of lignin and hemicellulose.

3.3.2 FTIR spectrum of the untreated and treated sample

The functional group and its properties are specifically revealed by the FTIR spectrum. The treated sample's FTIR spectra (Fig. 3.9) showed a broad, highly intense peak spanning 3500–3200 cm^{-1} that indicated N–H stretching of primary amines, an increase in free O–H bonds as a result of extractives being removed, and the presence of cellulose (Lu and Hsieh, 2010). When the sample is left untreated, an ester bond-related band at 1740 cm^{-1} indicates that the polymer within is mutually interconnected; however, when the sample is treated with alkali, xylose solubilization takes place and the band vanishes. (Sills and Gossett, 2012). The treated sample exhibits more intense peaks at 1430 cm^{-1} and 1320 cm^{-1}, which correspond to C-H_2 and C-H bending, respectively, about cellulose (Sombatpraiwan et al., 2019). The hemicellulose acetyl ester's stretching vibration is reduced in the treated sample, indicating the removal of the xyloglucan acetyl group with the COOH group from the lignin hemicellulose matrix. This is represented by the peak at 1231 cm^{-1} (Trevorah and Othman, 2015) 2015. The peak at 1024 cm^{-1} represents polysaccharides. The treated sample has high intensity at this region because of C-O, C=C, and C-C-O stretching for polysaccharides, which correlate with hemicellulose decomposition (Bano and Irfan, 2019). The aromatic band ranges from 950 to 700 cm^{-1}, which corresponds to the β-glycosidic linkage in cellulose and hemicellulose units (Gusain and Suthar, 2017). Due to C-O-C stretching in the β-glycosidic linkage in cellulose and hemicellulose, which makes cellulose accessible to enzymes, the intensity of the peak at 880 cm^{-1} increases in the treated sample (Deng et al., 2019).

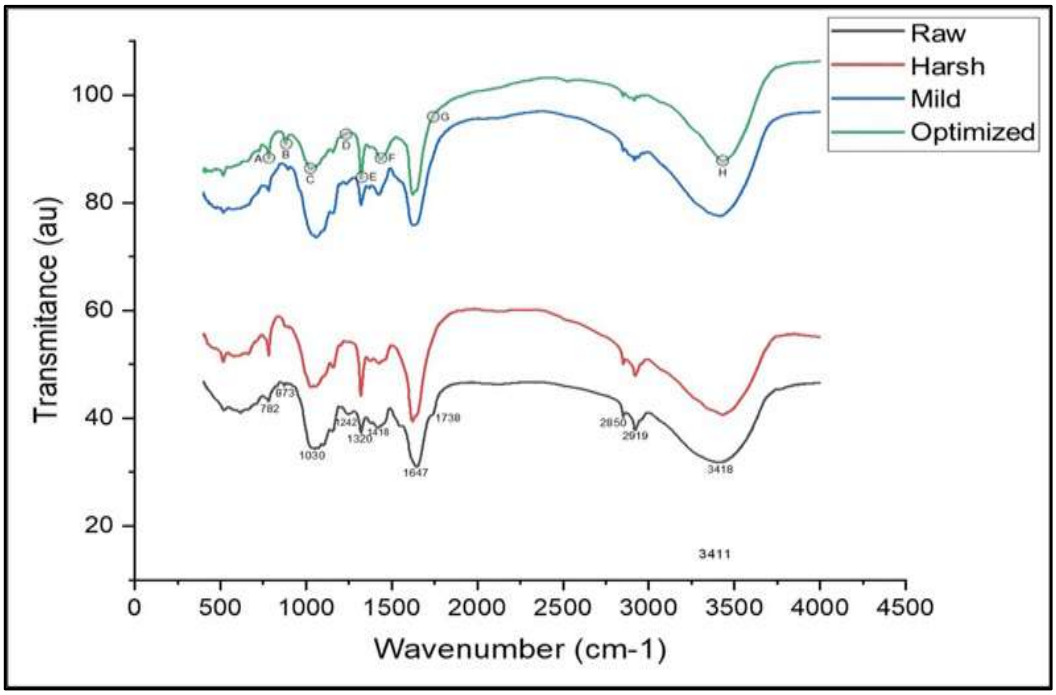


Fig. 3.9 FTIR spectra for untreated (Raw), mild, harsh and optimized conditions. The intensity of the signal in the form of peaks in different wavelengths shows the presence of relevant molecular bonds found in the polymers of samples

3.3.3 Thermogravimetric analysis (TGA) and Differential thermogravimetric analysis (DTG)

Fig. 3.10 shows the thermogravimetric analysis of the untreated sample (raw), mild (0.5 % NaOH conc., 30 min and 60 ºC), harsh (3 % NaOH Conc., 60 min and 120 ºC), and optimised pre-treated condition (2.47 % NaOH conc., 60 min and 120 ºC) to determine their degradation characteristics at 0-600 ºC. During the TGA analysis shown in Fig. 3.10, four distinct types of weight reduction were observed in *Pistia stratiotes*: moisture, cellulose, hemicellulose, and lignin reduction. Water evaporation causes weight loss to begin at 100 ºC. At 200–300 ºC, hemicellulose depolymerizes and breaks down its cellulosic glycosidic bonds. Water loss causes a mild, raw sample to lose 10–12 % of its weight between 100 and 200 ºC. Unexpected weight loss of approximately 50 % was noted in all samples between 250 and 350 ºC as a result of hemicellulose, cellulose, and lignin degradation. Lignin breaks down between 200 and 500 ºC. The primary thermal degradation region was observed to be between ~325 and 345 ºC, indicating the degradation of cellulose and lignin in the sample (Basak et al., 1993). The range

of 200 to 500 ºC was where the optimised condition's 55 % weight loss occurred. This indicates the sample's delignification and is the result of the alkaline pretreatment. The optimised sample's DTG curves show a peak at 345 ºC, respectively, with the hydrolyzed sample showing the strongest signal because of its higher cellulose content. The raw material's maximum thermal stability, which corresponds to its non-cellulosic content, was measured at 325 ºC. Fig. 3.11 illustrates the optimised sample's high thermal stability, with the DTG curve indicating a maximum stability temperature of 345 ºC. The optimised sample's degradation temperature is higher than that of other lignocellulosic materials that have been reported, such as napier grass (300 ºC) (Reddy et al., 2018), roselle fibres (326 ºC)(Kian et al., 2017) and kans grass (340 ºC) (Baruah et al.,2020).

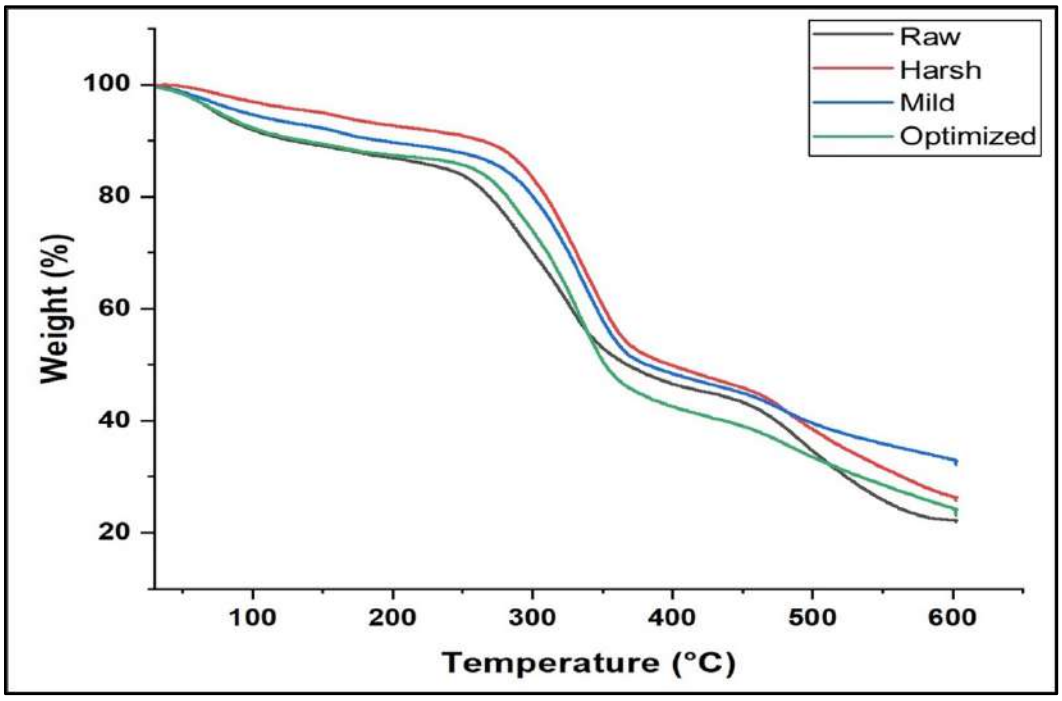


Fig. 3.10 Thermal profile for untreated (Raw), mild, harsh and optimized condition thermogravimetric analysis (TGA) curves represent the thermal degradation of the areas of the major compounds present in the samples

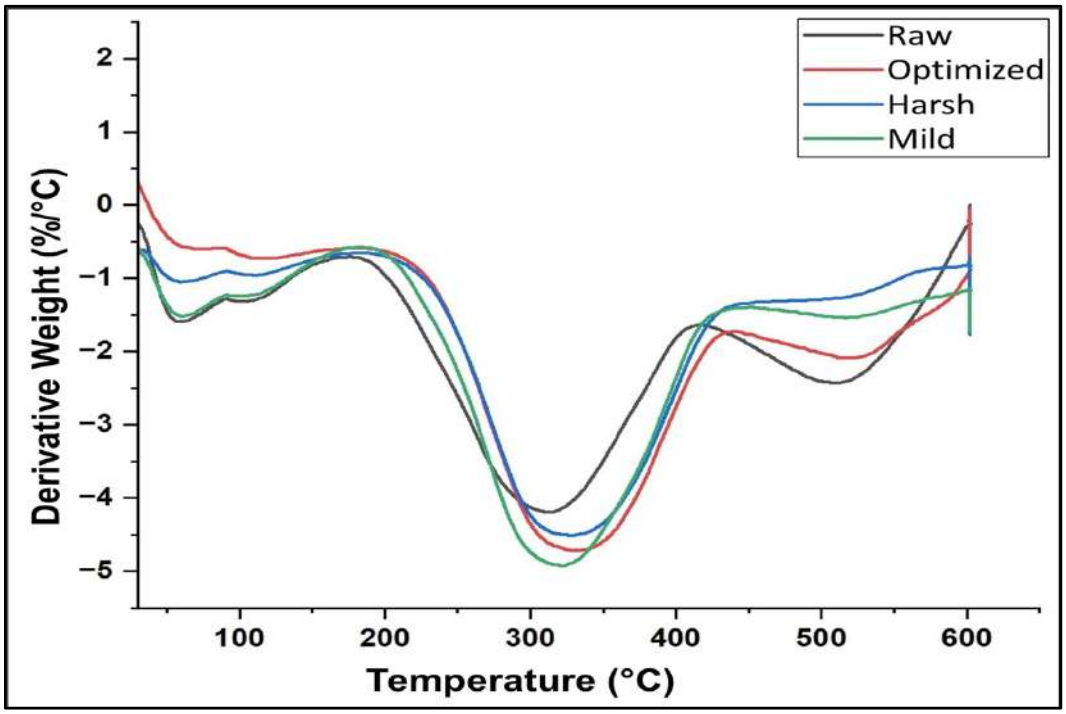


Fig. 3.11 Differential thermogravimetric (DTG) curves represent the rate of thermal degradation of treated and untreated samples.

3.4 Effect of cellulase loading on enzymatic hydrolysis

A comparison of the pre-treated and raw samples' enzymatic hydrolysis shows promising results following pretreatment. 25 FPU, 50 FPU, and 75 FPU cellulase loadings are used to hydrolyse the raw and pre-treated samples in a shaker at 150 rpm and 50 ºC. After hydrolysis reduced sugar was estimated by the DNSA method as shown in Fig. 3.12. After 72 hours, all samples were in equilibrium. The sample that was pre-treated and hydrolyzed by 50 FPU of cellulase is in equilibrium after 72 hours, displaying a maximum concentration of 25 g/L of sugar reduction Fig. 3.14. With an enzymatic activity of 75 FPU, the pre-treated sample's maximum reduced sugar of 33.62 g/L was formed in 72 hours. After 72 hours, the pre-treated sample's maximum sugar yield was discovered, with 75 FPU enzymatic activity of 60.53% Fig. 14. In contrast, under the same conditions, the raw sample yields 6.68 g/L of reducing sugar with 50 FPU in 72 hours following hydrolysis. With 75 FPU, the maximum reduced sugar of 11.56 g/L was formed in 96 hours. After 96 hours, the raw sample's maximum sugar yield of 20.80 % was formed, with an enzymatic activity of 75 FPU shown in Fig. 3.13. It demonstrates how successful the pretreatment is when hydrolysis occurs. The Mass balance of

the complete study is determined by the total product formation from the substrate (Njoku et al., 2013)**.** The mass balance is shown in Fig. 3.15.

(a)

(b)

Fig. 3.12 Reduced sugar after enzymatic hydrolysis (a) glucose standard (b) reduced sugar in a sample

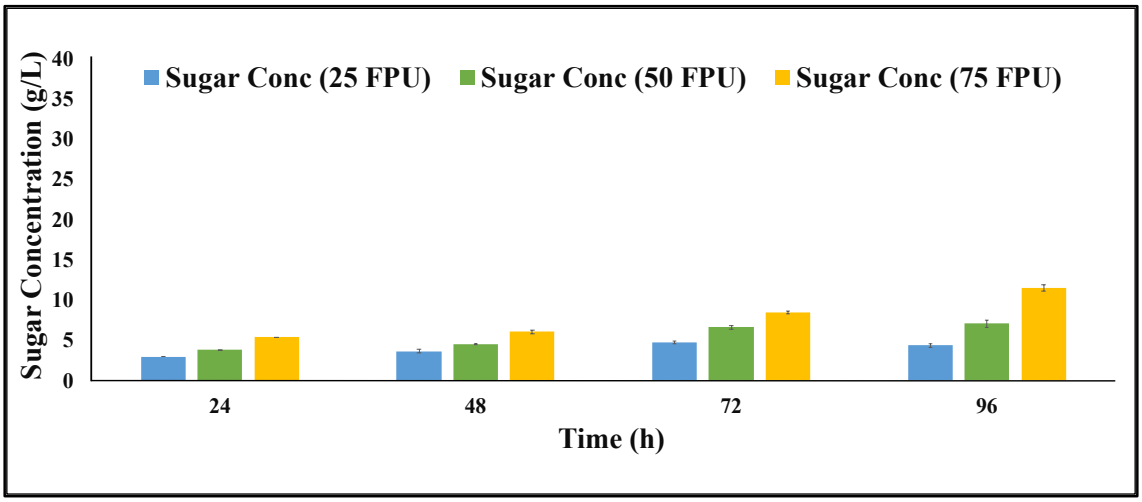


Fig. 3.13 Profile of reducing sugar concentration at the time of enzymatic hydrolysis of a raw sample of *P. stratiotes* at different initial enzyme loading. (50 ºC, pH 4.8, rpm 150 with 5 % biomass loading)

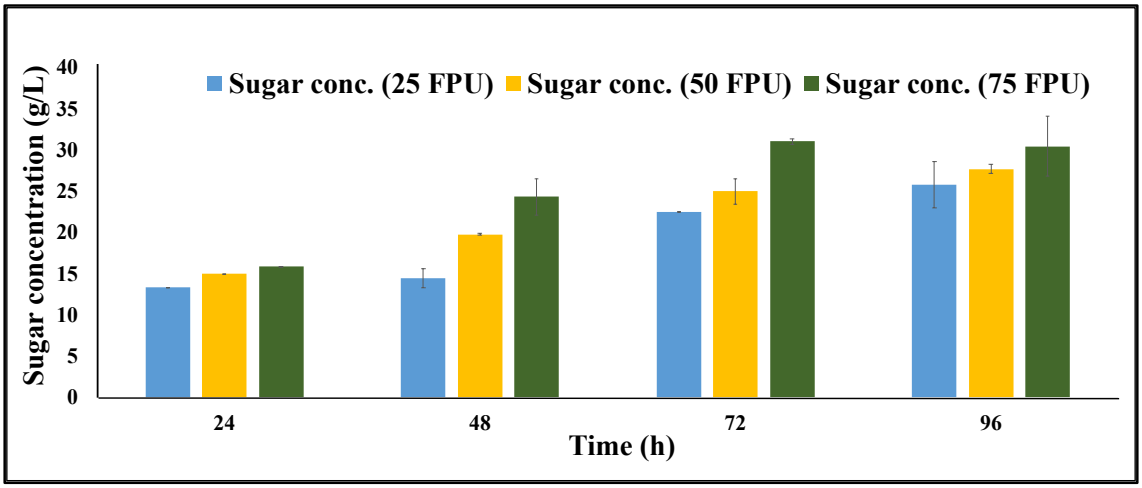


Fig. 3.14 The profile of reducing sugar concentration at the enzymatic hydrolysis optimized pre-treated sample at different enzyme loading conditions (50 ºC, pH 4.8, 150 rpm and 5 % pre-treated solid content)

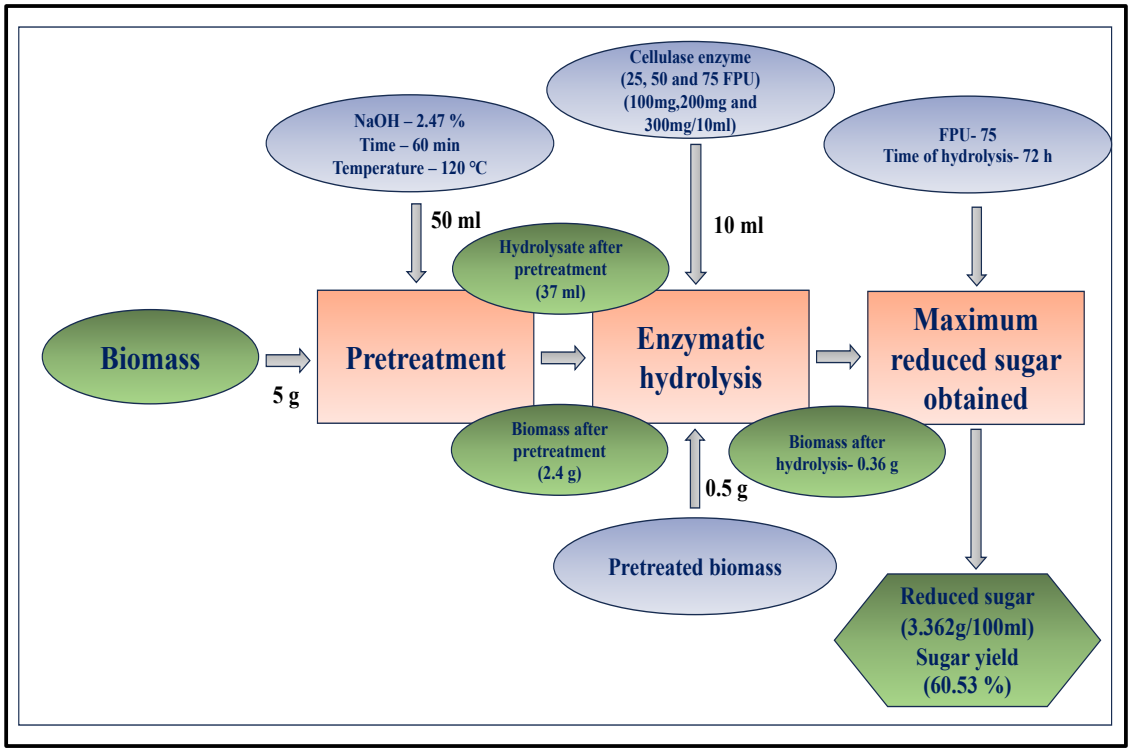


Fig. 3.15 The mass balance diagram of the complete process from pretreatment to enzymatic hydrolysis

3.5 Optimization of Acidic Pretreatment by RSM and validation

Sulphuric acid pretreatment has a high impact on the biomass for depolymerization of cellulose and hemicellulose with the maximum amount of sugar found, which is further utilized in the fermentation process for ethanol production. After performing all the experiments, maximum sugar was found at 3.1% acid concentration. After performing the optimization conditions, the range of sugar content was 3.07% to 24.01%, and the target was to find out 23% sugar content. Similarly, the range of cellulose content was 4.91% to 24.55%. After the optimization, the best condition was achieved as 2.5% sulphuric acid, 120 °C with a duration of 15 min. by autoclave. The outcome of this experiment was 23.44% sugar with 68% hydrolysate.

All the details of independent variables and responses from each experiment in experimental design are mentioned in Table 3.4.

Table 3.4 Experimental design with three independent factors (time, temperature and acid conc.) with cellulose and sugar (dependent factors)

Experiments	**Acid conc. (%)**	**Time (min)**	**Temperature (℃)**	**Cellulose %)**	**Sugar (%)**
1	3.1	55	110	19.81 ± 0.47	24.01 ± 3.87
2	0.75	30	120	10.88 ± 1.35	10.36 ± 1.21
3	2.5	80	120	24.55 ±1.98	19.86 ± 0.0
4	1.63	12.96	110	14.46 ± 0.43	12.75 ± 0.74
5	2.5	30	120	22 ± 3.14	19.88 ± 1.31
6	1.63	55	110	16.02 ± 1.22	15.89 ± 0.34
7	0.75	80	100	9.31 ± 0.58	6.76 ± 0.13
8	1.63	55	110	17.7 ± 1.27	14.99 ± 0.27
9	2.5	30	100	12.84 ± 1.38	13.54 ± 3.82
10	1.63	55	126.82	17.6 ± 1.37	17.02 ± 0.89
11	1.63	55	110	14.9 ± 4.17	16.49 ± 0.52
12	1.63	55	93.18	10.93 ± 2.02	4.47 ± 0.16
13	1.63	55	110	15.56 ± 1.80	14.72 ± 0.28
14	0.75	80	120	14.1 ± 1.51	8.53 ± 0.35
15	1.63	55	110	16.63 ± 0.25	15.33 ± 0.18
16	1.63	97.04	110	15.2 ± 1.25	11.79 ± 0.10
17	2.5	80	100	15.17 ± 0.86	11.22 ± 0.15
18	0.75	30	100	8.45 ± 1.19	4.1 ± 0.11

19	1.63	55	110	16.79 ± 2.48	15.75 ± 0.50
20	0.15	55	110	4.91 ± 0.67	3.07 ± 0.02

3.5.1 Analysis of Variance (ANOVA)

The coefficient of determination and statistical significance were estimated in the current study. The significance of the model was represented by coded coefficients and analysis of variance shown in Table 3.5 and 3.6. The optimization model is significant based on the P value. Model terms acid concentration, temp., square, time*time and temp.*temp were most significant in the optimization of sugar ($p<0.05$). This regression model is performed with an alpha value of 1.68.

The R^2 (coefficient of determination) values are used to determine the goodness of fit of this statistical optimization model. In this model, the lack of fit F values is 8.26 for optimization of the dependent variable (i.e. sugar), which is non-significant. This non-significance of lack of fit suggests our model is reliable. The regression model in this study gives a regression equation that determines the relationship between the response (i.e. sugar) and the independent variable uncoded values (acid con., temp. and time). The regression equation is shown in Eqn. 3.2.

Table 3.5 Coded Coefficients

Term	**Coef**	**SE Coef**	**T-Value**	**P-Value**	**VIF**
Constant	15.535	0.587	26.48	**0.000**	
Acid conc.	5.123	0.389	13.16	**0.000**	1.00
Time	-0.229	0.389	-0.59	0.569	1.00
Temp.	3.230	0.389	8.30	**0.000**	1.00
Acid conc.*Acid conc.	-0.745	0.379	-1.97	0.075	1.02
Time*Time	-1.194	0.379	-3.15	**0.009**	1.02
Temp.*Temp.	-1.734	0.379	-4.57	**0.001**	1.02
Acid conc.*Time	-0.396	0.509	-0.78	0.452	1.00

Acid conc.*Temp.	0.869	0.509	1.71	0.116	1.00

Table 3.6 Analysis of variance for coded coefficients

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	8	570.888	71.361	34.48	**0.000**
Linear	3	501.680	167.227	80.80	**0.000**
Acid conc.	1	358.453	358.453	173.20	**0.000**
Time	1	0.715	0.715	0.35	0.569
Temp.	1	142.512	142.512	68.86	**0.000**
Square	3	61.914	20.638	9.97	**0.002**
Acid conc.*Acid conc.	1	8.007	8.007	3.87	0.075
Time*Time	1	20.559	20.559	9.93	**0.009**
Temp.*Temp.	1	43.310	43.310	20.93	**0.001**
2-Way Interaction	2	7.294	3.647	1.76	0.217
Acid conc.*Time	1	1.256	1.256	0.61	0.452
Acid conc.*Temp.	1	6.038	6.038	2.92	0.116
Error	11	22.765	2.070		
Lack-of-Fit	6	20.678	3.446	8.26	0.018
Pure Error	5	2.087	0.417		
Total	19	593.653			

3.5.2 Model summery

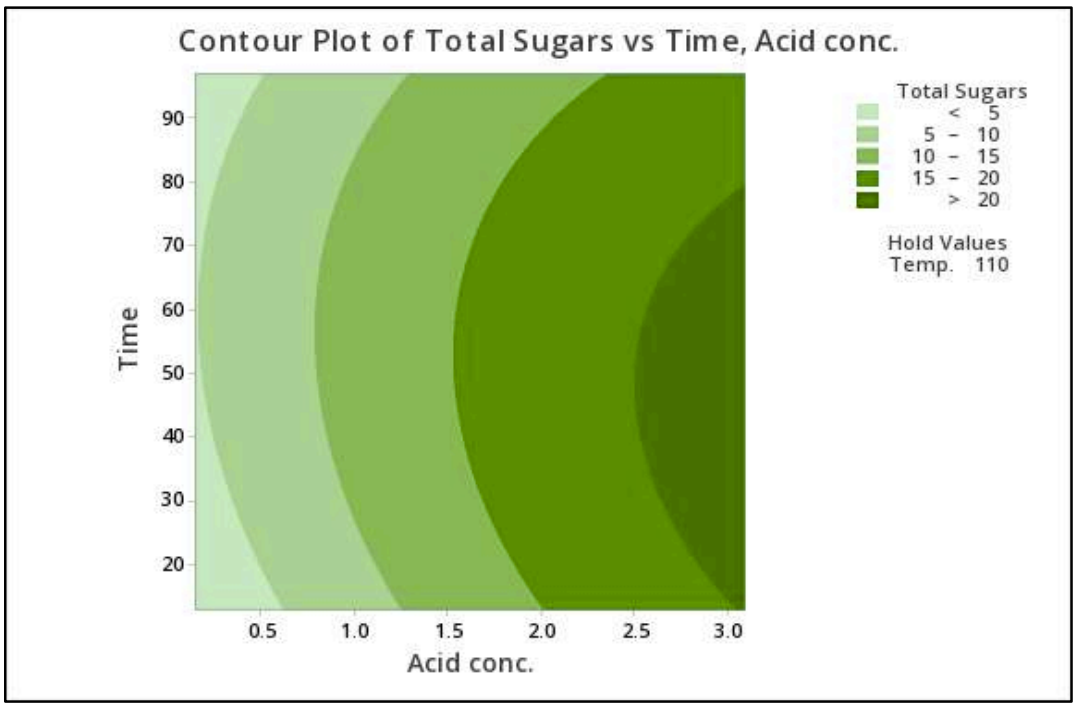
The R^2 value is 96.17% **(**R squared is a statistical measurement of data that shows how close the data is to the regression line) with 93.38% adjusted R^2 value. From R^2 and R^2 (adj.) value R^2 (pred.) predicted value was confirmed at 80.34% which indicates the regression model predicts the responses in new findings very well. The regression equation after performing this model was shown by Eqn. 3.2.

$$\boldsymbol{Total\ Sugars} = -231.0 - (0.91 \times Acid\ conc.) + (0.2305 \times Time) + (3.976 \times Temp.) - (0.974 \times Acid\ conc. \times Acid\ conc.) - (0.001911 \times Time \times Time) - (0.01734 \times Temp. \times Temp.) - (0.0181 \times Acid\ conc. \times Time) + (0.0993 \times Acid\ conc. \times Temp.) \qquad (Eq.\ 3.2)$$

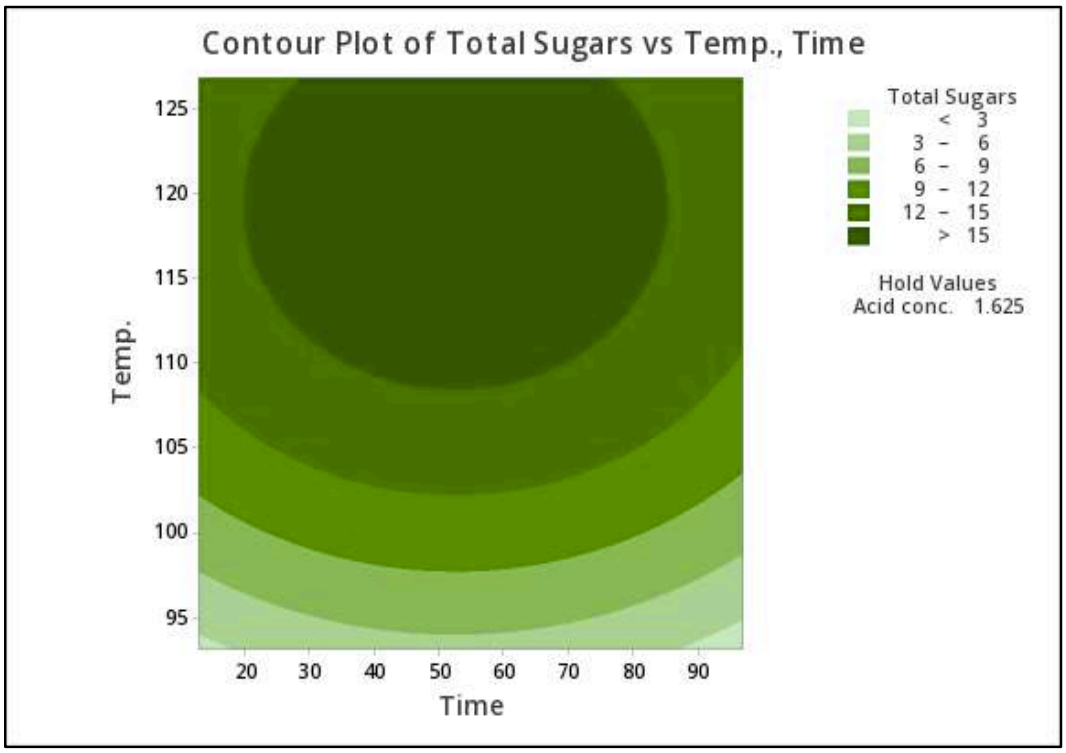
Equation no. 1: Regression equation in uncoded unit

3.5.3 Contour plot

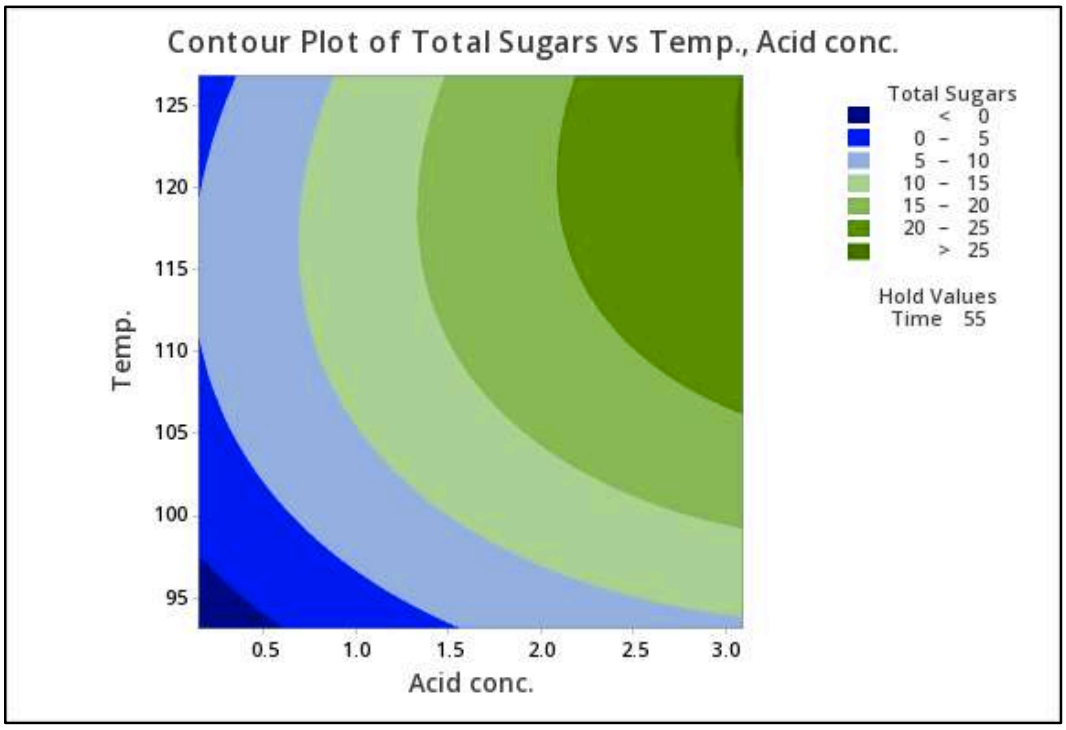
The contour plot represents the two-dimensional (2D) image of factors and responses shows the relationship between the independent variable and dependent variables (Fig. 3.16). While keeping the temperature constant, sugar (>20%) was obtained at a combination of acid concentration (2.5-2.6%) and time (0-75 min) (a). While keeping the acid conc. constant, sugar conc. (>15%) was obtained at the temperature (110-125 °C) with time (20-85 min) (b). Similarly, while keeping the time constant, sugar conc. (20-25%) was obtained at acid concentration (2.2-3.0%) and temperature (107-125 °C) Fig. 3.16 (c).



(a)


Contour Plot of Total Sugars vs Temp., Time
Total Sugars
< 3
3 – 6
6 – 9
9 – 12
12 – 15
> 15
Hold Values
Acid conc. 1.625
Temp.
125
120
115
110
105
100
95
20
30
40
50
60
70
80
90
Time

(b)

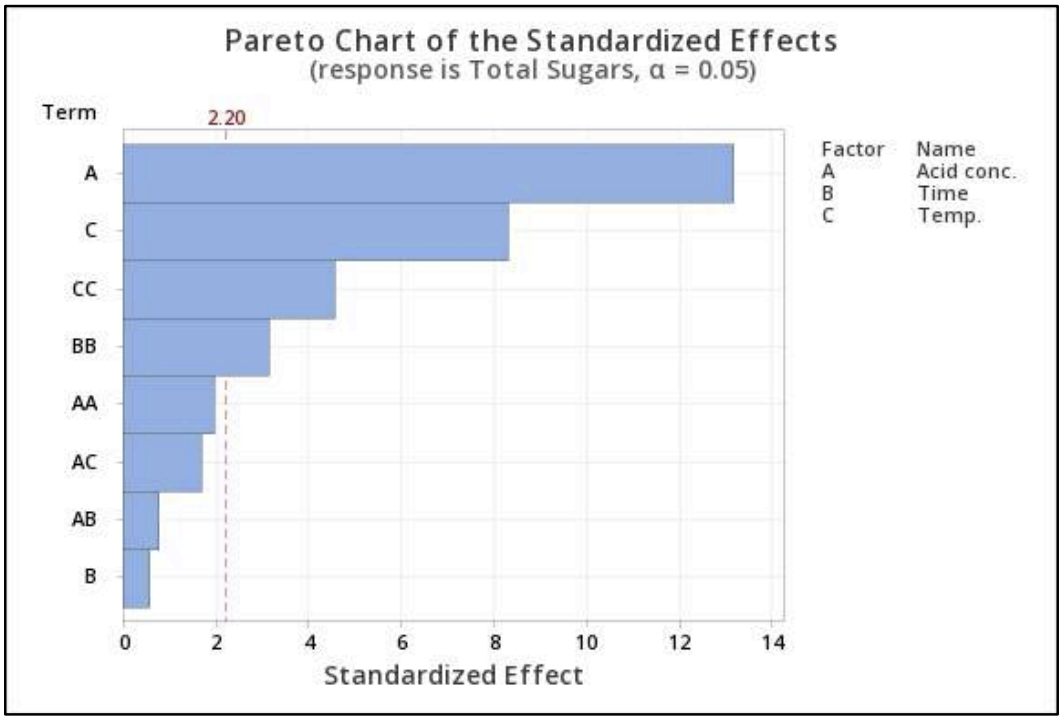

Contour Plot of Total Sugars vs Temp., Acid conc.
Total Sugars
< 0
0 – 5
5 – 10
10 – 15
15 – 20
20 – 25
> 25
Hold Values
Time 55
Temp.
125
120
115
110
105
100
95
0.5
1.0
1.5
2.0
2.5
3.0
Acid conc.

(c)

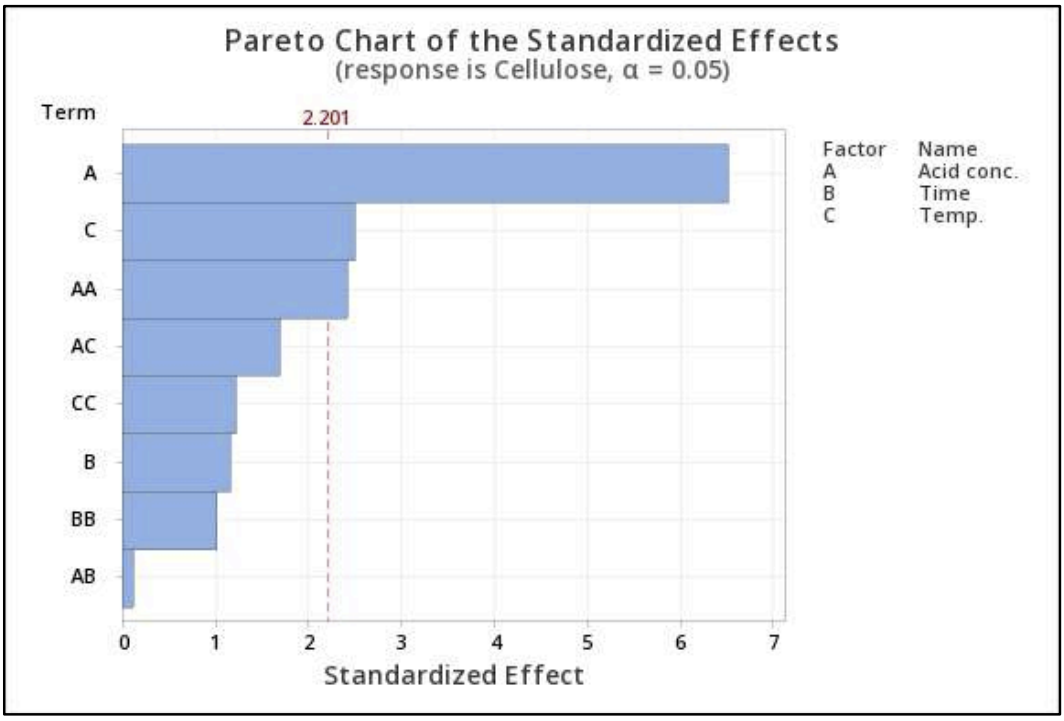
Fig. 3.16 Contour Plot shows the detailed information of independent and dependent variable correlation **(a)** total sugar vs time and acid concentration at constant temperature **(b)** total sugar vs temp. and time at constant acid conc. **(c)** total sugar vs temp. and acid conc. at constant time.

3.5.4 Pareto chart

The Pareto chart determines the significance of the effect of independent variables on total sugar and cellulose. This pareto chart gives all details of the independent variable in pictorial form. In the pareto chart of the standardized effect for sugar, the reference line was at 2.20 whereas for cellulose it was at 2.201. the parameters crossing the reference line were statistically significant. The outcome of the chart determines the acid conc. (A), temperature(C), temperature*temperature(CC) and time*time (BB) are statistically significant at 0.05 level. On the other hand significant effect of the independent variable on the cellulose shown in chart no. (b) in Fig. 3.17. shows acid conc. (A), temperature (C) and acid conc.*acid conc. (AA) are statistically significant. This statistical significance of the independent variable proves that this model is valid for the optimization of pretreatment.



(a)



(b)

Fig. 3.17 Pareto chart shows the standardized effect of independent factors on sugar and cellulose. The parameter of the independent variable combination crossing the reference line shown by the dotted red line is statistically significant.

3.5.5 Response outcome after multiple response prediction

RSM was used to determine the response (total sugar content) outcome, and it fit 22.043 % of total sugar content with a standard error of 2.18. After statistical analysis of the optimisation model, the target total sugar content was 23 %, and the objective was to maximise the total sugar outcome with a lower sugar content of 3.07 %. The ideal condition was discovered at 2.8 % acid concentration with 15 min at 120 ºC temperature after multiple responses.

3.5.6 Optimization Plot

After performing the optimization of acidic pretreatment, an optimized condition was found in which independent variables are acid conc. 2.8%, time 15 min with 120 °C. This condition is performed separately to find out the reliability of the condition. After performing the optimized condition maximum sugar was 23.44% in the hydrolysate which shows the reliability of the model. Minitab software creates a model in which independent variables are added to find the target response. Optimization is calculated by combined desirability ranges from 0-1 (Yosrey et al., 2021)(Auwal et al., 2018). For the optimization experiment, the separate desirability for the entire data is shown in Fig. 3.18. Sugar has an independent desirability result of 0.94448 as the predicted feedback of 21.8936 which is very close to the target of 23. Cellulose has a moderate desirability outcome is 0.78804 as the predicted outcome of 20.3871 which is less than the target of 24.55 as mentioned in the parameter table. The composite desirability of 0.8627 is a good score which represents that both the responses were very close in their absolute settings. The optimization of responses did not give an ideal composite desirability score as the sugar and cellulose contents were not in absolute settings but in a justifiable range. The conditions found after this RSM model are mentioned in the Fig. 3.18. Texture and colour of the sample changed after performing the pretreated condition shown in Fig. 3.19.

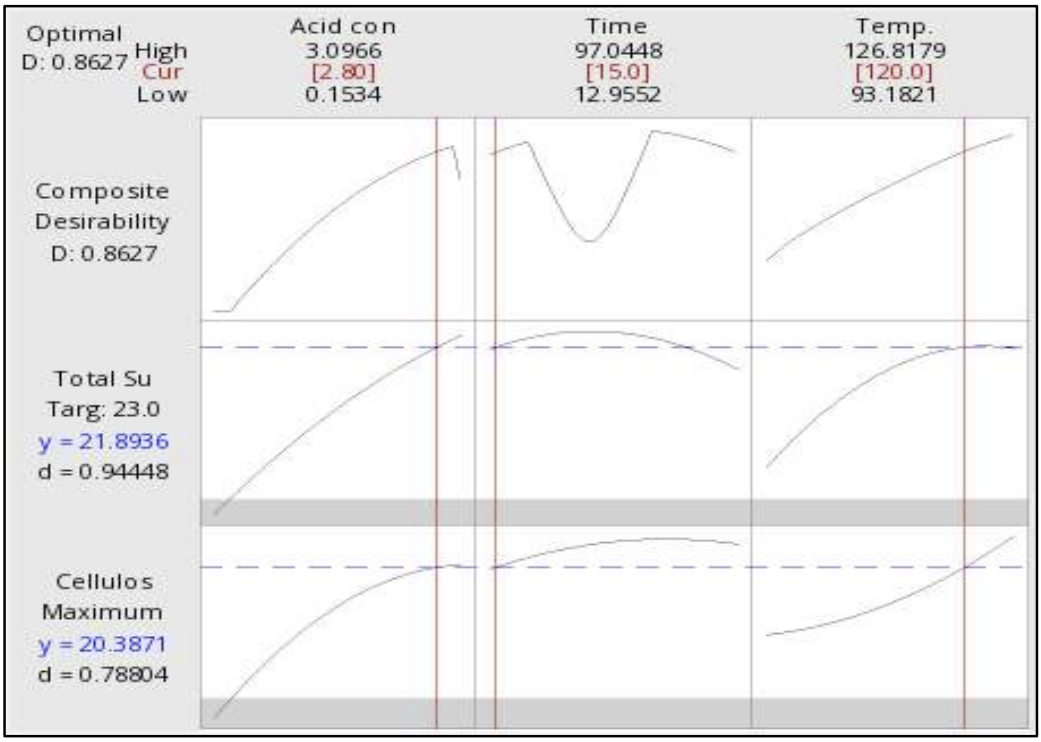


Fig. 3.18 Optimized condition after performing RSM shows composite desirability score is 0.8627 which is good define both responses are very close to the entire things in the model. Sugar has high desirability as compared to cellulose shown by the score, of 0.94448 for sugar and 0.78804 for cellulose. The outcome of the result was found with 2.8 % acid at 120 ºC for 15 min.

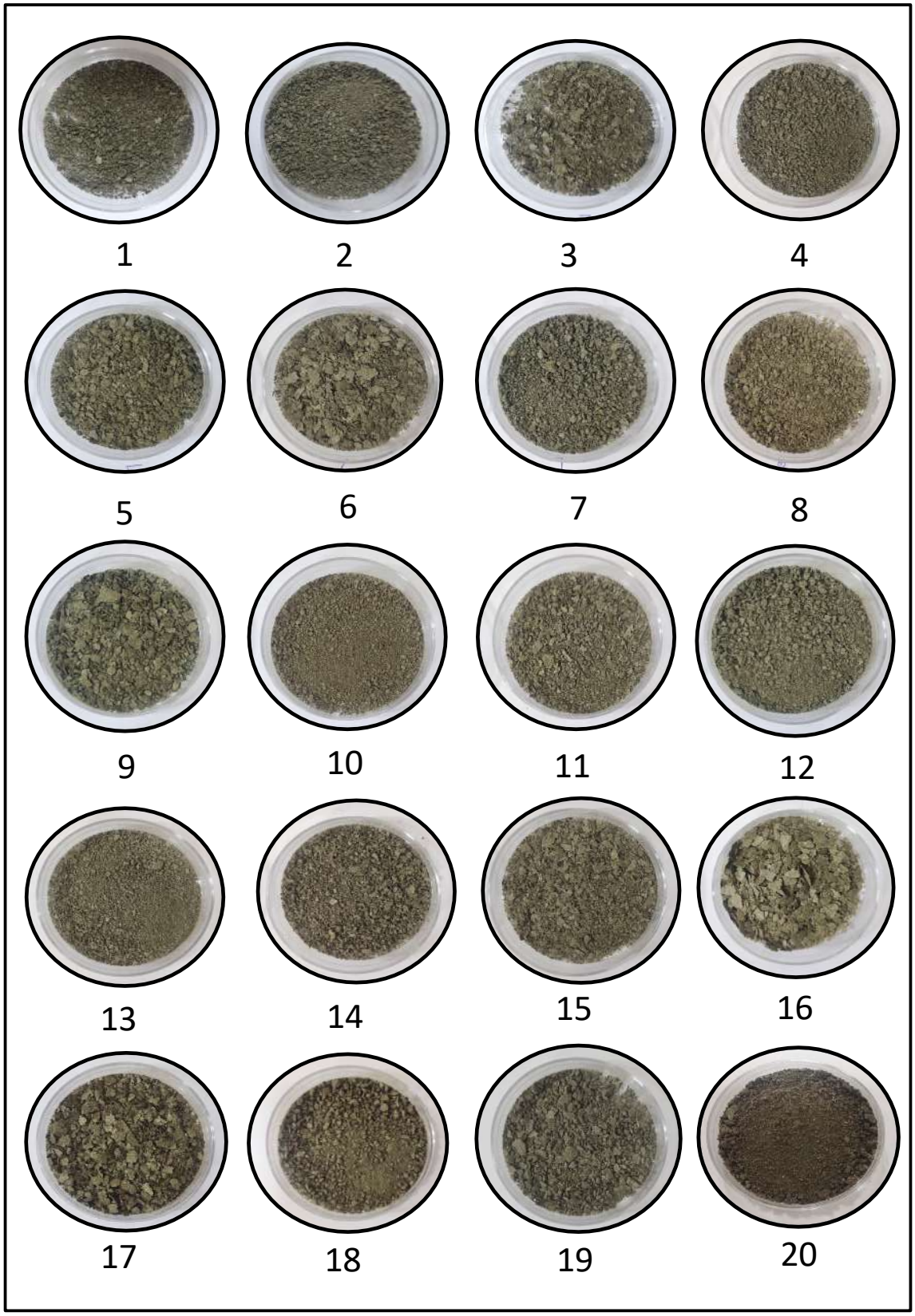
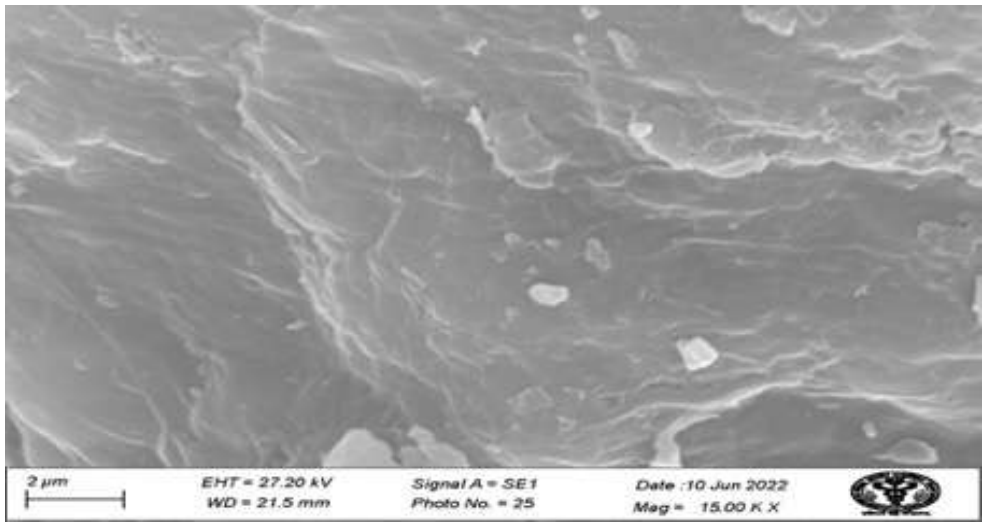


Fig. 3.19 Acidic pre-treated sample according to RSM conditions

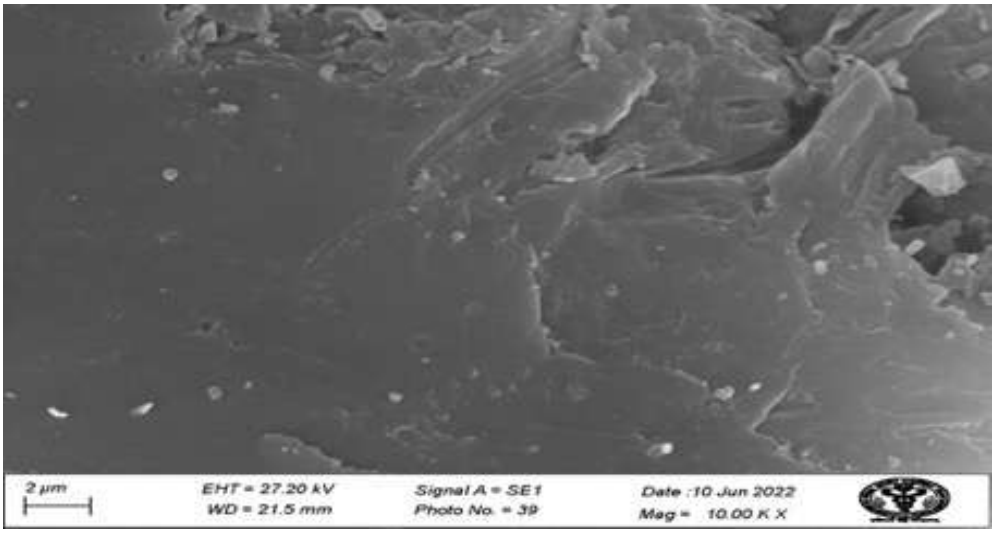
3.6 Characterization of raw and Acidic pre-treated biomass

3.6.1 Morphological changes in biomass after acidic pretreatment (SEM analysis)

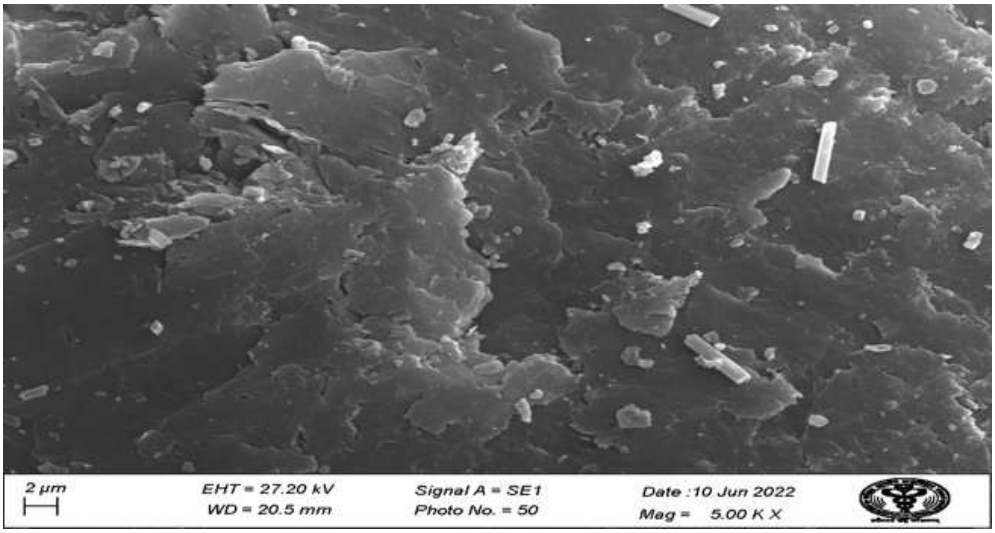
The morphology of *P. stratiotes* changes after acidic pretreatment. It is more destructive after providing acidic pretreatment to the sample as compared to the raw material. Maximum disruption was observed in optimized acidic conditions which is denoted by Fig. 3.20 (d). However harsh condition (2.5% acid conc.) also show a high amount of disruptions denoted by Fig. 3.20 (b). Mild condition (0.75% acid conc.) in pretreatment is shown in Fig. 3.20 (c). These disruptions are due to the effect of temperature and acid by which the smooth surface of the biomass is converted into the a rough surface which shows the bonds between cellulose and hemicellulose are broken and the removal of lignin from the sample which increases the surface area of the biomass for increase the accessibility to the enzymes for saccharification. This type of structural change is seen in the case of elephant grasses when treated with sulphuric acid (Kataria et al., 2017)**.**



(a)


2 µm
EHT = 27.20 kV
WD = 21.5 mm
Signal A = SE1
Photo No. = 39
Date :10 Jun 2022
Mag = 10.00 K X

(b)


2 µm
EHT = 27.20 kV
WD = 20.5 mm
Signal A = SE1
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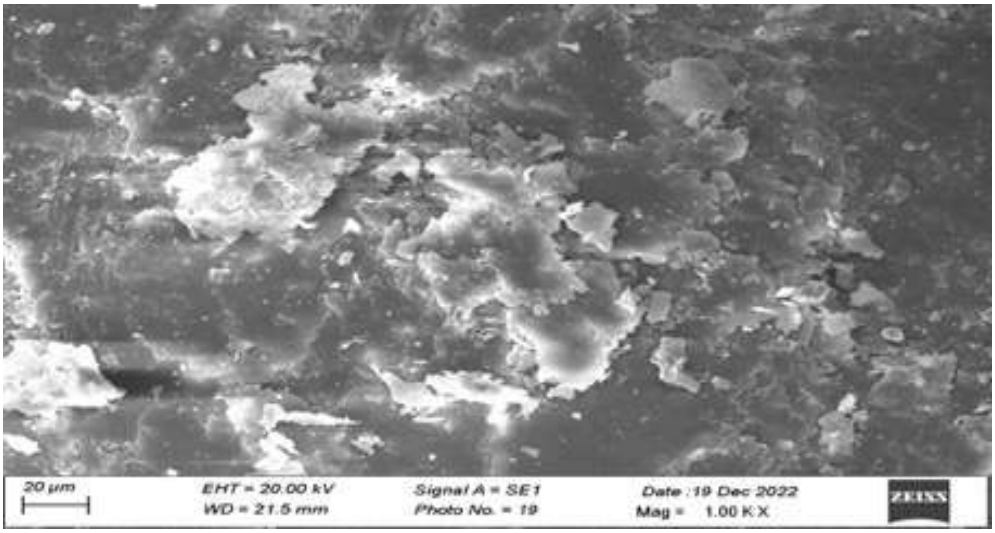
Date :10 Jun 2022
Mag = 5.00 K X

(c)



(d)

Fig. 3.20 SEM images of **(a)** Raw biomass and **(b)** Harsh pretreatment conditions (2.5% acid, 80 min. with 120 °C **(c)** Mild pretreatment conditions (0.75 % acid, 30 min. with 100 °C) and **(d)** Optimized condition

3.6.2 FTIR spectrum of raw sample and acidic Pre-treated sample

The FTIR spectrum provides detailed information regarding the functional group and its properties. The FTIR spectra of raw, mild (0.75% acid, 30 min, 100 °C), harsh (2.5% acid, 80 min, 120 °C) and optimized pre-treated samples of *P. stratiotes* (Fig. 3.21) showed a broad peak (H) around 3500-3200 cm^{-1} in the optimized condition, which shows N-H starching of primary amines and free O-H stretching of the OH group present in cellulose (Lu and Hsieh, 2010). By acidic pretreatment, these O-H bands were reduced (Dahunsi et al., 2019). The peak in between 2848-2851 cm^{-1} in each pre-treated and raw sample represents the characteristic C-H stretching, which represents the vibration of cellulose (Luzi et al., 2019). The peak observed at 1640 cm^{-1} (G) shows water absorption and in the optimized pre-treated condition the intensity of the peak decreased due to aromatic skeletal vibration giving information regarding lignin removal (Mandal and Chakrabarty, 2011). In the pre-treated condition, the peak at 1515 cm^{-1} (F) relates to the aromatic skeletal stretching. Acidic pretreatment reduced the adsorption of bands, but acid soluble lignin occurred in the solid fraction of the pre-treated sample. The intensity of the peak at 1374 cm^{-1} (E) increased due to breakage in ether groups

present in lignin (Hsu et al., 2010) As compared to the pre-treated sample, the peak at 1316 cm^{-1} (D) in the raw sample is more prominent due to C-H ring vibration, indicating the presence of cellulose. CH_2 bending, which represents the cellulose with skeletal vibration of the C-C bond, shows the presence of cellulose (Jmel et al., 2019). The peak between 1200-1000 cm^{-1} (C) displays the presence of cellulose and hemicellulose in the sample with C-O starching and C-H rocking vibration correlating with cellulose structure (Alemdar and Sain, 2008). The range of the aromatic band is 950-700 cm^{-1} corresponding to the β-glycosidic linkage between sugar units of cellulose and hemicellulose (Gusain and Suthar, 2017). FTIR indicate the effective removal of other contents apart from cellulose by acidic treatment of *P. stratiotes*. The appearance of a peak at 894 cm^{-1} (B), the absorption peak for cellulose, in the pre-treated sample shows the C-O-C stretching in β-glycosidic linkage in cellulose and hemicellulose (Deng et al., 2019). The peak intensity at 781 cm^{-1} (A) is decreased in the pre-treated sample as compared to the raw sample, showing the removal of lignin by the acidic treatment (Malik et al., 2020a).

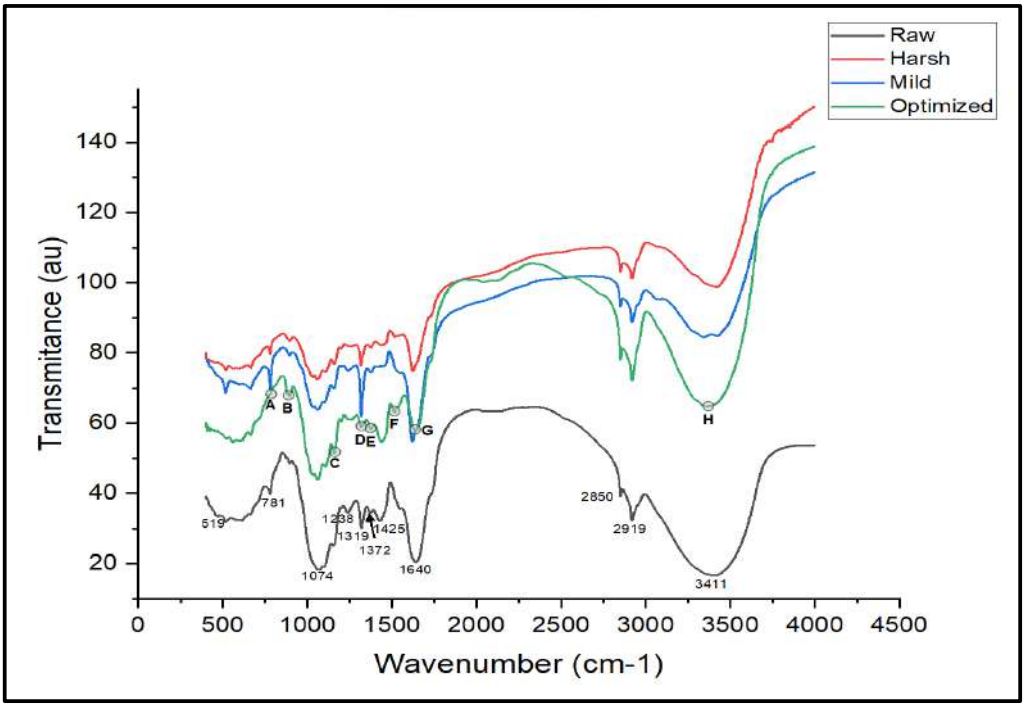


Fig. 3.21 FTIR spectra of raw, Mild (0.75% acid, 30 min, 100 °C), Harsh (2.5% acid, 80 min, 120 °C)and Optimized pre-treated sample. This spectra show the changes in the functional

groups after pretreatment (A-H) denotes the changes in the peaks which shows the breakdown of the cellulose and removal of lignin

3.6.3 Thermogravimetric analysis (TGA)

Raw sample, mild (0.75% acid and 30 min at 100 °C), harsh (2.5% acid and 80 min at 120 °C), and optimized pre-treated sample (acid conc. 2.5% and 15 min. at 120 °C) were thermogravimetrically analysed to differentiate their degradation characteristics. *P. stratiotes* biomass contains four types of weight which can be degraded (moisture, cellulose, hemicellulose and lignin) during the TGA shown in Fig. 3.22. (Kataria et al., 2018a). In all samples, initial weight loss occurred at 100 °C due to evaporation of water. Temperature from 200 °C - 300 °C leads the way to depolymerization of hemicellulose and breakage of glycosidic bonds in cellulose. Unexpected loss of weight in mild condition and untreated raw material at 250 °C is due to degradation of hemicellulose. This was primarily degraded due to the presence of acetyl group as compared to cellulose and lignin. Lignin degradation occurs simultaneously at the temperature range 200 °C- 500 °C and the range 250 °C - 400 °C is confirmed for cellulose degradation. Maximum weight loss of the pre-treated sample of approximately 53% occurs in the range 250 °C – 400 °C due to degradation of cellulose. It is much higher as compared to the raw sample (Kale et al., 2019)(Umesh et al., 2022b) on the other hand optimized and harsh conditions degradation goes simultaneously as the same pattern. Degradation in the optimized conditions is seen maximum in Fig. 3.22.

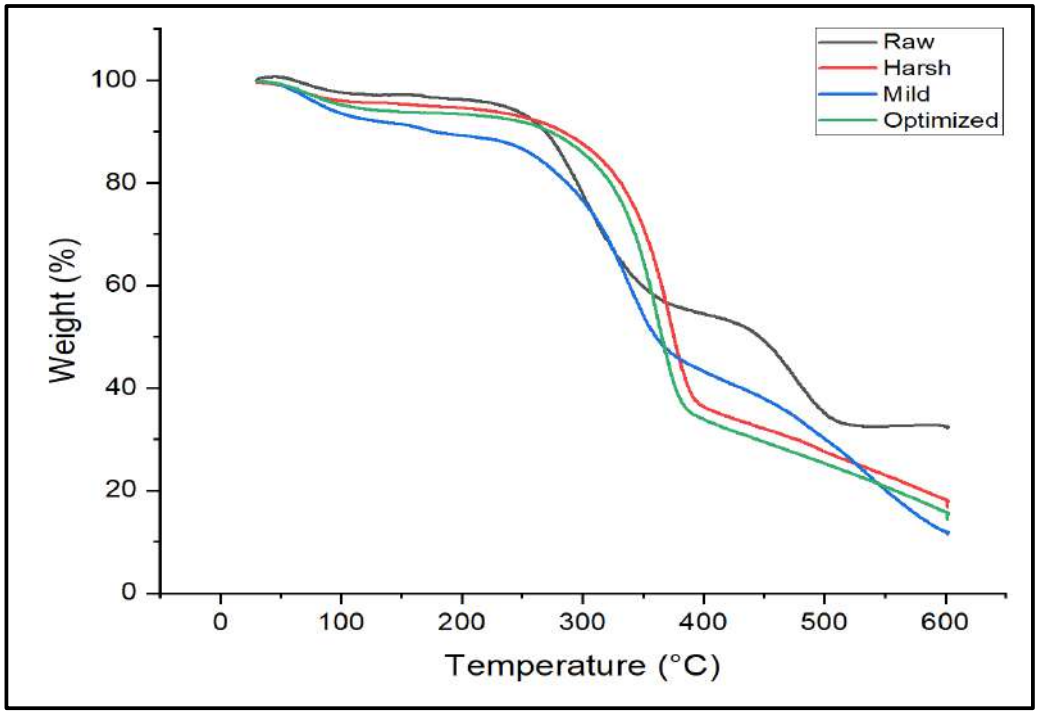


Fig. 3.22 Thermogravimetric analysis of Raw (untreated), Mild (0.75% acid, 30 min, 100 °C), Harsh (2.5% acid, 80 min,120 °C) and Optimized pre-treated samples confirm the degradation of the compounds in the material or lose the stability of material at a particular temperature optimized condition have high degradation of cellulose and lignin

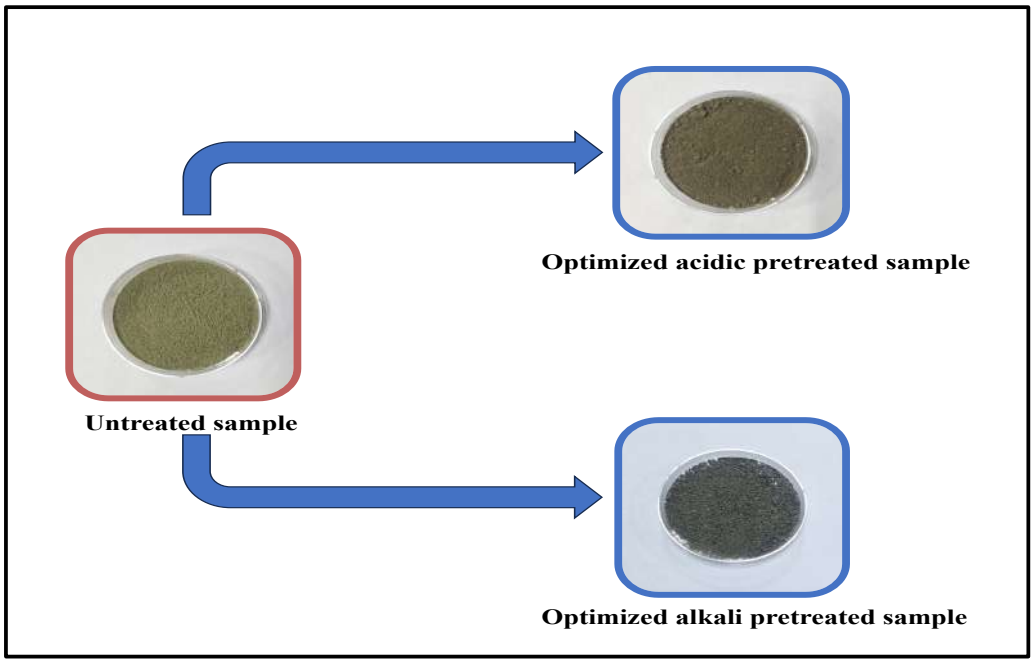


Fig.3.23 Variations in the raw sample after pretreatment

A difference was observed between the texture and colour of the sample when the sample was treated with optimized acidic and alkali conditions as shown in Fig. 3.23.

3.7 Fermentation by *Saccharomyces cerevisiae* and *Pichia stipites*

3.7.1 Analysis of ethanol by Gas chromatography (GC- FID)

The retention time for the standard of ethanol was 3.61 min with a complete 10 min cycle. *S. cerevisiae* used in fermentation have the ability of utilise hexose for ethanol production, while *P. stipitis* has the capability for the usage of pentose and hexose for ethanol production. The fermentation was done with *S. cerevisiae* NCIM 3594 and *P. stipitis* NCIM 3498 separately to find out the maximum outcomes of the fermentation process. Fermentation done by *S. cerevisiae* and *P. stipitis* utilizes all sugar within 52 h. *P. stratiotes* hydrolysate after pretreatment contained a maximum of 23.44% sugar, which was further used in the process of fermentation by *S. cerevisiae* and *P. stipitis* . Maximum glucose was consumed within 48 hours and converted into ethanol. Starting sugar was taken 10.04 g/L. The fermentation condition was carried out at 30 °C at 150 rpm. In the fermentation maximum of 86% sugar was consumed from the hydrolysate by the *S. cerevisiae* while 88% sugar was consumed by *P. stipites*. During the initial stage of fermentation, intake of sugar by the strains was slower and cell growth increased after 14 h when sugar consumption increased. Maximum ethanol concentration was found by the *S. cerevisiae* in synthetic media in 30 h while in hydrolysate it comes in 36 h (3.25g/L) with 0.37 g/g ethanol yield. On the other hand *P. stipitis* strain utilizes both pentose and hexose sugars and the maximum ethanol concentration in synthetic media comes in 32 h while in hydrolysate it comes in 36 h (3.57 g/L) with 0.39 g/g ethanol yield (Table 3.7 and Fig. 3.24). The ethanol concentration in the hydrolysate was approximately similar the synthetic media because of detoxification of hydrolysate which removes the high amount of toxic compound from the hydrolysate by which production of ethanol increased. In the Sunwoo IY et al. 2019 (Sunwoo et al., 2019) (Table 3.8) study, *S. cerevisiae* gave less ethanol yield (0.32g/g) as compared to the present study (0.37g/g), and *P. stipitis* gives higher production (0.39g/g) as compared to *S. cerevisiae*. This determines that both the stains give a very good amount of ethanol by this aquatic weed. The kinetic parameters of our study are comparable just like another study on t sugarcane molasses (Sulaiman et al., 2022).

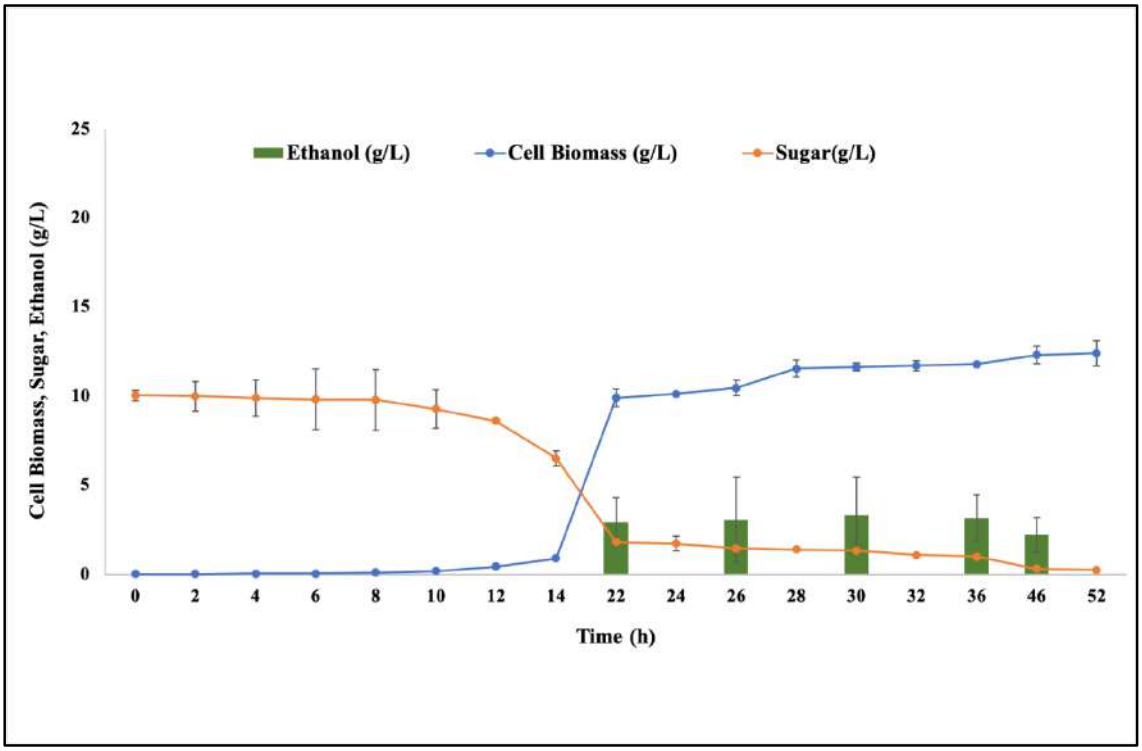
Table 3.7 Kinetic parameters for ethanol production by *S. cerevisiae* and *P. stipitis* stains with synthetic media and hydrolysate

Kinetic parameters	***Saccharomyces cerevisiae* in Synthetic media**	***Saccharomyces cerevisiae* in hydrolysate**	***Pichia stipitis* in synthetic media**	***Pichia stipitis* in hydrolysate**
Initial total sugar(g/l)	10.04 ± 0.29	10.04 ± 1.36	9.97 ± 0.76	9.99 ±0.21
Maximum ethanol conc.(g/l)	3.32 ± 0.96	3.26 ± 0.71	3.57 ± 0.31	3.57 ± 2.02
Maximum time (hours)	30	36	32	36
Maximum sugar consumed (%)	97.6 ± 0.29	85.9 ± 1.34	94.9 ± 0.74	87.9 ± 0.21
Ethanol productivity(g/l/h)	0.11 ± 0.04	0.09 ± 0.02	0.10 ± 0.01	0.09 ± 0.06
Growth rate(g cells/l/h)	0.38 ± 0.01	0.30 ± 0.004	0.68 ± 0.03	0.56 ± 0.01
Maximum sugar consumption rate(g/l/h)	0.29 ± 0.01	0.24 ± 0.002	0.24 ± 0.001	0.24 ± 0.00
Ethanol yield coefficient (g/g)	0.39 ± 0.02	0.37 ± 0.06	0.41± 0.03	0.39 ± 0.01

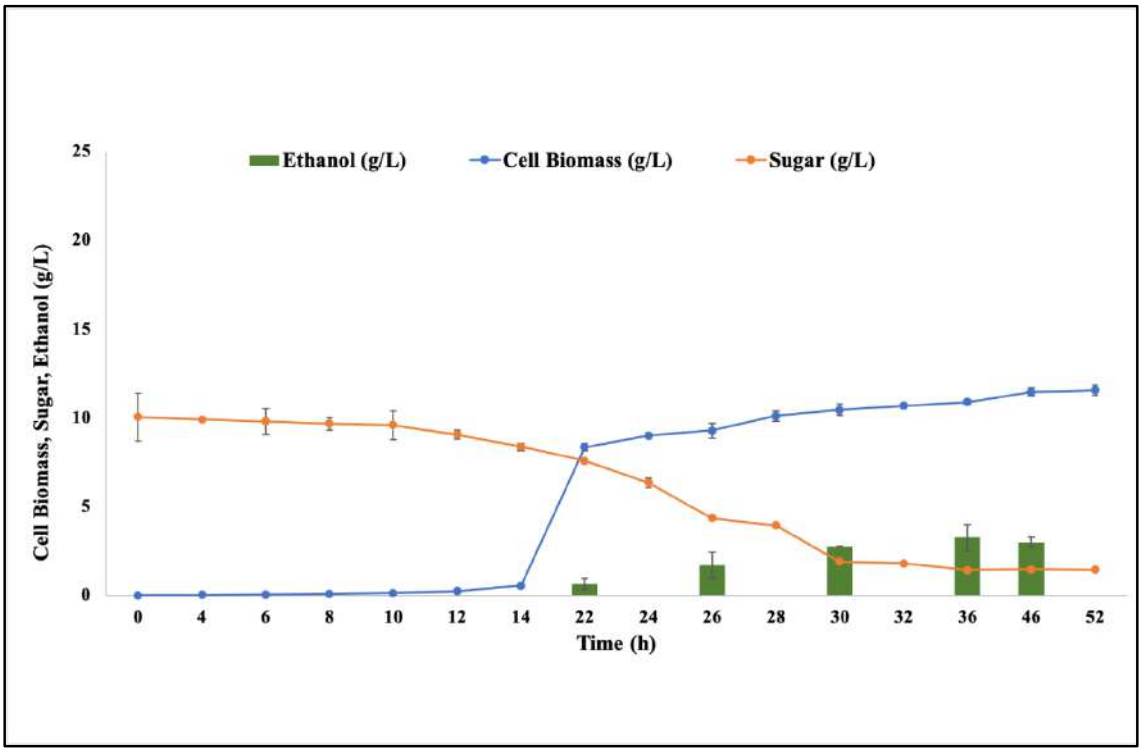
Table 3.8 Comparison of different lignocellulosic biomass capabilities of conversion into ethanol

Biomass	Strains	Ethanol yield (g/g)	Reference
Lignocellulosic biomass	Engineered *Saccharomyces cerevisiae*	0.43	(Tran et al., 2023)
Oak	*S. cerevisiae*, SXA-R2P-E	0.43	(Ko et al., 2016)
Rice straw	*S. cerevisiae*, SXA-R2P-E	0.46	(Ko et al., 2016)
Wheat straw	*S. cerevisiae* VS3	0.44	(Govumoni et al., 2013)
Corn stover	*Escherichia coli* FBR 5	0.49	(Saha et al., 2013)
Water hyacinth	*S. cerevisiae , P. stipitis* and *C. lusitaniae*	0.32 0.44 0.49	(Sunwoo et al., 2019)
Lemna minor	S. cerevisiae	0.258	(Bayrakci and Koçar, 2014)
Rubberwood waste	S. cerevisiae	0.14	(Nunui et al., 2022)
Sugarcane bagasse	*S. cerevisiae*	0.41	(Irfan et al., 2014)
Sugarcane bagasse	*Scheffersomyces shehatae* UFMG HM 52.2 and *Scheffersomyces stipitis* NRRL Y-7124	0.42 and 0.16	(Dussán et al., 2016)
Kans grass	*S. cerevisiae*	0.46	(Kataria and Ghosh, 2011)
W. globosa	*S. cerevisiae*	170g/kg	(Soda et al., 2015)

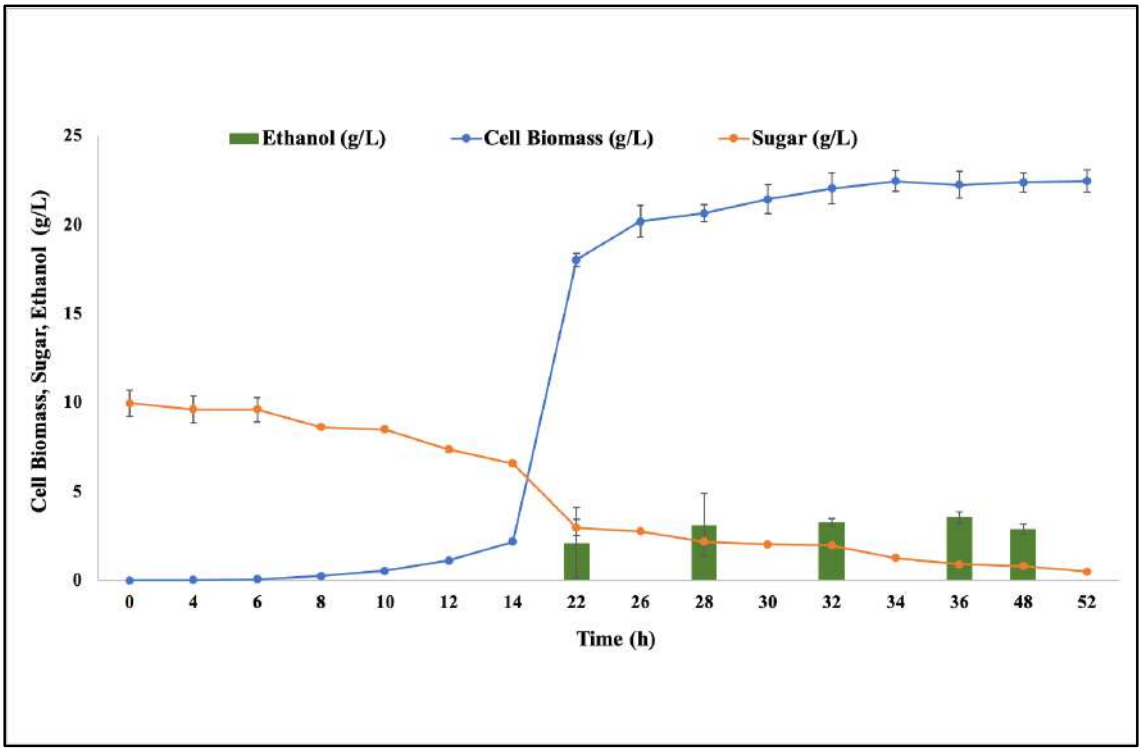
Lemna minor	Self-flocculating yeast strain SPSC01 and *Saccharomyces cerevisiae* ATCC 24859	0.44 - 0.47	(Ge et al., 2012)
Pistia stratiotes	*S. cerevisiae* and *P. stipites*	0.37 and 0.39	Present study



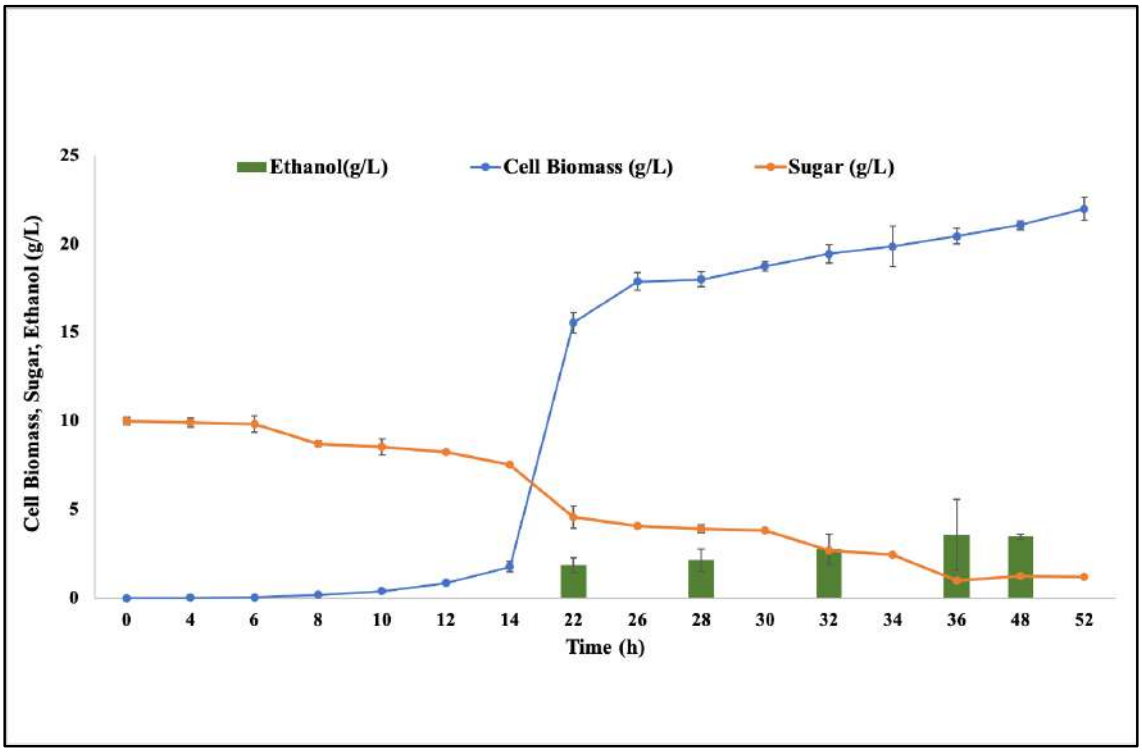
(a)


Ethanol (g/L)
Cell Biomass (g/L)
Sugar (g/L)
Cell Biomass, Sugar, Ethanol (g/L)
25
20
15
10
5
0
0 4 6 8 10 12 14 22 24 26 28 30 32 36 46 52
Time (h)

(b)


Ethanol (g/L)
Cell Biomass (g/L)
Sugar (g/L)
Cell Biomass, Sugar, Ethanol (g/L)
25
20
15
10
5
0
0 4 6 8 10 12 14 22 26 28 30 32 34 36 48 52
Time (h)

(c)



(d)

Fig. 3.24 The outcome of the fermentation process in four different conditions using *Saccharomyces cerevisiae* **(a)** with synthetic sugar **(b)** with hydrolysate, by using *Pichia stipitis* **(c)** with synthetic sugar **(d)** with hydrolysate, was done by calculation of cell biomass, sugar consumption and ethanol production

3.8 Discussion

Biofuels can be produced from biomass, providing an environmentally friendly way to mitigate the energy crisis. The sustainable development goal is aided by the bioconversion of lignocellulosic biomass into biogas and biofuel, which lessens the carbon footprints. Lignocellulosic biomass is composed of lignin, hemicellulose, and crystalline cellulose. Unlike the first generation, which used sugar directly, intrinsic biomass recalcitrance significantly hinders lignocellulose's bioconversion by protecting carbohydrates from degradation. The recalcitrance of wrapped cellulose significantly hinders the recovery of fermentable sugar from it. This type of biomass degrades slowly, so effective pre-treatment and enzyme dosage are necessary (Hu et al., 2023a). Pre-treatment is a crucial stage in the bioprocess technology process as a whole. In modern biorefineries, various pre-treatments such as physical, chemical, and biological are used to overcome the recalcitration of lignocellulosic biomass (Mankar et al., 2021). Physical pretreatment is used to reduce the size of particles and increase the surface area of lignocellulosic biomass by disrupting of structure of cellulose, hemicellulose and lignin for accessibility of enzymes e.g. grinding (Ji et al., 2017) Chemical pretreatment is more effective for increasing the hydrolysis and it is a simple procedure with high efficiency. Different chemicals are used in this process like acids, alkali, organic solvents and ionic liquids, all of these can break down the lignocellulosic biomass. Acidic pretreatment is specific for lignin degradation by using different concentrations of acid, reaction time and temperature. The disadvantage of this pretreatment is its ineffectiveness in the lignin removal and stopping its hindrance effect on cellulose (Rodrigues Gurgel da Silva et al., 2019). Alkali pretreatment is helpful for lignin and hemicellulose removal and increases the accessibility to the enzymes for hydrolysis with a high reduced sugar yield. The expensive neutralization and secondary contamination are its drawbacks (Malik et al., 2020b) Ionic liquids are advantageous over acids with non-flammability, thermal stability and chemical adjustability (Abushammala and Mao, 2020). Biological methods use fungi and bacteria, its advantage over other methods is the low cost of the downstream process, simple procedure, and low energy consumption. The drawback is its low-efficiency limit and time-consuming process (Rezania et al., 2020)(X. Li et al., 2022).

One abundant and renewable feedstock for making biofuels is grass. Its growth cycle is short, with low lignin content, and its crystallinity is also less. However, getting a high yield of biofuels from grass is difficult due to the stiff cellulose-hemicellulose-lignin network that resists enzyme attack. Consequently, various pre-treatment methods have been applied to

different types of grass to boost the yield of biofuels (Jin et al., 2015). To use the substrate going forward, it is first necessary to determine the precise composition of the biomass through composition analysis. Both terrestrial and aquatic forms of lignocellulosic biomass exist. The lignin content of aquatic biomass is lower than that of terrestrial biomass, and terrestrial biomass grows more slowly than aquatic biomass. For aquatic weeds to be more effective as a carbon source than other lignocellulosic materials. They are readily broken down to create bioenergy (Anand et al., 2017). Aquatic weeds are classified in the same category as grasses. Numerous aquatic weeds, such as *Eichhornia sp.*, *Pistia stratiotes*, *Lemna minor*, and *Lemna gibba*, can serve as good carbon sources for the synthesis of biofuel. The composition of *Lemna minor, Lemna gibba, Pistia stratiotes*, and *Eichhornia sp.* indicates that they are good carbon sources for bioethanol production, as demonstrated by the bioethanol production from these weeds in a previous study.

According to the proximate analysis, the following species have adequate ash content: *L. minor* (moisture, 3.5%; ash, 18.7%), *L. gibba* (moisture, 3.6%; ash, 19.46), *P. stratiotes* (moisture, 5%; ash, 22.7%), and *Eichhornia sp.* (moisture, 4.2%; ash, 24.9%). These four weeds: *L. minor, L. gibba, P. stratiotes,* and *Eichhornia sp.* have respective carbohydrate contents of 310.27 ± 3.82, 354 ± 25.19, 341.79 ± 7.58, and 311 ± 11.08 mg/g. The weeds had sufficient starch content, such as *L. minor* (290.90 ± 60.43), *L. gibba* (102.90 ± 32.25), *P. stratiotes* (229.49 ± 83.96), and *Eichhornia sp.* (217.81 ± 86.34 mg/g). On the other hand, the lipid content of these weeds was *L. minor* (12.60 ± 0.18), *L. gibba* (17.60 ± 0.08), *P. stratiotes* (4.11 ± 0.19), and *Eichhornia sp.* (16.79 ± 0.07). These results indicate that *L. gibba* had the highest starch and carbohydrate content, while *L. minor* had the highest lipid content. In our study, the ash content and starch content were lower in *P. stratiotes* as compared to the previous study (Gusain and Suthar, 2017). Each of these weeds has a different composition. According to a previous study, duckweed (*Lemna minor*) contains cellulose 55.2%, hemicellulose 32.6 %, and lignin 12.2% (Yadav et al., 2017). The composition of water hyacinth before pre-treatment showed 1.77% acid-soluble lignin, 6.33% acid-insoluble lignin, 32.84% cellulose, and 24.7% hemicellulose. The results of the thermal pre-treatment were different: by using the autoclave technique, 1.58% acid-soluble lignin, 8.35 % acid-insoluble lignin, 27.8% hemicellulose, 29.26% cellulose were formed (Barua and Kalamdhad, 2017). *Pistia stratiotes* contains 49.4 % carbohydrates, 17.8% fibres, and 16.5% protein with 23.8% ash content (Pantawong R., Chuanchai A., Thipbunrat P., Unpaprom Y., 2015). *Pistia stratiotes L*. had an ash content of

25.12%, which was substantially higher than the biomass of fruit shells, wood, crop straw, and herbal medicine that had been reported in the literature; the biomass of these other materials was typically less than 20% (Yang et al., 2022). The total ash content determined by another study was 21.85 ± 0.19 %, which was higher than our study due to changes in the environmental condition from where we collected the sample (Tripathi et al., 2024).

Any aquatic weed's chemical makeup is correlated with the water's composition where it grows. Conversely, the chemical makeup of river sediments influences the composition of certain weeds, such as hippo grass. The water's chemical makeup has an impact on the water hyacinth. While the river next to the human settlement has aquatic weeds that are highly concentrated in certain macronutrients, the river next to the industry has weeds that are full of heavy metals (Kapembwa et al., 2024). A different prior study on the composition of *Pistia stratiotes* found that the total volatile solid was 86 ± 6.44 % and the ash content was 14 ± 5.37, which was lower than what we found in our study. The biochemical analysis revealed that the total protein content was 9.7 ± 1.3% and the total starch content was 10 ± 2%. Except of starch content, the total cellulose, hemicellulose, and lignin contents were 9.7 ± 0.31%, 12 ± 1.3, and 10 ± 2.1, respectively, lower than in our study. The impact of the environment can alter the chemical makeup of any weed (Jacob and Banerjee, 2016).

Lignocellulosic biomass includes corn stover, which undergoes enzymatic hydrolysis and pre-treatment before being transformed into a value-added product (Li et al., 2017). Organic waste also includes rice straw that is used to optimise the alkali pre-treatment process. The temperature, time, NaOH concentration, and solid loading were the independent variables. The sugar reached its peak at 121°C, 40 minutes, 2% w/v NaOH concentration, and 5% loading. With 50.5 g/L of glucose, 13.5 g/L of xylose, and 1.3 g/L of arabinose, the maximum sugar content was 65.3 g/L.(Valles et al., 2021). A comparative investigation of diverse chemical pretreatment was performed on sorghum straw. Six different pretreatment were applied to it, namely, 2 % (w/v) NaOH, 2 % H_2SO_4, 2 % (w/v) Na_2CO_3, 2 % (w/v) oxalic acid, 2.15 % (w/v) H_2O_2 and 95% (v/v) glycerol pretreatment. In this study, 2 % NaOH pretreatment was most effective for lignin removal and enzyme-mediated hydrolysis process (Bhati and Sharma, 2023). Another optimization of alkaline pretreatment by central composite design with enzymatic hydrolysis study was performed on the cocoa pod husk. The optimal condition was 5%(w/v) NaOH for 30 min at 120 °C increased the content of cellulose from 27.68 (untreated)

to 57 % and then enzymatic hydrolysis was performed resulting in 66.80g/L reducing sugar yield up to 98.75 % (Hernández-Mendoza et al., 2021) which is higher from our study.

The cellulose content of *Pistia stratiotes* was extracted in a previous study under various conditions using bleaching agents. Four distinct conditions were used to optimise the pre-treatment: (1) 2% sodium chlorite + 2% glacial acetic acid; (2) 4% sodium hypochlorite; (3) 4% hydrogen peroxide + 4% sodium hydroxide; and (4) 4% soapnut solution. The results of all the conditions in the form of cellulose yield as a percentage were 38.92, 25.70, 10.7 and none in the fourth condition (Umesh et al., 2022a). In another study, 1% alkali (NaOH) and 1% H_2O_2 were used to treat *Pistia stratiotes* and water hyacinth. Following this treatment, the cellulose results in water hyacinth increased from 19.7 to 34.2% and in *Pistia* from 16.5 to 28.4% (Mishima et al., 2008a). After the Pre-treatment, besides ethanol, other solid-liquid products are also produced like foliar fertilizers, jet fuels and catalysts from the *Pistia stratiotes* (Yang et al., 2022). Twenty distinct pretreatment methods were carried out to enhance the enzymatic hydrolysis and identify the reduced sugar. Sodium hydroxide and hydrogen peroxide treatment of the water lettuce and water hyacinth was found to be an effective chemical pretreatment. Compared to water hyacinth, the reduced sugar content in water lettuce was 1.8 times higher. Therefore, it appears that water lettuce is a more effective substrate for the production of bioethanol than water hyacinth (Mishima et al., 2006). A study shows how beneficial the alkali pretreatment was in helping aquatic weeds produce valuable products. It used chopped fresh forms of French weed, para grass, water lettuce, and sledge as the substrate. These weeds underwent co-digestion, both with and without an alkali pretreatment, producing CH_3. That study's conclusion was satisfactory. Substrate, cow dung and Water were in a ratio of 10:10:80 . The results of methane with French weed, para grass, water lettuce, and sledge increased by 15.77%, 16.52%, 4.22% and 31.48% following pretreatment (Sinbuathong, 2019).

The optimization of pre-treatment was done on water hyacinth. Different acids like HCL/H_2SO_4/HCOOH (2%v/v) with varied concentrations were used such as 1%, 2%, 3% and 4%v/v to obtain a high amount of reduced sugar. Also, 3% v/v NaOH was used for alkali pre-treatment. The observed results gave maximum reduced sugar at 37.89 mg/100 ml filtrate with 4 % H_2SO_4 (dilute acid pre-treatment). With the alkali pre-treatment, 3% NaOH gave 17.185 mg/100ml sugar concentration (Awasthi et al., 2013). In contrast, the highest amount of reduced sugar detected in our study was 33.62 g/L following 72 hours of enzymatic hydrolysis.

The pre-treatment of lignocellulosic biomass with enzymatic hydrolysis is crucial. The saccharification of cellulosic biomass or the low degradation of lignocellulosic biomass for the production of valuable products is the bottleneck in this field, necessitating the use of economically used enzymes that are effective in the saccharification process. Enzymes ought to be less expensive to produce at the industrial scale. The cost of using sugars from the lignocellulosic biomass reaches 25–30% of the total cost of producing biofuel (Hu et al., 2023b). Either commercially available or in-house-produced cellulase enzymes can be used for enzymatic hydrolysis. The primary source of fermentable sugars is plant cell walls. Certain bacteria possess the ability to break down cellulose, hemicellulose, and lignin. For example, the bacterium, *Trabulsiella sp*. is capable of efficiently breaking down the lignin model compound, guaiacylglycerol-β-guaiacyl ether (Suman et al., 2016). *Clostridium thermocellum* is an isolated bacteria that is capable of degradation of cellulose and is used for the saccharification process. It is isolated by goat rumen and is a thermophilic bacteria that is active at the temperature of 50-70 °C and works on agro-industrial waste (Hamann et al., 2015).

Apart from bacteria, the fungus is also used for the production of enzymes. Different types of fungi, like *Aspergillus sp. A1C2-06*, *Talaromyces verruculosus* A1C2-05 (Fontes et al., 2023), *Aspergillus niger* (Sulyman et al., 2020) and *Trichoderma ressei* (Wu et al., 2019), are used for the production of cellulase enzyme. In the previous study, the *A. niger* produced enzymes by using the waste of *C. oleifera* as a substrate and the conditions were 50 °C at 200 rpm for 24 hours and produced 20.58 g/L sugar (Dessie et al., 2024). Another study shows that using the same fungus stain and conditions were different than was at 50 °C at 150 rpm for 48 hours on the wheat straw after thermal pretreatment produced 32.90 g/L sugar (Infanzón-Rodríguez et al., 2022). When alkaline pretreatment was done on the *Brassica juncea* with a low concentration of NaOH (0.5 M) at 160 °C for 30 minutes with 10 % loading. The delignification occurred in the sample and then enzymatic hydrolysis was done by the mixture of cellulase obtained from *A. niger* MTCC284, *T. harzianum*MTCC8230 and *F. incarnatum*KU377454. Then 62.35 mg/ml reduced sugar was formed after hydrolysis in 48 hours at 50 °C with 15 % loading (Pant et al., 2021). Commercially available cellulase enzymes that are suitable for the saccharification process are also reasonably priced. We employed the inexpensive, readily available commercial cellulase enzyme in our investigation to achieve successful saccharification results.

Pistia stratiotes was treated with acid (H_2SO_4) in the prior study. The H_2SO_4 solution of (v/v) 1.5, 2.5, and 3.5% was used as the optimization's parameters, and it was left at a constant temperature of 25 °C for 15, 30, and 60 minutes. The full optimisation conditions are composed of two factors with three levels. Following treatment, the hydrolysate is filtered using grade 6 Whatman filter paper to analyse the sugar content. The result demonstrates that by raising the H_2SO_4 concentration by about 3% (v/v) and treating for 40 minutes, the sugar yield increased to 122.2±5.2 mg/g. The sugar yield dropped when both parameters were raised because sugar breaks down into harmful substances (Mthethwa et al., 2018) In our study, the hydrolysate was filtered through a vacuum filter, which effectively removed even the smallest impurities. CaCO3 was then used to perform the detoxification process. In a different investigation, the *Pistia stratiotes* contained 36% cellulose, 23% hemicellulose, and 3% lignin. For 45 minutes, this raw sample was hydrolysed at a concentration of 2.5% H_2SO_4 and 1% w/v biomass to acid ratio (Mthethwa et al., 2019). *Pistia stratiotes* was given a hydrothermal pretreatment at 473 K for 30 minutes. A notable sugar yield of 23.70 ± 0.52 g/kg dry mass was observed (Luo et al., 2011).

Pistia stratiotes and water hyacinth are free-floating weeds that were the subject of an investigation into the fermentation-based production of bioethanol. *Pistia stratiotes* and water hyacinth had the same sugar content, except arabinose. In *Pistia stratiotes*, there is more starch than cellulose and hemicellulose. The fermentation of *S. cerevisiae* was used to measure the concentration of ethanol; the results for water hyacinth and *Pistia stratiotes* were 14.4 g/L and 14.9 g/L, respectively. Nevertheless, 16.2 and 16.9 g/L of ethanol were produced when recombinant *E. coli KO11* was used to perform simultaneous saccharification and fermentation. It demonstrates that saccharification and fermentation done simultaneously were more efficient than saccharification and hydrolysis done separately. The yield of ethanol was compared to agricultural waste: for water hyacinth, it was 0.14-0.17 g/g dry, and 0.15- 0.16 g/g dry biomass of *Pistia stratiotes* (Mishima et al., 2008b). Water hyacinth and water lettuce are two examples of aquatic weeds that successfully produce bioethanol. To boost output, malt and barley extract were added to the newly taken substrate during the fermentation process. The concentration was different in different conditions. The water hyacinth and water lettuce samples had the highest sugar content (0.283 mg/L and 0.228 mg/L) on the second day. In comparison to water lettuce, the water hyacinth produced the most ethanol on the second day. The fermentation process was carried out at 30°C in the dark. In comparison to other enhancer mixtures, 1.019

mg/L of ethanol was produced when 10% barley was used as an enhancer during the process (Rezania et al., 2014).

Similar to water lettuce, water hyacinth is classified as an aquatic weed. After providing an acidic pretreatment, this weed produced ethanol through fermentation using four distinct yeast strains: *S. cerevisiae* NRRLY-12632, *Candida intermedia* NRRLY-12854, *Pachysolan tannophilus* NRRLY-2460, *Pichia stipitis*, and a fungal strain called *Trichoderma reesei* NRRL-3652 used to produce cellulase enzyme for enzymatic hydrolysis. The pretreatment was carried out at 121°C for one hour using 0.1%, 0.5%, 1%, 1.5%, and 2% H_2SO_4. Additionally, the solid biomass produced used for enzymatic hydrolysis by *T. reesei's* in a shaking flask at 30 °C was subjected to enzymatic hydrolysis. The fermentation process lasted 15 minutes at 28 °C and 125 rpm. Using *P. tannophilus*, the highest ethanol concentration of 0.043g/g was achieved and by *S. cerevisiae* 0.015 g/g (Manivannan and Narendhirakannan, 2015). Potential aquatic weed *Spirodela polyrhiza* is grown on sewage and is being considered as feedstock for bioethanol production. It has a high starch content that is useful for certain purposes. This starch is additionally utilised during the fermentation process to produce ethanol. The enzymatic hydrolysis of starch by *B. amyloliquefaciens* crude enzyme extract was a necessary step before fermentation. Enzymatically hydrolysed 40 g/L of reduced sugar was used to begin the production of ethanol. *S. cerevisiae* (109 CFU/ml) was used for the fermentation, which was conducted for 48 hours at 28 °C in an incubator with a shaker set at 150 rpm. A tonne of biomass can produce 79.7-80.4 kg of starch. the ethanol yield was obtained at 8.98 % and 0.032 g/g waste biomass (Patel and Bhatt, 2021). The procedure followed in our study is mentioned in Fig. 3.25.

A programme called PRADHAN MANTRI JI-VAN YOJANA uses lignocellulosic biomass and other renewable feedstock to finance integrated bioethanol projects. This programme is suitable for second-generation (2G) biofuel production. The Indian government has launched this programme to improve the nation's energy security and lessen its reliance on imports. The 2018 announcement of the National Policy on Biofuels aims to accelerate the production of biofuel and blend it 20% with petrol and 5% with diesel by 2030. 2G bioethanol is produced using agricultural residues, organic waste (such as woody and grassy waste), and waste materials as a substrate combined with biodegradable industrial and municipal waste. About 12–16 crore tonnes of these materials are available annually in India. The projected 2500–3000 crore litres of bioethanol produced annually have the potential to

lessen reliance on imported crude oil. This policy has the benefit of generating additional valuable products like compost, liquid CO2, and biogas. This programme improves citizen health by reducing pollution of the environment, soil, and water. The socioeconomic growth of rural India along with the formation of other possible by-products like furfural, Xylitol, high fructose, and L-arabinose, raised the process's profitability. India would be the first time in the country to use 2G ethanol ("Refinery Division - Second Generation (2G) Ethanol | Ministry of Petroleum and Natural Gas | Government of India," n.d.). Our weed is also included in the 2G feedstock for the production of bioethanol.

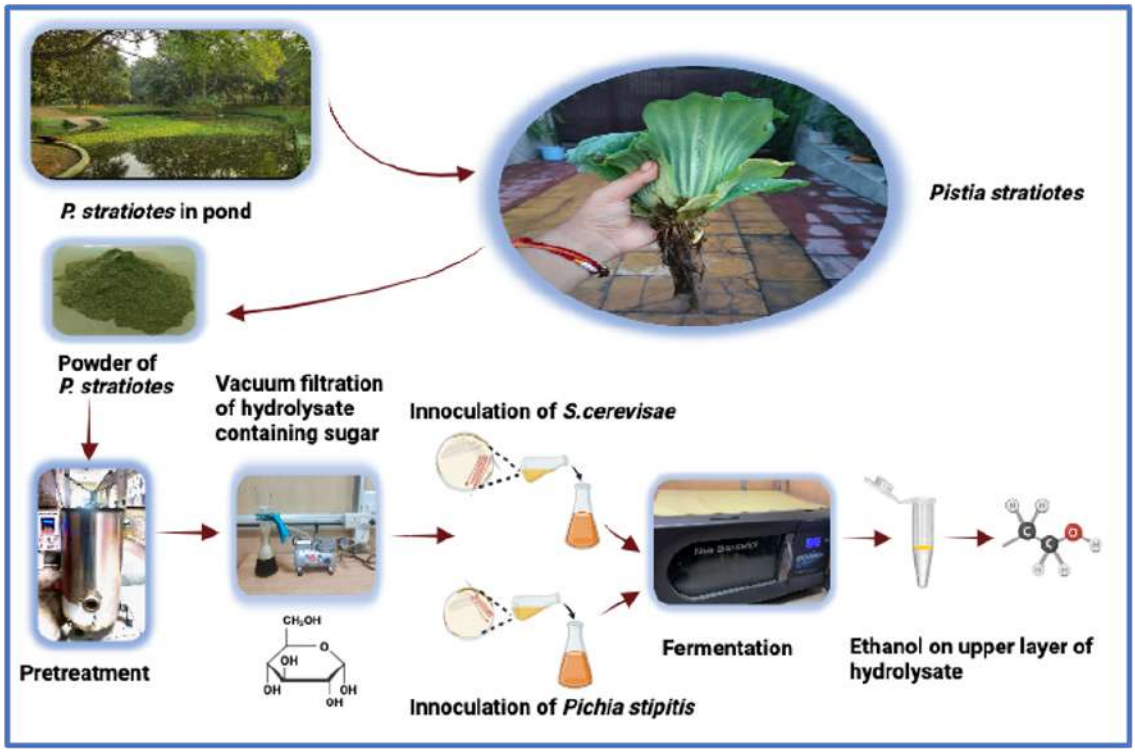


Fig.3.25 Overall process of ethanol production from the *Pistia stratiotes* in the study

CHAPTER – 4

SUMMARY, CONCLUSION AND FUTURE SCOPE

CHAPTER (4)

SUMMARY, CONCLUSION AND FUTURE SCOPE

4.1 Summary

Pistia stratiotes was chosen from all the aquatic weeds for the production of ethanol after reviewing the literature. *Pistia stratiotes* (water lettuce) is an efficient carbon source because of its composability. It contains a high amount of cellulose and hemicellulose which makes it suitable for the formation of valuable products. It is easily available and it can grow within a week and spread like a network in freshwater bodies. It is counted as a weed because it spreads in the water bodies and consumes all the nutrients in the water bodies which is harmful to the water ecosystem. The second region of selection of this weed was that less research was conducted on this weed as compared to other aquatic weeds. *Pistia stratiotes* was collected from Hauz Rani city forest, Delhi in the early winter season and monsoon season. It is about 25 kg in weight whenever we collected it, then washed it 2 to 3 times to remove extra dirt from it with tap water. The washed sample was dried at room temperature for 24 hours and then dried in the oven for 24 hours at 40 °C. The sample was crushed by a grinder and stored in air-tight bags for further use. After the preparation of the sample, composition analysis was the initial step in our study by which we detected the exact carbon content in our weed. The composition analysis was done with the standardized protocols. Ash content, total solids, moisture content and lignin content were determined by the NREL methods which is very common in the renewable energy protocols. Protein analysis was done by Foss Nitrogen analyzer. Starch was detected by the anthrone method. Cellulose and hemicellulose estimation was done by up-degraff and detergent method. The composition proves the statement that it is a good source of ethanol production. 25.90 % cellulose and 18.44 % hemicellulose content give its weightage to use as a carbon source. Whenever we did the composition the next step was the extraction of sugar from the raw sample. This extraction of sugar was done by the optimization of alkali and acidic pretreatment methods. This optimization was done by Minitab software with Response surface methodology (RSM). In this RSM the independent variables were taken which have an impact on the dependent variables (responses). In the alkali pretreatment optimization, we have taken independent variables like alkali concentration, time and temperature. The range of independent variables was 0.5, 1.75, 3 % NaOH concentration,

30, 45 and 60 min and 60, 90 and 120 °C. 20 different conditions were formed by central composite design in RSM. These 20 conditions were performed in the laboratory and set in the model by which one single optimized condition was formed and this optimized condition (2.47 % NaOH concentration, 60 min at 120° C) was performed individually to reconfirm the results. After performing the alkali optimization sample was neutralized with diluted sulfuric acid sulphuric acid. The solid sample was used to detect the cellulose content in the sample. Maximum cellulose content was formed by the optimized condition and this is further used for the enzymatic hydrolysis process. In the alkali-optimized condition, the maximum is 51.67 % cellulose content. This proves that alkali optimized condition increases double fold of cellulose content from the raw sample. The hydrolysis was done by commercialized cellulase enzyme which was purchased by India Mart. These commercially available cellulase enzymes make the process cost-effective. Initially, the filter paper unit was measured by the Whatman filter paper 1. Whenever the FPU (247 FPU/g) was determined then further hydrolysis was done by different concentrations of 25 FPU, 50 FPU and 75 FPU. In the initial step, enzymatic hydrolysis was done in a 10 ml solution with a 0.5 g sample (cellulose). It is performed in triplicate and three different samples are used for hydrolysis (pure cellulose, raw sample and hydrolyzed sample) with the addition of 2 % sodium (w/v) to protect the sample from microbial growth. The maximum hydrolysis was done by 50 FPU within.

In the acidic optimization, different parameters were taken and 20 different conditions were performed with the help of an autoclave. In the central composite design, α value was also taken. The independent variables with α values were 0.15, 0.75, 1.63, 2.5 and 3.1 % H_2SO_4 concentration, 12.96, 30, 55, 80 97.04 min and 93.18, 100,110,120 and 126.82 °C. After performing all the experiments, the hydrolysate contains many toxic compounds which restrict microbes during the fermentation process. For the detoxification of hydrolysate, the hydrolysate was neutralized by $CaCO_3$ and then stored at -20 °C for further use. The hydrolysate contains reduced sugar which is used for the production of ethanol with the help of microbes. The reduced sugar was determined by DNSA, miller 1959 method. In the acidic optimization process, both cellulose content and reduced sugar content are carried out as a response. When the outcomes were put in the model then an optimized condition was obtained. The acidic optimized condition (2.8 % H_2SO_4 concentration, 15 min at 120°C). when the optimized condition was performed individually then it gives 23.44 % reduced sugar which proves that our model is fit according to the observation. For the fermentation process, two strains were procured from NCIM, Pune (India). *Saccharomyces cerevisiae* (ATCC 834)

(NCIM 3594) and *Pichia Stipitis* (NCIM 3498) were in the form of active culture. Initially, they were revived on the MGYP media as mentioned in the instructions in the pamphlet provided by NCIM (Pune) along with strains. *S. cerevisiae* convert hexose sugars into ethanol on the other hand *P. stipitis* converts both hexose and pentose sugars into ethanol. So both of these stains are model strains for the production of bioethanol. Initially, the strains were maintained in the petri plate for storage. The inoculum was prepared and the growth came within 12 hours. Initially, 10 g/L of reduced sugar was taken in the hydrolysate and the synthetic media for the fermentation process. The hydrolysate amount was taken as 150 ml in the conical flask. Inoculate the culture 5 % of total hydrolysate and then fermentation was done and take out the hydrolysate in every 12 hours for taking the growth rate of *S. cerevisiae* and *P. stipitis* the ethanol concentration in the media. The quantification of ethanol was done by Gas Chromatography. The retention time of ethanol was 10 minutes. The graph was plotted cell growth, ethanol concentration vs time.

4.2 Conclusion

P. stratiotes is used as a biomass for ethanol production because of its high carbohydrate content with the maximum amount of cellulose. To create a high amount of reduced sugar and better enzymatic hydrolysis and form more reduced sugar for the production of valuable products, acidic and alkali pretreatment optimisation is helpful. Thus, we used Response Surface Methodology (RSM) analysis using Minitab Statistical Software to optimize the condition of acidic and alkali pretreatment. It was discovered that the ideal conditions were 2.47 % alkali content, 60 minutes, and 120 ºC for alkali pretreatment optimization. Once optimised, the highest percentage of cellulose obtained was 51.66 %. The maximum amount of reduced sugar with 75 FPU/g solid biomass is obtained after performing the enzymatic hydrolysis by a commercially used enzyme, which is thought to be cost-effective. This is equivalent to 31.06 g/L in 72 hours as opposed to hydrolysing the raw sample with the same amount of enzyme, which is 8.51 g/L. Acidic pre-treatment enhances the sugar content accessible to the microbes used in the fermentation process in the sample. In previous studies that used *P. stratiotes*, the optimization of acidic pre-treatment wasn't performed. Acidic pre-treatment condition to obtain maximum sugar that is affected by three independent variables, viz. acid concentration, time and temperature. The optimized condition (2.5 % acid conc. at 15 min and 120 °C) was used to obtain hydrolysate with 23.44% reduced sugar, which was further detoxified with calcium carbonate and subsequently used for ethanol production. *S. cerevisiae* can only use hexose sugar for fermentation, while *P. stipitis* can use both pentose and hexose sugar. *S. cerevisiae* produced ethanol conc. 3.32 g/L with 0.39 g/g ethanol yield from the synthetic media and in the hydrolysate, ethanol conc. 3.25 g/L with 0.37 g/g ethanol yield was found. *P. stipitis* produced 3.57 g/L ethanol conc. with 0.41 g/g ethanol yield from the synthetic media while in hydrolysate, 0.39 g/g ethanol yield was calculated. Bioethanol from lignocellulosic biomass is a sustainable approach as it is a requirement to replace fossil fuels. The current study's novelty is in lowering operating costs while achieving
the maximum amount of reduced sugar formation with techno-economical results. The environmental, and socioeconomic impacts need to be evaluated. This is necessary to scale up the entire biorefinery process and produce valuable products at a higher and more affordable rate.

4.3 Future scope

- Aquatic weed used as biofuel substrates could give way for wastewater treatment and renewable energy production.
- Phytoremediation characteristics of aquatic weeds, and constructed water bodies could be economical alternatives to the cultivation of aquatic weeds.
- The production of other valuable products could be possible by aquatic weeds like lactic acids, enzymes and bioplastic.
- Metagenomic sequencing research could give insight into growth patterns, and their response to the biotic and abiotic factors and find out the particular gene involved in the carbohydrate production. This technique gives the direction for higher cellulose and hemicellulose content by gene editing technique and gives a novel way of bioenergy production.
- To increase biofuel yields, process augmentation and other hybrid pretreatment techniques, such as biological ones, can be used.
- • Aquatic weed must be fully utilised to achieve economic sustainability. Under an integrated biorefinery approach, residual biomass after the production of biofuel could be used for other industrial applications.
- It is necessary to conduct extensive life cycle analyses and risk assessments of aquatic weeds to develop and expand biorefineries for a viable future them.

REFERENCES

Aarti, C., Khusro, A., Agastian, P., Kuppusamy, P., Al Farraj, D.A., 2022. Synthesis of gold nanoparticles using bacterial cellulase and its role in saccharification and bioethanol production from aquatic weeds. J. King Saud Univ. - Sci. 34, 101974. https://doi.org/10.1016/J.JKSUS.2022.101974

Abdel-Banat, B.M.A., Hoshida, H., Ano, A., Nonklang, S., Akada, R., 2010. High-temperature fermentation: How can processes for ethanol production at high temperatures become superior to the traditional process using mesophilic yeast? Appl. Microbiol. Biotechnol. 85, 861–867. https://doi.org/10.1007/s00253-009-2248-5

Abushammala, H., Mao, J., 2020. A Review on the Partial and Complete Dissolution and Fractionation of Wood and Lignocelluloses Using Imidazolium Ionic Liquids. Polymers (Basel). 12, 195. https://doi.org/10.3390/polym12010195

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment: Fundamentals toward application. Biotechnol. Adv. 29, 675–685. https://doi.org/10.1016/J.BIOTECHADV.2011.05.005

Aguilar-Reynosa, A., Romaní, A., Ma. Rodríguez-Jasso, R., Aguilar, C.N., Garrote, G., Ruiz, H.A., 2017. Microwave heating processing as alternative of pretreatment in second-generation biorefinery: An overview. Energy Convers. Manag. 136, 50–65. https://doi.org/10.1016/j.enconman.2017.01.004

Ahmed, F., Yan, Z., Bao, J., 2019. Dry biodetoxification of acid pretreated wheat straw for cellulosic ethanol fermentation. Bioresour. Bioprocess. 6. https://doi.org/10.1186/s40643-019-0260-x

Alabdalall, A.H., Almutari, A.A., Aldakeel, S.A., Albarrag, A.M., Aldakheel, L.A., Alsoufi, M.H., Alfuraih, L.Y., Elkomy, H.M., 2023. Bioethanol Production from Lignocellulosic Biomass Using Aspergillus niger and Aspergillus flavus Hydrolysis Enzymes through Immobilized S. cerevisiae. Energies 16. https://doi.org/10.3390/en16020823

Alam, S.N., Khalid, Z., Guldhe, A., Singh, B., Korstad, J., 2021a. Harvesting and pretreatment techniques of aquatic macrophytes and macroalgae for production of biofuels. Environ. Sustain. 2021 42 4, 299–316. https://doi.org/10.1007/S42398-021-00178-6

Alam, S.N., Khalid, Z., Guldhe, A., Singh, B., Korstad, J., 2021b. Harvesting and pretreatment techniques of aquatic macrophytes and macroalgae for production of biofuels. Environ. Sustain. 4, 299–316. https://doi.org/10.1007/S42398-021-00178-6

Alemdar, A., Sain, M., 2008. Isolation and characterization of nanofibers from agricultural

residues - Wheat straw and soy hulls. Bioresour. Technol. 99, 1664–1671. https://doi.org/10.1016/j.biortech.2007.04.029

Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., Cantarella, M., 2000. Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw. J. Ind. Microbiol. Biotechnol. 25, 184–192. https://doi.org/10.1038/SJ.JIM.7000054

Ali, C.H., Mbadinga, S.M., Liu, J.-F., Yang, S.-Z., Gu, J.-D., Mu, B.-Z., 2015. Significant enhancement of Pseudomonas aeruginosa FW_SH-1 lipase production using response surface methodology and analysis of its hydrolysis capability. J. Taiwan Inst. Chem. Eng. 52, 7–13. https://doi.org/https://doi.org/10.1016/j.jtice.2015.02.001

Ali, S., Kaviraj, A., 2018. Aquatic weed Ipomoea aquatica as feed ingredient for rearing Rohu, Labeo rohita (Hamilton). https://doi.org/10.1016/j.ejar.2018.09.004

Alonso, D.M., Bond, J.Q., Dumesic, J.A., 2010. Catalytic conversion of biomass to biofuels. Green Chem. 12, 1493–1513. https://doi.org/10.1039/c004654j

Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour. Technol. 101, 4851–4861. https://doi.org/10.1016/J.BIORTECH.2009.11.093

Ampese, L.C., Sganzerla, W.G., Di Domenico Ziero, H., Mudhoo, A., Martins, G., Forster-Carneiro, T., 2022. Research progress, trends, and updates on anaerobic digestion technology: A bibliometric analysis. J. Clean. Prod. 331, 130004. https://doi.org/10.1016/J.JCLEPRO.2021.130004

Anand, S., Bharti, S.K., Dviwedi, N., Barman, S.C., Kumar, N., 2017. Macrophytes for the reclamation of degraded waterbodies with potential for bioenergy production, in: Phytoremediation Potential of Bioenergy Plants. Springer Singapore, pp. 333–351. https://doi.org/10.1007/978-981-10-3084-0_13

Andrić, P., Meyer, A.S., Jensen, P.A., Dam-Johansen, K., 2010. Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: I. Significance and mechanism of cellobiose and glucose inhibition on cellulolytic enzymes. Biotechnol. Adv. https://doi.org/10.1016/j.biotechadv.2010.01.003

Appenroth, K.J., Sree, K.S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., Jahreis, G., 2017. Nutritional value of duckweeds (Lemnaceae) as human food. Food Chem. 217, 266–273. https://doi.org/10.1016/J.FOODCHEM.2016.08.116

Asghar, U., Irfan, M., Iram, M., Huma, Z., Nelofer, R., Nadeem, M., Syed, Q., 2015. Effect of alkaline pretreatment on delignification of wheat straw. Nat. Prod. Res. 29, 125–131. https://doi.org/10.1080/14786419.2014.964712

Auwal, S.M., Zarei, M., Tan, C.P., Basri, M., Saari, N., 2018. Enhanced physicochemical stability and efficacy of angiotensin I-converting enzyme (ACE) - inhibitory biopeptides by chitosan nanoparticles optimized using Box-Behnken design. Sci. Reports 2018 81 8, 1–11. https://doi.org/10.1038/s41598-018-28659-5

Awasthi, M., Kaur, J., Rana, S., 2013. Bioethanol Production Through Water Hyacinth, Eichhornia Crassipes Via Optimization of the Pretreatment Conditions. Int. J. Emerg. Technol. Adv. Eng. 3, 42–46.

Awoyale, A.A., Lokhat, D., 2021. Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production. Sci. Rep. 11, 1–16. https://doi.org/10.1038/s41598-020-78105-8

Baadhe, R.R., Potumarthi, R., Mekala, N.K., 2014. Influence of dilute acid and alkali pretreatment on reducing sugar production from corncobs by crude enzymatic method: A comparative study. Bioresour. Technol. 162, 213–217. https://doi.org/10.1016/j.biortech.2014.03.117

Bak, J.S., 2014. Electron beam irradiation enhances the digestibility and fermentation yield of water-soaked lignocellulosic biomass. Biotechnol. Reports 4, 30–33. https://doi.org/10.1016/J.BTRE.2014.07.006

Bano, A., Irfan, M., 2019. Alkali pretreatment of cotton stalk for bioethanol. Bangladesh J. Sci. Ind. Res. 54, 73–82. https://doi.org/10.3329/BJSIR.V54I1.40733

Barua, V.B., Kalamdhad, A.S., 2017. Effect of various types of thermal pretreatment techniques on the hydrolysis, compositional analysis and characterization of water hyacinth. Bioresour. Technol. 227, 147–154. https://doi.org/10.1016/J.BIORTECH.2016.12.036

Baruah, J., Deka, R., Kalita, E., 2020. Greener production of microcrystalline cellulose (MCC) from Saccharum spontaneum (Kans grass): Statistical optimization. Int. J. Biol. Macromol. 154, 672–682.

Basak, R.K., Saha, S.G., Sarkar, A.K., Saha, M., Das, N.N., Mukherjee, A.K., 1993. Thermal Properties of Jute Constituents and Flame Retardant Jute Fabrics. Text. Res. J. 63, 658–666. https://doi.org/10.1177/004051759306301107

Başar, İ.A., Çoban, Ö., Göksungur, M.Y., Eskicioğlu, Ç., Perendeci, N.A., 2021. Enhancement of lignocellulosic biomass anaerobic digestion by optimized mild alkaline hydrogen peroxide pretreatment for biorefinery applications. J. Environ. Manage. 298, 113539. https://doi.org/10.1016/J.JENVMAN.2021.113539

Bauer, S., Ibáñez, A.B., 2014. Rapid determination of cellulose. Biotechnol. Bioeng. 111,

2355–2357. https://doi.org/10.1002/BIT.25276

Bayrakci, A.G., Koçar, G., 2014. Second-generation bioethanol production from water hyacinth and duckweed in Izmir: A case study. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2013.10.011

Ben Bader, N., Germec, M., Turhan, I., 2022. Repeated-batch fermentation of Scheffersomyces stipitis in biofilm reactor for ethanol production from the detoxified and glucose- or xylose-enriched rice husk hydrolysate and its kinetic modeling. Fuel 326. https://doi.org/10.1016/j.fuel.2022.125053

Bhati, N., Sharma, A.K., 2023. Comparative study of different chemical pretreatments for enhanced enzymatic hydrolysis of sorghum straw. Biomass Convers. Biorefinery 1, 1–9. https://doi.org/10.1007/S13399-023-05185-7/FIGURES/4

Bokinsky, G., Peralta-Yahya, P.P., George, A., Holmes, B.M., Steen, E.J., Dietrich, J., Lee, T.S., Tullman-Ercek, D., Voigt, C.A., Simmons, B.A., Keasling, J.D., 2011. Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using engineered Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. 108, 19949–19954. https://doi.org/10.1073/PNAS.1106958108

Cai, C., Zhang, C., Li, N., Liu, H., Xie, J., Lou, H., Pan, X., Zhu, J.Y., Wang, F., 2023. Changing the role of lignin in enzymatic hydrolysis for a sustainable and efficient sugar platform. Renew. Sustain. Energy Rev. 183, 113445. https://doi.org/10.1016/J.RSER.2023.113445

Castañón-Rodríguez, J.F., Torrestiana-Sánchez, B., Montero-Lagunes, M., Portilla-Arias, J., De León, J.A.R., Aguilar-Uscanga, M.G., 2013. Using high pressure processing (HPP) to pretreat sugarcane bagasse. Carbohydr. Polym. 98, 1018–1024. https://doi.org/10.1016/J.CARBPOL.2013.06.068

Cavalaglio, G., Gelosia, M., Giannoni, T., Barros Lovate Temporim, R., Nicolini, A., Cotana, F., Bertini, A., 2021. Acid-catalyzed steam explosion for high enzymatic saccharification and low inhibitor release from lignocellulosic cardoon stalks. Biochem. Eng. J. 174, 108121. https://doi.org/10.1016/J.BEJ.2021.108121

Cesaro, A., Cieri, V., Belgiorno, V., 2021. Press-extrusion pretreatment of the organic fraction of municipal solid waste for enhanced methane production. J. Mater. Cycles Waste Manag. 23, 130–138. https://doi.org/10.1007/S10163-020-01105-3/FIGURES/6

Chauhan, A., Singh, V.K., Kwatra, Y., 2020. Saccharification of Water Hyacinth Biomass for Bioethanol Production: Optimization of Sulfuric Acid Pretreatment. J. Biofuels 11, 1. https://doi.org/10.5958/0976-4763.2020.00001.X

Chen, K.Y., Zheng, Y., Cheng, Y.S., 2015. Integrated alkali pretreatment and preservation of wet lettuce (Pistia stratiotes) by lactic acid bacteria for fermentable sugar production. Biomass and Bioenergy 81, 249–255. https://doi.org/10.1016/J.BIOMBIOE.2015.07.007

Chung, D., Cha, M., Guss, A.M., Westpheling, J., 2014. Direct conversion of plant biomass to ethanol by engineered Caldicellulosiruptor bescii. Proc. Natl. Acad. Sci. U. S. A. 111, 8931–8936. https://doi.org/10.1073/PNAS.1402210111

Cotana, F., Cavalaglio, G., Gelosia, M., Coccia, V., Petrozzi, A., Ingles, D., Pompili, E., 2015. A comparison between SHF and SSSF processes from cardoon for ethanol production. Ind. Crops Prod. 69, 424–432. https://doi.org/10.1016/j.indcrop.2015.02.064

Culaba, A.B., Mayol, A.P., San Juan, J.L.G., Vinoya, C.L., Concepcion, R.S., Bandala, A.A., Vicerra, R.R.P., Ubando, A.T., Chen, W.H., Chang, J.S., 2022. Smart sustainable biorefineries for lignocellulosic biomass. Bioresour. Technol. 344, 126215. https://doi.org/10.1016/J.BIORTECH.2021.126215

Dahunsi, S.O., 2019. Liquefaction of pineapple peel: Pretreatment and process optimization. Energy 185, 1017–1031. https://doi.org/10.1016/J.ENERGY.2019.07.123

Dahunsi, S.O., Adesulu-Dahunsi, A.T., Izebere, J.O., 2019. Cleaner energy through liquefaction of Cocoa (Theobroma cacao) pod husk: Pretreatment and process optimization. J. Clean. Prod. 226, 578–588. https://doi.org/10.1016/j.jclepro.2019.04.112

Dai, L., He, C., Wang, Y., Liu, Y., Yu, Z., Zhou, Y., Fan, L., Duan, D., Ruan, R., 2017. Comparative study on microwave and conventional hydrothermal pretreatment of bamboo sawdust: Hydrochar properties and its pyrolysis behaviors. Energy Convers. Manag. 146, 1–7. https://doi.org/10.1016/J.ENCONMAN.2017.05.007

Das, N., Jena, P.K., Padhi, D., Kumar Mohanty, M., Sahoo, G., 2021. A comprehensive review of characterization, pretreatment and its applications on different lignocellulosic biomass for bioethanol production. Biomass Convers. Biorefinery 2021 1–25. https://doi.org/10.1007/S13399-021-01294-3

Das, S.P., Gupta, A., Das, D., Goyal, A., 2016. Enhanced bioethanol production from water hyacinth (Eichhornia crassipes) by statistical optimization of fermentation process parameters using Taguchi orthogonal array design. Int. Biodeterior. Biodegrad. 109, 174–184. https://doi.org/10.1016/j.ibiod.2016.01.008

Deng, Z., Xia, A., Liao, Q., Zhu, X., Huang, Y., Fu, Q., 2019. Laccase pretreatment of wheat straw: Effects of the physicochemical characteristics and the kinetics of enzymatic hydrolysis. Biotechnol. Biofuels 12. https://doi.org/10.1186/S13068-019-1499-3

Dessie, W., Xiao, J., Tang, J., An, B., Luo, X., Wang, M., Liao, Y., Wahab, R., Li, C., Qin, Z.,

2024. Maximizing fermentable feedstocks from Camellia oleifera seed oil extraction residues: Green pretreatment and enzymatic hydrolysis for effective valorization. Arab. J. Chem. 17, 105815. https://doi.org/10.1016/j.arabjc.2024.105815

Duque, A., Manzanares, P., Ballesteros, M., 2017. Extrusion as a pretreatment for lignocellulosic biomass: Fundamentals and applications. Renew. Energy 114, 1427–1441. https://doi.org/10.1016/J.RENENE.2017.06.050

Dussán, K.J., Silva, D.D.V., Perez, V.H., da Silva, S.S., 2016. Evaluation of oxygen availability on ethanol production from sugarcane bagasse hydrolysate in a batch bioreactor using two strains of xylose-fermenting yeast. Renew. Energy 87, 703–710. https://doi.org/10.1016/J.RENENE.2015.10.065

Eisenmenger, M.J., Reyes-De-Corcuera, J.I., 2009. High pressure enhancement of enzymes: A review. Enzyme Microb. Technol. 45, 331–347. https://doi.org/10.1016/J.ENZMICTEC.2009.08.001

Escobar, J.C., Lora, E.S., Venturini, O.J., Yáñez, E.E., Castillo, E.F., Almazan, O., 2009. Biofuels: Environment, technology and food security. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2008.08.014

Fillat, Ú., Ibarra, D., Eugenio, M.E., Moreno, A.D., Tomás-Pejó, E., Martín-Sampedro, R., 2017. Laccases as a Potential Tool for the Efficient Conversion of Lignocellulosic Biomass: A Review. Ferment. 2017, Vol. 3, Page 17 3, 17. https://doi.org/10.3390/FERMENTATION3020017

Fontes, D.I., Bezerra, T.S., de Freitas, E.P.B., de Oliveira, M.N., Silva, S. da C., Silva, S.Y.S., Albino, U.B., Santos, D. de A., 2023. Production of cellulases from Amazonian fungi and their application in babassu cellulose hydrolysis. Int. Biodeterior. Biodegradation 182, 105631. https://doi.org/10.1016/J.IBIOD.2023.105631

Froese, A.G., Nguyen, T.N., Ayele, B.T., Sparling, R., 2020. Digestibility of Wheat and Cattail Biomass Using a Co-culture of Thermophilic Anaerobes for Consolidated Bioprocessing. Bioenergy Res. 13, 325–333. https://doi.org/10.1007/S12155-020-10103-0

Galbe, M., Zacchi, G., 2012. Pretreatment: The key to efficient utilization of lignocellulosic materials. Biomass and Bioenergy 46, 70–78. https://doi.org/10.1016/J.BIOMBIOE.2012.03.026

Galbe, M., Zacchi, G., 2002. A review of the production of ethanol from softwood. Appl. Microbiol. Biotechnol. 59, 618–628. https://doi.org/10.1007/S00253-002-1058-9/METRICS

Gao, L., Gu, J.D., 2021. A new unified conceptual framework involving maintenance energy,

metabolism and toxicity for research on degradation of organic pollutants. Int. Biodeterior. Biodegradation 162, 105253. https://doi.org/10.1016/J.IBIOD.2021.105253

Gaurav, G.K., Mehmood, T., Cheng, L., Klemeš, J.J., Shrivastava, D.K., 2020. Water hyacinth as a biomass: A review. J. Clean. Prod. 277, 122214. https://doi.org/10.1016/J.JCLEPRO.2020.122214

Ge, X., Zhang, N., Phillips, G.C., Xu, J., 2012. Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol. Bioresour. Technol. 124, 485–488. https://doi.org/10.1016/J.BIORTECH.2012.08.050

Gedye, R., Smith, F., Westaway, K., Ali, H., Baldisera, L., Laberge, L., Rousell, J., 1986. The use of microwave ovens for rapid organic synthesis. Tetrahedron Lett. 27, 279–282. https://doi.org/10.1016/S0040-4039(00)83996-9

Gonçalves, F.A., Ruiz, H.A., Silvino dos Santos, E., Teixeira, J.A., de Macedo, G.R., 2016. Bioethanol production by Saccharomyces cerevisiae, Pichia stipitis and Zymomonas mobilis from delignified coconut fibre mature and lignin extraction according to biorefinery concept. Renew. Energy 94, 353–365. https://doi.org/10.1016/J.RENENE.2016.03.045

Goswami, R.K., Sharma, J.G., Shrivastav, A.K., Kumar, G., Glencross, B.D., Tocher, D.R., Chakrabarti, R., 2022. Effect of Lemna minor supplemented diets on growth, digestive physiology and expression of fatty acids biosynthesis genes of Cyprinus carpio. Sci. Rep. 12. https://doi.org/10.1038/s41598-022-07743-x

Govumoni, S.P., Koti, S., Kothagouni, S.Y., Venkateshwar, S., Linga, V.R., 2013. Evaluation of pretreatment methods for enzymatic saccharification of wheat straw for bioethanol production. Carbohydr. Polym. 91, 646–650. https://doi.org/10.1016/J.CARBPOL.2012.08.019

Grabowski, C., 2015. The Impact of Electron Beam Pretreatment on the Fermentation of Wood-based Sugars. Honor. These 1–24.

Gu, J.D., 2020. On environmental biotechnology of bioremediation. Appl. Environ. Biotechnol. 5, 3–8. https://doi.org/10.26789/AEB.2020.02.002

Gu, J.D., 2016. Biodegradation testing: so many tests but very little new innovation. Appl. Environ. Biotechnol. 1, 92–95. https://doi.org/10.26789/AEB.2016.01.007

Gu, Y.M., Kim, H., Sang, B.-I., Lee, J.H., 2018. Effects of water content on ball milling pretreatment and the enzymatic digestibility of corn stover. Water-Energy Nexus 1, 61–65. https://doi.org/10.1016/J.WEN.2018.07.002

Gunaraj, V., Murugan, N., 1999. Application of response surface methodology for predicting

weld bead quality in submerged arc welding of pipes. J. Mater. Process. Technol. 88, 266–275. https://doi.org/10.1016/S0924-0136(98)00405-1

Gundupalli, M.P., Tantayotai, P., Panakkal, E.J., Chuetor, S., Kirdponpattara, S., Thomas, A.S.S., Sharma, B.K., Sriariyanun, M., 2022. Hydrothermal pretreatment optimization and deep eutectic solvent pretreatment of lignocellulosic biomass: An integrated approach. Bioresour. Technol. Reports 17, 100957. https://doi.org/10.1016/J.BITEB.2022.100957

Gunst, R.F., Myers, R.H., Montgomery, D.C., 1996. Response Surface Methodology: Process and Product Optimization Using Designed Experiments, Technometrics. Wiley. https://doi.org/10.2307/1270613

Gusain, R., Suthar, S., 2017. Potential of aquatic weeds (Lemna gibba, Lemna minor, Pistia stratiotes and Eichhornia sp.) in biofuel production. Process Saf. Environ. Prot. 109, 233–241. https://doi.org/10.1016/J.PSEP.2017.03.030

Haldar, D., Purkait, M.K., 2021. A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: Mechanistic insight and advancements. Chemosphere 264. https://doi.org/10.1016/J.CHEMOSPHERE.2020.128523

Hamann, P.R. V., Serpa, D.L., Barreto da Cunha, A.S., de Camargo, B.R., Osiro, K.O., Valle de Sousa, M., Felix, C.R., Miller, R.N.G., Noronha, E.F., 2015. Evaluation of plant cell wall degrading enzyme production by Clostridium thermocellum B8 in the presence of raw agricultural wastes. Int. Biodeterior. Biodegradation 105, 97–105. https://doi.org/10.1016/J.IBIOD.2015.08.013

Han, S.F., Jin, W., Yang, Q., El-Fatah Abomohra, A., Zhou, X., Tu, R., Chen, C., Xie, G.J., Wang, Q., 2019. Application of pulse electric field pretreatment for enhancing lipid extraction from Chlorella pyrenoidosa grown in wastewater. Renew. Energy 133, 233–239. https://doi.org/10.1016/J.RENENE.2018.10.034

Hassan, S.S., Williams, G.A., Jaiswal, A.K., 2018. Emerging technologies for the pretreatment of lignocellulosic biomass. Bioresour. Technol. 262, 310–318. https://doi.org/10.1016/J.BIORTECH.2018.04.099

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100, 10–18. https://doi.org/10.1016/j.biortech.2008.05.027

Hernández-Mendoza, A.G., Saldaña-Trinidad, S., Martínez-Hernández, S., Pérez-Sariñana, B.Y., Láinez, M., 2021. Optimization of alkaline pretreatment and enzymatic hydrolysis of cocoa pod husk (Theobroma cacao L.) for ethanol production. Biomass and Bioenergy

154, 106268. https://doi.org/10.1016/J.BIOMBIOE.2021.106268

Hinkelmann, K., 2012. Design and Analysis of Experiments, Design and Analysis of Experiments. https://doi.org/10.1002/9781118147634

Hjorth, M., Gränitz, K., Adamsen, A.P.S., Møller, H.B., 2011. Extrusion as a pretreatment to increase biogas production. Bioresour. Technol. 102, 4989–4994. https://doi.org/10.1016/j.biortech.2010.11.128

Holtzapple, M.T., 2003. HEMICELLULOSES, in: Caballero, B.B.T.-E. of F.S. and N. (Second E. (Ed.), . Academic Press, Oxford, pp. 3060–3071. https://doi.org/https://doi.org/10.1016/B0-12-227055-X/00589-7

Hsu, T.C., Guo, G.L., Chen, W.H., Hwang, W.S., 2010. Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. Bioresour. Technol. 101, 4907–4913. https://doi.org/10.1016/j.biortech.2009.10.009

Hu, Y., Priya, A., Chen, C., Liang, C., Wang, W., Wang, Q., Lin, C.S.K., Qi, W., 2023a. Recent advances in substrate-enzyme interactions facilitating efficient biodegradation of lignocellulosic biomass: A review. Int. Biodeterior. Biodegrad. 180. https://doi.org/10.1016/j.ibiod.2023.105594

Hu, Y., Priya, A., Chen, C., Liang, C., Wang, W., Wang, Q., Lin, C.S.K., Qi, W., 2023b. Recent advances in substrate-enzyme interactions facilitating efficient biodegradation of lignocellulosic biomass: A review. Int. Biodeterior. Biodegradation 180, 105594. https://doi.org/10.1016/J.IBIOD.2023.105594

Huang, Y.F., Chiueh, P. Te, Kuan, W.H., Lo, S.L., 2016. Microwave pyrolysis of lignocellulosic biomass: Heating performance and reaction kinetics. Energy 100, 137–144. https://doi.org/10.1016/j.energy.2016.01.088

Infanzón-Rodríguez, M.I., Ragazzo-Sánchez, J.A., del Moral, S., Calderón-Santoyo, M., Aguilar-Uscanga, M.G., 2022. Enzymatic hydrolysis of lignocellulosic biomass using native cellulase produced by Aspergillus niger ITV02 under liquid state fermentation. Biotechnol. Appl. Biochem. 69, 198–208. https://doi.org/10.1002/BAB.2097

Irfan, M., Nadeem, M., Syed, Q., 2014. Ethanol production from agricultural wastes using Sacchromyces cervisae. Brazilian J. Microbiol. 45, 457. https://doi.org/10.1590/S1517-83822014000200012

Jacob, S., Banerjee, R., 2016. Modeling and optimization of anaerobic codigestion of potato waste and aquatic weed by response surface methodology and artificial neural network coupled genetic algorithm. Bioresour. Technol. 214, 386–395. https://doi.org/10.1016/j.biortech.2016.04.068

Jayanth, K.P., 2000. Biological Control of Weeds in India, Biocontrol Potential and its Exploitation in Sustainable Agriculture. CSIRO Publishing. https://doi.org/10.1007/978-1-4615-4209-4_15

Ji, G., Han, L., Gao, C., Xiao, W., Zhang, Y., Cao, Y., 2017. Quantitative approaches for illustrating correlations among the mechanical fragmentation scales, crystallinity and enzymatic hydrolysis glucose yield of rice straw. Bioresour. Technol. 241, 262–268. https://doi.org/10.1016/j.biortech.2017.05.062

Jin, M., Gunawan, C., Balan, V., Dale, B.E., 2012. Consolidated bioprocessing (CBP) of AFEX^{TM}-pretreated corn stover for ethanol production using Clostridium phytofermentans at a high solids loading. Biotechnol. Bioeng. 109, 1929–1936. https://doi.org/10.1002/BIT.24458

Jin, S., Zhang, G., Zhang, P., Jin, L., Fan, S., Li, F., 2015. Comparative study of high-pressure homogenization and alkaline-heat pretreatments for enhancing enzymatic hydrolysis and biogas production of grass clipping. Int. Biodeterior. Biodegrad. 104, 477–481. https://doi.org/10.1016/j.ibiod.2015.08.005

Jmel, M.A., Anders, N., Ben Messaoud, G., Marzouki, M.N., Spiess, A., Smaali, I., 2019. The stranded macroalga Ulva lactuca as a new alternative source of cellulose: Extraction, physicochemical and rheological characterization. J. Clean. Prod. 234, 1421–1427. https://doi.org/10.1016/j.jclepro.2019.06.225

John, R.P., Anisha, G.S., Nampoothiri, K.M., Pandey, A., 2011. Micro and macroalgal biomass: A renewable source for bioethanol. Bioresour. Technol. 102, 186–193. https://doi.org/10.1016/j.biortech.2010.06.139

Joy, S.P., Krishnan, C., 2022. Modified organosolv pretreatment for improved cellulosic ethanol production from sorghum biomass. Ind. Crops Prod. 177, 114409. https://doi.org/10.1016/J.INDCROP.2021.114409

Kale, R.D., Taye, M., Chaudhary, B., 2019. Extraction and characterization of cellulose single fiber from native Ethiopian Serte (Dracaena steudneri Egler) plant leaf. J. Macromol. Sci. Part A Pure Appl. Chem. 56, 837–844. https://doi.org/10.1080/10601325.2019.1612252

Kapembwa, C., Shitumbanuma, V., Yengwe, J., Schoustra, S., De Deyn, G.B., 2024. Impact of river water and sediment properties on the chemical composition of water hyacinth and hippo grass. Environ. Challenges 14, 100851. https://doi.org/10.1016/J.ENVC.2024.100851

Karunanithy, C., Muthukumarappan, K., 2011. Optimization of alkali, big bluestem particle size, and extruder parameters for maximum enzymatic sugar recovery using response

surface methodology. BioResources 6, 762–790. https://doi.org/10.15376/biores.6.1.762-790

Kataria, R., Ghosh, S., 2011. Saccharification of Kans grass using enzyme mixture from Trichoderma reesei for bioethanol production. Bioresour. Technol. 102, 9970–9975. https://doi.org/10.1016/j.biortech.2011.08.023

Kataria, R., Mol, A., Schulten, E., Happel, A., Mussatto, S.I., 2017. Bench scale steam explosion pretreatment of acid impregnated elephant grass biomass and its impacts on biomass composition, structure and hydrolysis. Ind. Crops Prod. 106, 48–58. https://doi.org/10.1016/j.indcrop.2016.08.050

Kataria, R., Woods, T., Casey, W., Cerrone, F., Davis, R., O'Connor, K., Ruhal, R., Babu, R., 2018a. Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production. Ind. Crops Prod. 111, 625–632. https://doi.org/10.1016/j.indcrop.2017.11.029

Kataria, R., Woods, T., Casey, W., Cerrone, F., Davis, R., O'Connor, K., Ruhal, R., Babu, R., 2018b. Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production. Ind. Crops Prod. 111, 625–632. https://doi.org/10.1016/J.INDCROP.2017.11.029

Kaur, M., Kumar, M., Sachdeva, S., Puri, S.K., 2018. Aquatic weeds as the next generation feedstock for sustainable bioenergy production. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.11.082

Kaur, M., Kumar, M., Singh, D., Sachdeva, S., Puri, S.K., 2019. A sustainable biorefinery approach for efficient conversion of aquatic weeds into bioethanol and biomethane. Energy Convers. Manag. 187, 133–147. https://doi.org/10.1016/j.enconman.2019.03.018

Khounani, Z., Abdul Razak, N.N., Hosseinzadeh-Bandbafha, H., Madadi, M., Sun, F., Mohammadi, P., Mahlia, T.M.I., Aghbashlo, M., Tabatabaei, M., 2024. Biphasic pretreatment excels over conventional sulfuric acid in pinewood biorefinery: An environmental analysis. Environ. Res. 248. https://doi.org/10.1016/j.envres.2024.118286

Kian, L., Jawaid, M., Ariffin, H., Alothman, O., 2017. Isolation and characterization of microcrystalline cellulose from roselle fibers. Int. J. Biol. Macromol. 103, 931–940.

Kim, I., Han, J.I., 2012. Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass and Bioenergy 46, 210–217. https://doi.org/10.1016/J.BIOMBIOE.2012.08.024

Ko, J.K., Um, Y., Woo, H.M., Kim, K.H., Lee, S.M., 2016. Ethanol production from lignocellulosic hydrolysates using engineered Saccharomyces cerevisiae harboring xylose

isomerase-based pathway. Bioresour. Technol. 209, 290–296. https://doi.org/10.1016/J.BIORTECH.2016.02.124

Kothari, R., Vashishtha, A., Singh, H.M., Pathak, V. V., Tyagi, V. V., Yadav, B.C., Ashokkumar, V., Singh, D.P., 2020. Assessment of Indian bioenergy policy for sustainable environment and its impact for rural India: Strategic implementation and challenges. Environ. Technol. Innov. 20. https://doi.org/10.1016/j.eti.2020.101078

Kovačić, Đ., Rupčić, S., Kralik, D., Jovičić, D., Spajić, R., Tišma, M., 2021. Pulsed electric field: An emerging pretreatment technology in a biogas production. Waste Manag. 120, 467–483. https://doi.org/10.1016/J.WASMAN.2020.10.009

Kucera, D., Benesova, P., Ladicky, P., Pekar, M., Sedlacek, P., Obruca, S., 2017. Production of Polyhydroxyalkanoates Using Hydrolyzates of Spruce Sawdust: Comparison of Hydrolyzates Detoxification by Application of Overliming, Active Carbon, and Lignite. Bioeng. 2017, Vol. 4, Page 53 4, 53. https://doi.org/10.3390/BIOENGINEERING4020053

Kumar, A., Singh, L.K., Ghosh, S., 2009. Bioconversion of lignocellulosic fraction of water-hyacinth (Eichhornia crassipes) hemicellulose acid hydrolysate to ethanol by Pichia stipitis. Bioresour. Technol. 100, 3293–3297. https://doi.org/10.1016/j.biortech.2009.02.023

Kumar, M., Turner, S., 2015. Protocol: A medium-throughput method for determination of cellulose content from single stem pieces of Arabidopsis thaliana. Plant Methods 11. https://doi.org/10.1186/S13007-015-0090-6

Kumar, R., Tabatabaei, M., Karimi, K., Horváth, I.S., 2016. Recent updates on lignocellulosic biomass derived ethanol - A review. Biofuel Res. J. 3, 347–356. https://doi.org/10.18331/BRJ2016.3.1.4

Kumar, V., Nanda, M., Joshi, H.C., Singh, A., Sharma, S., Verma, M., 2018. Production of biodiesel and bioethanol using algal biomass harvested from fresh water river. Renew. Energy 116, 606–612. https://doi.org/10.1016/J.RENENE.2017.10.016

Kunwer, R., Ranjit Pasupuleti, S., Sureshchandra Bhurat, S., Kumar Gugulothu, S., Rathore, N., 2022. Blending of ethanol with gasoline and diesel fuel – A review. Mater. Today Proc. 69, 560–563. https://doi.org/10.1016/j.matpr.2022.09.319

Li, F., Zhang, P., Zhang, G., Tang, X., Wang, S., Jin, S., 2017. Enhancement of corn stover hydrolysis with rumen fluid pretreatment at different solid contents: Effect, structural changes and enzymes participation. Int. Biodeterior. Biodegrad. 119, 405–412. https://doi.org/10.1016/j.ibiod.2016.10.038

Li, X., Shi, Y., Kong, W., Wei, J., Song, W., Wang, S., 2022. Improving enzymatic hydrolysis of lignocellulosic biomass by bio-coordinated physicochemical pretreatment—A review. Energy Reports. https://doi.org/10.1016/j.egyr.2021.12.015

Li, Y., Xu, Y., Xue, Y., Yang, S., Cheng, Y., Zhu, W., 2022. Ethanol production from lignocellulosic biomass by co-fermentation with Pecoramyces sp. F1 and Zymomonas mobilis ATCC 31821 in an integrated process. Biomass and Bioenergy 161. https://doi.org/10.1016/j.biombioe.2022.106454

Liu, C.G., Xiao, Y., Xia, X.X., Zhao, X.Q., Peng, L., Srinophakun, P., Bai, F.W., 2019. Cellulosic ethanol production: Progress, challenges and strategies for solutions. Biotechnol. Adv. 37, 491–504. https://doi.org/10.1016/J.BIOTECHADV.2019.03.002

Liu, Q., Li, W., Ma, Q., An, S., Li, M., Jameel, H., Chang, H.M., 2016. Pretreatment of corn stover for sugar production using a two-stage dilute acid followed by wet-milling pretreatment process. Bioresour. Technol. 211, 435–442. https://doi.org/10.1016/J.BIORTECH.2016.03.131

Liu, Y., Guo, L., Wang, L., Zhan, W., Zhou, H., 2017. Irradiation pretreatment facilitates the achievement of high total sugars concentration from lignocellulose biomass. Bioresour. Technol. 232, 270–277. https://doi.org/10.1016/J.BIORTECH.2017.01.061

Liu, Y., Zhou, H., Wang, L., Wang, S., Fan, L., 2016. Improving Saccharomyces cerevisiae growth against lignocellulose-derived inhibitors as well as maximizing ethanol production by a combination proposal of γ-irradiation pretreatment with in situ detoxification. Chem. Eng. J. 287, 302–312. https://doi.org/10.1016/J.CEJ.2015.10.086

Liu, Y., Zhou, H., Wang, S., Wang, K., Su, X., 2015. Comparison of γ-irradiation with other pretreatments followed with simultaneous saccharification and fermentation on bioconversion of microcrystalline cellulose for bioethanol production. Bioresour. Technol. 182, 289–295. https://doi.org/https://doi.org/10.1016/j.biortech.2015.02.009

Lu, P., Hsieh, Y. Lo, 2010. Preparation and properties of cellulose nanocrystals: Rods, spheres, and network. Carbohydr. Polym. 82, 329–336. https://doi.org/10.1016/j.carbpol.2010.04.073

Łukajtis, R., Rybarczyk, P., Kucharska, K., Konopacka-Łyskawa, D., Słupek, E., Wychodnik, K., Kamiński, M., 2018. Optimization of Saccharification Conditions of Lignocellulosic Biomass under Alkaline Pre-Treatment and Enzymatic Hydrolysis. Energies 2018, Vol. 11, Page 886 11, 886. https://doi.org/10.3390/EN11040886

Luo, G., Shi, W., Chen, X., Ni, W., Strong, P.J., Jia, Y., Wang, H., 2011. Hydrothermal conversion of water lettuce biomass at 473 or 523 K. Biomass and Bioenergy 35, 4855–

4861. https://doi.org/10.1016/J.BIOMBIOE.2011.10.002

Luzi, F., Puglia, D., Sarasini, F., Tirillò, J., Maffei, G., Zuorro, A., Lavecchia, R., Kenny, J.M., Torre, L., 2019. Valorization and extraction of cellulose nanocrystals from North African grass: Ampelodesmos mauritanicus (Diss). Carbohydr. Polym. 209, 328–337. https://doi.org/10.1016/j.carbpol.2019.01.048

Lynd, L.R., Van Zyl, W.H., McBride, J.E., Laser, M., 2005. Consolidated bioprocessing of cellulosic biomass: an update. Curr. Opin. Biotechnol. 16, 577–583. https://doi.org/10.1016/J.COPBIO.2005.08.009

Mabazza, K.A.A., Requiso, P.J., Alfafara, C.G., Nayve, F.R.P., Ventura, J.R.S., 2020. Steam explosion and sequential steam explosion – dilute acid pretreatment optimization of banana pseudostem for polyhydroxybutyrate (Phb) production. Philipp. J. Sci. 149, 259–271. https://doi.org/10.56899/149.02.05

Mahalik, K., Sahu, J.N., Patwardhan, A. V., Meikap, B.C., 2010. Statistical modelling and optimization of hydrolysis of urea to generate ammonia for flue gas conditioning. J. Hazard. Mater. 182, 603–610. https://doi.org/10.1016/j.jhazmat.2010.06.075

Malik, K., Salama, E.S., Kim, T.H., Li, X., 2020a. Enhanced ethanol production by Saccharomyces cerevisiae fermentation post acidic and alkali chemical pretreatments of cotton stalk lignocellulose. Int. Biodeterior. Biodegrad. 147. https://doi.org/10.1016/j.ibiod.2019.104869

Malik, K., Salama, E.S., Kim, T.H., Li, X., 2020b. Enhanced ethanol production by Saccharomyces cerevisiae fermentation post acidic and alkali chemical pretreatments of cotton stalk lignocellulose. Int. Biodeterior. Biodegrad. 147. https://doi.org/10.1016/j.ibiod.2019.104869

Mandal, A., Chakrabarty, D., 2011. Isolation of nanocellulose from waste sugarcane bagasse (SCB) and its characterization. Carbohydr. Polym. 86, 1291–1299. https://doi.org/10.1016/j.carbpol.2011.06.030

Manivannan, A., Narendhirakannan, R.T., 2015. Bioethanol Production From Aquatic Weed Water Hyacinth (Eichhornia crassipes) by Yeast Fermentation. Waste and Biomass Valorization 6, 209–216. https://doi.org/10.1007/s12649-015-9347-6

Mankar, A.R., Pandey, A., Modak, A., Pant, K.K., 2021. Pretreatment of lignocellulosic biomass: A review on recent advances. Bioresour. Technol. 334, 125235. https://doi.org/10.1016/J.BIORTECH.2021.125235

Manmai, N., Unpaprom, Y., Ponnusamy, V.K., Ramaraj, R., 2020. Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar

production by chemical pretreatment and enzymatic degradation. Fuel 278. https://doi.org/10.1016/j.fuel.2020.118262

Martín-Lara, M.A., Chica-Redecillas, L., Pérez, A., Blázquez, G., Garcia-Garcia, G., Calero, M., 2020. Liquid Hot Water Pretreatment and Enzymatic Hydrolysis as a Valorization Route of Italian Green Pepper Waste to Delivery Free Sugars. Foods 2020, Vol. 9, Page 1640 9, 1640. https://doi.org/10.3390/FOODS9111640

Martinez, A., Rodriguez, M.E., Wells, M.L., York, S.W., Preston, J.F., Ingram, L.O., 2001. Detoxification of dilute acid hydrolysates of lignocellulose with lime. Biotechnol. Prog. 17, 287–293. https://doi.org/10.1021/BP0001720

Meehnian, H., Jana, A.K., Jana, M.M., 2017. Pretreatment of cotton stalks by synergistic interaction of Daedalea flavida and Phlebia radiata in co-culture for improvement in delignification and saccharification. Int. Biodeterior. Biodegradation 117, 68–77. https://doi.org/10.1016/J.IBIOD.2016.11.022

Mejica, G.F.C., Unpaprom, Y., Whangchai, K., Ramaraj, R., 2022. Cellulosic-derived bioethanol from Limnocharis flava utilizing alkaline pretreatment. Biomass Convers. Biorefinery 12, 1737–1743. https://doi.org/10.1007/S13399-020-01218-7

Mikulski, D., Kłosowski, G., 2023. Cellulose hydrolysis and bioethanol production from various types of lignocellulosic biomass after microwave-assisted hydrotropic pretreatment. Renew. Energy 206, 168–179. https://doi.org/10.1016/j.renene.2023.02.061

Miller, G.L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem. 31, 426–428. https://doi.org/10.1021/AC60147A030

Miranda, A.F., Biswas, B., Ramkumar, N., Singh, R., Kumar, J., James, A., Roddick, F., Lal, B., Subudhi, S., Bhaskar, T., Mouradov, A., 2016. Aquatic plant Azolla as the universal feedstock for biofuel production. SpringerAF Miranda, B Biswas, N Ramkumar, R Singh, J Kumar, A James, F Roddick, B LalBiotechnology biofuels, 2016•Springer 9, 221. https://doi.org/10.1186/s13068-016-0628-5

Mishima, D., Kuniki, M., Sei, K., Soda, S., Ike, M., Fujita, M., 2008a. Ethanol production from candidate energy crops: Water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes L.). Bioresour. Technol. 99, 2495–2500. https://doi.org/10.1016/J.BIORTECH.2007.04.056

Mishima, D., Kuniki, M., Sei, K., Soda, S., Ike, M., Fujita, M., 2008b. Ethanol production from candidate energy crops: Water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes L.). Bioresour. Technol. 99, 2495–2500. https://doi.org/10.1016/j.biortech.2007.04.056

Mishima, D., Tateda, M., Ike, M., Fujita, M., 2006. Comparative study on chemical pretreatments to accelerate enzymatic hydrolysis of aquatic macrophyte biomass used in water purification processes. Bioresour. Technol. 97, 2166–2172. https://doi.org/10.1016/j.biortech.2005.09.029

Mithra, M.G., Jeeva, M.L., Sajeev, M.S., Padmaja, G., 2018. Comparison of ethanol yield from pretreated lignocellulo-starch biomass under fed-batch SHF or SSF modes. Heliyon 4, 885. https://doi.org/10.1016/J.HELIYON.2018.E00885

Moerman, D.E., 1996. An analysis of the food plants and drug plants of native North America. J. Ethnopharmacol. 52, 1–22. https://doi.org/10.1016/0378-8741(96)01393-1

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96, 673–686. https://doi.org/10.1016/J.BIORTECH.2004.06.025

Mthethwa, N.P., Nasr, M., Bux, F., Kumari, S., 2018. Utilization of Pistia stratiotes (aquatic weed) for fermentative biohydrogen: Electron-equivalent balance, stoichiometry, and cost estimation. Int. J. Hydrogen Energy 43, 8243–8255. https://doi.org/10.1016/j.ijhydene.2018.03.099

Mthethwa, N.P., Nasr, M., Kiambi, S.L., Bux, F., Kumari, S., 2019. Biohydrogen fermentation from Pistia stratiotes (aquatic weed) using mixed and pure bacterial cultures. Int. J. Hydrogen Energy 44, 17720–17731. https://doi.org/10.1016/j.ijhydene.2019.05.152

Murciano Martínez, P., Bakker, R., Harmsen, P., Gruppen, H., Kabel, M., 2015. Importance of acid or alkali concentration on the removal of xylan and lignin for enzymatic cellulose hydrolysis. Ind. Crops Prod. 64, 88–96. https://doi.org/10.1016/J.INDCROP.2014.10.031

Naik, G.P., Poonia, A.K., Chaudhari, P.K., 2021. Pretreatment of lignocellulosic agricultural waste for delignification, rapid hydrolysis, and enhanced biogas production: A review. J. Indian Chem. Soc. 98, 100147. https://doi.org/10.1016/J.JICS.2021.100147

Nazari, M.T., Mazutti, J., Basso, L.G., Colla, L.M., Brandli, L., 2021. Biofuels and their connections with the sustainable development goals: a bibliometric and systematic review. Environ. Dev. Sustain. 23, 11139–11156. https://doi.org/10.1007/S10668-020-01110-4

Njoku, S.I., Ahring, B.K., Uellendahl, H., 2013. Tailoring wet explosion process parameters for the pretreatment of cocksfoot grass for high sugar yields. Appl. Biochem. Biotechnol. 170, 1574–1588. https://doi.org/10.1007/S12010-013-0299-7

Nomanbhay, S.M., Hussain, R., Palanisamy, K., Nomanbhay, S.M., Hussain, R., Palanisamy, K., 2013. Microwave-Assisted Alkaline Pretreatment and Microwave Assisted

Enzymatic Saccharification of Oil Palm Empty Fruit Bunch Fiber for Enhanced Fermentable Sugar Yield. J. Sustain. Bioenergy Syst. 3, 7–17. https://doi.org/10.4236/JSBS.2013.31002

Nong, H.T.T., Whangchai, K., Unpaprom, Y., Thararux, C., Ramaraj, R., 2022. Development of sustainable approaches for converting the agro-weeds Ludwigia hyssopifolia to biogas production. Biomass Convers. Biorefinery 12, 793–801. https://doi.org/10.1007/s13399-020-01083-4

Nunui, K., Boonsawang, P., Chaiprapat, S., Charnnok, B., 2022. Using organosolv pretreatment with acid wastewater for enhanced fermentable sugar and ethanol production from rubberwood waste. Renew. Energy 198, 723–732. https://doi.org/10.1016/J.RENENE.2022.08.068

Obeng, A., Premjet, D., Biomolecules, S.P.-, 2019. Combining Autoclaving with Mild Alkaline Solution as a Pretreatment Technique to Enhance Glucose Recovery from the Invasive Weed Chloris barbata. mdpi.com. https://doi.org/https://doi.org/10.3390/biom9040120

Ocreto, J.B., Chen, W.-H., Ubando, A.T., Park, Y.-K., Sharma, A.K., Ashokkumar, V., Ok, Y.S., Kwon, E.E., Rollon, A.P., De Luna, M.D.G., 2021. A critical review on second- and third-generation bioethanol production using microwaved-assisted heating (MAH) pretreatment. Renew. Sustain. Energy Rev. 152, 111679. https://doi.org/10.1016/J.RSER.2021.111679

Olatunji, K.O., Ahmed, N.A., Ogunkunle, O., 2021. Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: a review. Biotechnol. Biofuels 14. https://doi.org/10.1186/S13068-021-02012-X

Olson, D.G., McBride, J.E., Joe Shaw, A., Lynd, L.R., 2012. Recent progress in consolidated bioprocessing. Curr. Opin. Biotechnol. 23, 396–405. https://doi.org/10.1016/J.COPBIO.2011.11.026

Olsson, L., Jørgensen, H., Krogh, K., Roca, C., 2004. Bioethanol Production from Lignocellulosic Material, in: Polysaccharides. https://doi.org/10.1201/9781420030822.ch42

Pant, S., Ritika, Komesu, A., Penteado, E.D., Diniz, A.A.R., Rahman, M.A., Kuila, A., 2021. NaOH pretreatment and enzymatic hydrolysis of Brassica juncea using mixture of cellulases. Environ. Technol. Innov. 21, 101324. https://doi.org/10.1016/J.ETI.2020.101324

Pantawong R., Chuanchai A., Thipbunrat P., Unpaprom Y., R.R., 2015. Experimental

Investigation of Biogas Production from Water Lettuce, Pistia stratiotes L. Emer Life Sci Res 1, 41–46.

Parot, M., Rodrigue, D., Stevanovic, T., 2022. High purity softwood lignin obtained by an eco-friendly organosolv process. Bioresour. Technol. Reports 17, 100880. https://doi.org/10.1016/J.BITEB.2021.100880

Pashaei, H., Ghaemi, A., Nasiri, M., Karami, B., 2020. Experimental Modeling and Optimization of CO2 Absorption into Piperazine Solutions Using RSM-CCD Methodology. ACS Omega 5, 8432–8448. https://doi.org/10.1021/acsomega.9b03363

Patel, V.R., Bhatt, N., 2021. Aquatic weed Spirodela polyrhiza, a potential source for energy generation and other commodity chemicals production. Renew. Energy 173, 455–465. https://doi.org/10.1016/j.renene.2021.03.054

Pereira, S.C., Maehara, L., Machado, C.M.M., Farinas, C.S., 2015. 2G ethanol from the whole sugarcane lignocellulosic biomass. Biotechnol. Biofuels 8. https://doi.org/10.1186/S13068-015-0224-0

Phwan, C.K., Chew, K.W., Sebayang, A.H., Ong, H.C., Ling, T.C., Malek, M.A., Ho, Y.C., Show, P.L., 2019. Effects of acids pre-treatment on the microbial fermentation process for bioethanol production from microalgae. Biotechnol. Biofuels 12, 1–8. https://doi.org/10.1186/S13068-019-1533-5/TABLES/2

Pooja, N.S., Sajeev, M.S., Jeeva, M.L., Padmaja, G., 2018. Bioethanol production from microwave-assisted acid or alkali-pretreated agricultural residues of cassava using separate hydrolysis and fermentation (SHF). 3 Biotech 8. https://doi.org/10.1007/S13205-018-1095-4

Raina, N., Boonmee, R., Kirdponpattara, S., Narasingha, M., Sriariyanun, M., Phitsuwan, P., Chuetor, S., 2024. Process performance evaluation of different chemical pretreatments of lignocellulosic biomass for bioethanol production. Ind. Crops Prod. 211. https://doi.org/10.1016/j.indcrop.2024.118207

Ramaraj, R., Unpaprom, Y., 2019. Optimization of pretreatment condition for ethanol production from Cyperus difformis by response surface methodology. 3 Biotech 9. https://doi.org/10.1007/S13205-019-1754-0

Reddy, K.O., Maheswari, C.U., Dhlamini, M.S., Mothudi, B.M., Kommula, V.P., Zhang, Jinming, Zhang, Jun, Rajulu, A.V., 2018. Extraction and characterization of cellulose single fibers from native african napier grass. Carbohydr. Polym. 188, 85–91. https://doi.org/10.1016/j.carbpol.2018.01.110

Refinery Division - Second Generation (2G) Ethanol | Ministry of Petroleum and Natural Gas

| Government of India [WWW Document], n.d. URL https://mopng.gov.in/en/refining/second-generation-ethenol (accessed 5.14.24).

Rezania, S., Oryani, B., Cho, J., Talaiekhozani, A., Sabbagh, F., Hashemi, B., Rupani, P.F., Mohammadi, A.A., 2020. Different pretreatment technologies of lignocellulosic biomass for bioethanol production: An overview. Energy 199. https://doi.org/10.1016/j.energy.2020.117457

Rezania, S., Ponraj, M., Din, M.F.M., ..., 2014. True Potential of Aquatic plants (Eichhornia crassipes, Pistia stratiotes) in the production of bio-ethanol. … Young Water Prof. ….

RFA Releases 2023 Ethanol Industry Outlook and Pocket Guide: "Ready. Set. Go!" [WWW Document], 2023. URL https://ethanolrfa.org/media-and-news/category/news-releases/article/2023/02/rfa-releases-2023-ethanol-industry-outlook-and-pocket-guide-ready-set-go (accessed 11.21.23).

Rodrigues Gurgel da Silva, A., Giuliano, A., Errico, M., Rong, B.G., Barletta, D., 2019. Economic value and environmental impact analysis of lignocellulosic ethanol production: assessment of different pretreatment processes. Clean Technol. Environ. Policy 21, 637–654. https://doi.org/10.1007/s10098-018-01663-z

Saha, B.C., Qureshi, N., Kennedy, G.J., Cotta, M.A., 2016. Biological pretreatment of corn stover with white-rot fungus for improved enzymatic hydrolysis. Int. Biodeterior. Biodegrad. 109, 29–35. https://doi.org/10.1016/j.ibiod.2015.12.020

Saha, B.C., Yoshida, T., Cotta, M.A., Sonomoto, K., 2013. Hydrothermal pretreatment and enzymatic saccharification of corn stover for efficient ethanol production. Ind. Crops Prod. 44, 367–372. https://doi.org/10.1016/J.INDCROP.2012.11.025

Sahoo, A., Kumar, S., Mohanty, K., 2022. A comprehensive characterization of non-edible lignocellulosic biomass to elucidate their biofuel production potential. Biomass Convers. Biorefinery 12, 5087–5103. https://doi.org/10.1007/S13399-020-00924-6

SAJAD Rabani, M., 2019. Proximate Nutrient Content Analysis of Some Aquatic Weeds of Dal Lake Srinagar. https://doi.org/10.21276/ijpbs.2019.9.2.86

Saratale, G.D., Saratale, R.G., Varjani, S., Cho, S.K., Ghodake, G.S., Kadam, A., Mulla, S.I., Bharagava, R.N., Kim, D.S., Shin, H.S., 2020. Development of ultrasound aided chemical pretreatment methods to enrich saccharification of wheat waste biomass for polyhydroxybutyrate production and its characterization. Ind. Crops Prod. 150, 112425. https://doi.org/10.1016/J.INDCROP.2020.112425

Saravanan, A., Senthil Kumar, P., Jeevanantham, S., Karishma, S., Vo, D.V.N., 2022. Recent advances and sustainable development of biofuels production from lignocellulosic

biomass. Bioresour. Technol. 344, 126203. https://doi.org/10.1016/J.BIORTECH.2021.126203

Selvakumar, P., Adane, A.A., Zelalem, T., Hunegnaw, B.M., Karthik, V., Kavitha, S., Jayakumar, M., Karmegam, N., Govarthanan, M., Kim, W., 2022. Optimization of binary acids pretreatment of corncob biomass for enhanced recovery of cellulose to produce bioethanol. Fuel 321. https://doi.org/10.1016/j.fuel.2022.124060

Shao, X., Jin, M., Guseva, A., Liu, C., Balan, V., Hogsett, D., Dale, B.E., Lynd, L., 2011. Conversion for Avicel and AFEX pretreated corn stover by Clostridium thermocellum and simultaneous saccharification and fermentation: Insights into microbial conversion of pretreated cellulosic biomass. Bioresour. Technol. 102, 8040–8045. https://doi.org/10.1016/j.biortech.2011.05.021

Shrivastava, A., Sharma, R.K., 2023. Conversion of lignocellulosic biomass: Production of bioethanol and bioelectricity using wheat straw hydrolysate in electrochemical bioreactor. Heliyon 9. https://doi.org/10.1016/j.heliyon.2023.e12951

Sills, D.L., Gossett, J.M., 2012. Using FTIR spectroscopy to model alkaline pretreatment and enzymatic saccharification of six lignocellulosic biomasses. Biotechnol. Bioeng. 109, 894–903. https://doi.org/10.1002/BIT.24376

Sinbuathong, N., 2019. Predicting the increase of methane yield using alkali pretreatment for weeds prior to co-digestion. Energy Sources, Part A Recover. Util. Environ. Eff. 41, 1124–1131. https://doi.org/10.1080/15567036.2018.1544990

Singh, Y.D., Mahanta, P., Bora, U., 2017. Comprehensive characterization of lignocellulosic biomass through proximate, ultimate and compositional analysis for bioenergy production. Renew. Energy 103, 490–500. https://doi.org/10.1016/J.RENENE.2016.11.039

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Wolfe, J., 2008. Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples Laboratory Analytical Procedure (LAP) Issue Date: 3/31/2008.

Soda, S., Ohchi, T., Piradee, J., Takai, Y., Ike, M., 2015. Duckweed biomass as a renewable biorefinery feedstock: Ethanol and succinate production from Wolffia globosa. Biomass and Bioenergy 81, 364–368. https://doi.org/10.1016/j.biombioe.2015.07.020

Sombatpraiwan, S., Junyusen, T., Treeamnak, T., Junyusen, P., 2019. Optimization of microwave-assisted alkali pretreatment of cassava rhizome for enhanced enzymatic hydrolysis glucose yield. Food Energy Secur. 8. https://doi.org/10.1002/FES3.174

Sudhakar, M.P., Ravel, M., Perumal, K., 2021. Pretreatment and process optimization of

bioethanol production from spent biomass of Ganoderma lucidum using Saccharomyces cerevisiae. Fuel 306. https://doi.org/10.1016/j.fuel.2021.121680

Sulaiman, N.M.A., El –Hafez, A.E.A., Refaat, A.E.-M.A., El-Dogdog, K.A., 2022. Kinetics of Bio-ethanol production on the molasses-based medium by Saccharomyces cerevisiae. المجلة العربية للعلوم الزراعية 5, 131–158. https://doi.org/10.21608/asajs.2022.228846

Sulyman, A.O., Igunnu, A., Malomo, S.O., 2020. Isolation, purification and characterization of cellulase produced by Aspergillus niger cultured on Arachis hypogaea shells. Heliyon 6, e05668. https://doi.org/10.1016/J.HELIYON.2020.E05668

Suman, S.K., Dhawaria, M., Tripathi, D., Raturi, V., Adhikari, D.K., Kanaujia, P.K., 2016. Investigation of lignin biodegradation by Trabulsiella sp. isolated from termite gut. Int. Biodeterior. Biodegrad. 112, 12–17. https://doi.org/10.1016/j.ibiod.2016.04.036

Sun, J., Ding, S.-Y., Doran-Peterson, J., 2013. CHAPTER 1. Biomass and its Biorefinery: Novel Approaches from Nature-Inspired Strategies and Technology. books.google.com 1–13. https://doi.org/10.1039/9781849734738-00001

Sun, S.F., Yang, H.Y., Yang, J., Shi, Z.J., 2021. Structural characterization of poplar lignin based on the microwave-assisted hydrothermal pretreatment. Int. J. Biol. Macromol. 190, 360–367. https://doi.org/10.1016/J.IJBIOMAC.2021.08.230

Sunwoo, I.Y., Kwon, J.E., Nguyen, T.H., Jeong, G.T., Kim, S.K., 2019. Ethanol production from water hyacinth (Eichhornia crassipes) hydrolysate by hyper-thermal acid hydrolysis, enzymatic saccharification and yeasts adapted to high concentration of xylose. Bioprocess Biosyst. Eng. 42, 1367–1374. https://doi.org/10.1007/S00449-019-02136-3

Sutaryo, S., Sempana, A.N., Mulya, R.M., Sulistyaningrum, D., Ali, M.S., Damarjati, R.I., Purbowati, E., Adiwinarti, R., Purnomoadi, A., 2022. Methane Production of Pistia Stratiotes as a Single Substrate and as a Co-Substrate with Dairy Cow Manure. Ferment. 2022, Vol. 8, Page 736 8, 736. https://doi.org/10.3390/FERMENTATION8120736

Tavares, A.P.M., Gonçalves, M.J.A., Brás, T., Pesce, G.R., Xavier, A.M.R.B., Fernandes, M.C., 2022. Cardoon Hydrolysate Detoxification by Activated Carbon or Membranes System for Bioethanol Production. https://doi.org/10.3390/en15061993

Tharunkumar, J., Arosha, V.K., Bajhaiya, A.K., Rakesh, S., 2024. Optimizing alkaline pretreatment for delignification of paddy straw and sugarcane bagasse to enhance bioethanol production. Biomass Convers. Biorefinery. https://doi.org/10.1007/S13399-024-05458-9

THU THI NONG, H., UNPAPROM, Y., CHAICHOMPOO, C., RAMARAJ, R., 2021. Biomethane Potential of Invasive Aquatic Weed Water Primrose. Glob. J. Sci. Eng. 05,

1–5. https://doi.org/10.37516/global.j.sci.eng.2021.0025

Timung, R., Mohan, M., Chilukoti, B., Sasmal, S., Banerjee, T., Goud, V. V., 2015. Optimization of dilute acid and hot water pretreatment of different lignocellulosic biomass: A comparative study. Biomass and Bioenergy 81, 9–18. https://doi.org/10.1016/j.biombioe.2015.05.006

Tomás-Pejó, E., Oliva, J.M., Ballesteros, M., Olsson, L., 2008. Comparison of SHF and SSF processes from steam-exploded wheat straw for ethanol production by xylose-fermenting and robust glucose-fermenting Saccharomyces cerevisiae strains. Biotechnol. Bioeng. 100, 1122–1131. https://doi.org/10.1002/BIT.21849

Tran, P.H.N., Jung, J.H., Ko, J.K., Gong, G., Um, Y., Lee, S.-M., 2023. Co-production of ethanol and polyhydroxybutyrate from lignocellulosic biomass using an engineered Saccharomyces cerevisiae. Renew. Energy 212, 601–611. https://doi.org/10.1016/J.RENENE.2023.05.080

Trevorah, R.M., Othman, M.Z., 2015. Alkali Pretreatment and Enzymatic Hydrolysis of Australian Timber Mill Sawdust for Biofuel Production. J. Renew. Energy 2015, 1–9. https://doi.org/10.1155/2015/284250

Tripathi, R., Varsha, G., Saya, T., Pankaj, J., Rashmi, T., 2024. Evaluation of the Bioactive compounds and its Functional role in the Aquatic weed Pistia stratiotes. Res. Varsha, T Saya, J Pankaj, T RashmiResearch J. Pharm. Technol. 2024•researchgate.net 17, 87–95. https://doi.org/10.52711/0974-360X.2024.00014

Tse, T.J., Wiens, D.J., Reaney, M.J.T., 2021. Production of Bioethanol—A Review of Factors Affecting Ethanol Yield. Ferment. 2021, Vol. 7, Page 268 7, 268. https://doi.org/10.3390/FERMENTATION7040268

Umesh, M., Santhosh, A.S., Shanmugam, S., Thazeem, B., Alharbi, S.A., Almoallim, H.S., Chi, N.T.L., Pugazhendhi, A., 2022a. Extraction, characterization, and fabrication of cellulose biopolymer sheets from Pistia stratiotes as a biodegradative coating material: an unique strategy for the conversion of invasive weeds into value-added products. J. Polym. Environ. 30, 5057–5068. https://doi.org/10.1007/S10924-022-02511-4/FIGURES/9

Umesh, M., Santhosh, A.S., Shanmugam, S., Thazeem, B., Alharbi, S.A., Almoallim, H.S., Chi, N.T.L., Pugazhendhi, A., 2022b. Extraction, characterization, and fabrication of cellulose biopolymer sheets from Pistia stratiotes as a biodegradative coating material: an unique strategy for the conversion of invasive weeds into value-added products. J. Polym. Environ. 30, 5057–5068. https://doi.org/10.1007/S10924-022-02511-4

Valamonfared, J., Javanmard, A.S., Ghaedi, M., Bagherinasab, M., 2023. Bioethanol

production using lignocellulosic materials and thermophilic microbial hydrolysis. Biomass Convers. Biorefinery. https://doi.org/10.1007/S13399-023-03980-W

Valles, A., Capilla, M., Álvarez-Hornos, F.J., García-Puchol, M., San-Valero, P., Gabaldón, C., 2021. Optimization of alkali pretreatment to enhance rice straw conversion to butanol. Biomass and Bioenergy 150, 106131. https://doi.org/10.1016/J.BIOMBIOE.2021.106131

Van Thuoc, D., Chung, N.T., Hatti-Kaul, R., 2021. Polyhydroxyalkanoate production from rice straw hydrolysate obtained by alkaline pretreatment and enzymatic hydrolysis using Bacillus strains isolated from decomposing straw. Bioresour. Bioprocess. 8. https://doi.org/10.1186/s40643-021-00454-7

Wannapokin, A., Ramaraj, R., Whangchai, K., Unpaprom, Y., 2018. Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. 3 Biotech 8. https://doi.org/10.1007/S13205-018-1084-7

Whangchai, K., Inta, W., Unpaprom, Y., Bhuyar, P., Adoonsook, D., Ramaraj, R., 2021. Comparative analysis of fresh and dry free-floating aquatic plant Pistia stratiotes via chemical pretreatment for second-generation (2G) bioethanol production. Bioresour. Technol. Reports 14. https://doi.org/10.1016/j.biteb.2021.100651

Wolfrum, E.J., Lorenz, A.J., deLeon, N., 2009. Correlating detergent fiber analysis and dietary fiber analysis data for corn stover collected by NIRS. Cellulose 16, 577–585. https://doi.org/10.1007/S10570-009-9318-9/FIGURES/4

Wood, R.W., Loomis, A.L., 1927. XXXVIII. The physical and biological effects of high-frequency sound-waves of great intensity . London, Edinburgh, Dublin Philos. Mag. J. Sci. 4, 417–436. https://doi.org/10.1080/14786440908564348

Wu, H., Zhao, X., Adsul, M., Zhong, Y., Qian, Y., Zhong, L., Sun, Y., Sun, N., Zhang, L., Liu, W., Qu, Y., 2019. Enhancement of Cellulase Production in Trichoderma reesei via Disruption of Multiple Protease Genes Identified by Comparative Secretomics. Enhancement of Cellulase Production in Trichoderma reesei via Disruption of Multiple Protease Genes Identified by Comparative Secretomics 10, 2784. https://doi.org/10.3389/fmicb.2019.02784

Wyman, C.E., Yang, B., 2017. Combined severity factor for predicting sugar recovery in acid-catalyzed pretreatment followed by enzymatic hydrolysis. Hydrothermal Process. Biorefineries Prod. Bioethanol High Added-Value Compd. Second Third Gener. Biomass 161–180. https://doi.org/10.1007/978-3-319-56457-9_6

Xia, M., Valverde-Barrantes, O.J., Suseela, V., Blackwood, C.B., Tharayil, N., 2022. Characterizing natural variability of lignin abundance and composition in fine roots across

temperate trees: a comparison of analytical methods. New Phytol. https://doi.org/10.1111/NPH.18515

Yadav, D., Barbora, L., Bora, D., Mitra, S., Rangan, L., Mahanta, P., 2017. An assessment of duckweed as a potential lignocellulosic feedstock for biogas production. Int. Biodeterior. Biodegradation 119, 253–259. https://doi.org/10.1016/J.IBIOD.2016.09.007

Yadav, K.K., Krishnan, S., Gupta, N., Prasad, S., Amin, M.A., Cabral-Pinto, M.M.S., Sharma, G.K., Marzouki, R., Jeon, B.H., Kumar, S., Singh, N., Kumar, A., Rezania, S., Islam, S., 2021. Review on Evaluation of Renewable Bioenergy Potential for Sustainable Development: Bright Future in Energy Practice in India. ACS Sustain. Chem. Eng. 9, 16007–16030. https://doi.org/10.1021/ACSSUSCHEMENG.1C03114

Yan, D., Ji, Q., Yu, X., Li, M., Abiola Fakayode, O., Yagoub, A.E.G.A., Chen, L., Zhou, C., 2021. Multimode-ultrasound and microwave assisted natural ternary deep eutectic solvent sequential pretreatments for corn straw biomass deconstruction under mild conditions. Ultrason. Sonochem. 72, 105414. https://doi.org/10.1016/J.ULTSONCH.2020.105414

Yang, M., Zhang, Xiaoliang, Wang, K., Zhu, S., Ye, Z., Sheng, K., Zhang, Ximing, 2022. Investigation of cascade valorization of Pistia stratiotes L. by hydrothermal treatment. Fuel 324, 124473. https://doi.org/10.1016/J.FUEL.2022.124473

Yosrey, E., Elmansi, H., Sheribah, Z.A., El-Sayed Metwally, M., 2021. Factorial design-assisted spectroscopic determination of oxybutynin hydrochloride. R. Soc. Open Sci. 8. https://doi.org/10.1098/RSOS.211027

Yuan, X., Chen, X., Shen, G., Chen, S., Yu, J., Zhai, R., Xu, Z., Jin, M., 2022. Densifying lignocellulosic biomass with sulfuric acid provides a durable feedstock with high digestibility and high fermentability for cellulosic ethanol production. Renew. Energy 182, 377–389. https://doi.org/10.1016/j.renene.2021.10.015

Zabed, H., Sahu, J.N., Boyce, A.N., Faruq, G., 2016a. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. Renew. Sustain. Energy Rev. 66, 751–774. https://doi.org/10.1016/j.rser.2016.08.038

Zabed, H., Sahu, J.N., Boyce, A.N., Faruq, G., 2016b. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. Renew. Sustain. Energy Rev. 66, 751–774. https://doi.org/10.1016/j.rser.2016.08.038

Zanellati, A., Spina, F., Bonaterra, M., Dinuccio, E., Varese, G.C., Scarpeci, T.E., 2021. Screening and evaluation of phenols and furans degrading fungi for the biological pretreatment of lignocellulosic biomass. Int. Biodeterior. Biodegradation 161, 105246. https://doi.org/10.1016/J.IBIOD.2021.105246

Zhang, F., Lan, W., Li, Z., Zhang, A., Tang, B., Wang, H., Wang, X., Ren, J., Liu, C., 2021. Co-production of functional xylo-oligosaccharides and fermentable sugars from corn stover through fast and facile ball mill-assisted alkaline peroxide pretreatment. Bioresour. Technol. 337, 125327. https://doi.org/10.1016/J.BIORTECH.2021.125327

Zhang, Y., Han, D., Xiong -, R., A Jusri, N.A., Azizan, A., Ibrahim, N., Mohd Salleh, R., Abd Rahman, M.F., Nuklear Malaysia, A., 2018. Pretreatment of Cellulose By Electron Beam Irradiation Method. IOP Conf. Ser. Mater. Sci. Eng. 358, 012006. https://doi.org/10.1088/1757-899X/358/1/012006

Zhong, L., Wang, C., Yang, G., Chen, J., Xu, F., Geun Yoo, C., Lyu, G., 2022. Rapid and efficient microwave-assisted guanidine hydrochloride deep eutectic solvent pretreatment for biological conversion of castor stalk. Bioresour. Technol. 343, 126022. https://doi.org/10.1016/J.BIORTECH.2021.126022

Zhou, Y., Zheng, J., Gan, R.-Y., Zhou, T., Xu, D.-P., Li, H.-B., Cravotto, G., Choi, Y.H., 2017. Optimization of Ultrasound-Assisted Extraction of Antioxidants from the Mung Bean Coat. Molecules. https://doi.org/10.3390/molecules22040638

Zhuang, X., Wang, W., Yu, Q., Qi, W., Wang, Q., Tan, X., Zhou, G., Yuan, Z., 2016. Liquid hot water pretreatment of lignocellulosic biomass for bioethanol production accompanying with high valuable products. Bioresour. Technol. 199, 68–75. https://doi.org/10.1016/J.BIORTECH.2015.08.051

Zorić, A.S., Morina, F., Toševski, I., Tosti, T., Jović, J., Krstić, O., Veljović-Jovanović, S., 2019. Resource allocation in response to herbivory and gall formation in Linaria vulgaris. Plant Physiol. Biochem. 135, 224–232. https://doi.org/10.1016/J.PLAPHY.2018.11.032

PUBLICATIONS

- Mann, S., Sharma, J. G., & Kataria, R. (2024). Microbial accumulation of bioplastics from waste stream: recent advancements and applications. *International Journal of Environmental Science and Technology*, *21*(2), 2279-2306, Impact factor- 3

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REVIEW

Microbial accumulation of bioplastics from waste stream: recent advancements and applications

S. Mann[1] · J. G. Sharma[1] · R. Kataria[1]

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Abstract

The demand for biodegradable and biocompatible polymers rises because of the environmentally hazardous property of petroleum-based plastic. Polyhydroxyalkanoates is an advantageous alternative to synthetic plastics. Biodegradable property of this biopolymer makes it suitable for the environment, human, and animal health. For production of polyhydroxyalkanoates, several challenges are associated like production cost and use of hazardous chemical during extraction and treatment which oppose the scale up of the process. To overcome these issues, many strategies such as utilization of cheap carbon source, optimization of processes, and modified microbial strains are being used for economical production. The use of organic waste is a suitable option to produce polyhydroxyalkanoates in sustainable manner, which is cost-effective and provide solution of its management. Modification in downstream process makes it economic and sustainable by which it can be implemented on the industrial scale. In the application part of these biopolymer, it can be made prominent in medical, agriculture, and tissue engineering fields by blending option.

Keywords Biodegradable · Environment · Organic waste · Polyhydroxyalkanoates · Sustainable · Waste management

Introduction

Petroleum-based plastic is commonly used because of its durability and availability; however, the main problem associated with plastic waste is its degradation and accumulation. It takes decades to degrade, and it affects the water resources, soil as well as ecosystem. Plastics accumulate in form of microplastics by the bioaccumulation process in humans as well as animals, which causes several health problems. Commonly used conventional plastics are polyethylene terephthalates (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC) and polystyrene (PS), and all of these conventional plastic cause many diseases like birth defects, skin diseases, cancer, ulcers, liver dysfunction.(Saravanan et al. 2022).

Landfilling and incineration are cost-effective processes to manage the plastic waste. To resolve these problems of fossil fuel-based plastic, another alternative approach is employed, which is to produce biodegradable plastic. These are biodegradable and biocompatible in nature. This degradation formed in two steps. First step, monomers, dimers, and oligomers are formed depending upon the depolymerase, and in the second step hydrolase enzymes degrade dimer and oligomers into the monomers (Jadhav et al. 2022). Biodegradation nature of bioplastic was evaluated by optical properties, swelling behavior, and moisture content, which has a potential to be a sustainable material for various bioplastic applications (Rohadi et al. 2022). An addition of the plasticizers to bioplastic can enhance the tensile strength and it can reduce swelling and water retaining capability of organic waste bioplastics. (Ng et al. 2022).

These biodegradable plastics are mechanically strong, water resistant, and thermally stable with recycling and biodegradable properties. Hence these may be an alternative to fossil fuel-based plastic. It can be degraded rapidly in water and recycled (Xia et al. 2021). The biodegradable and biocompatible nature of PHAs makes them significant in packaging, paints, and the medical area (Khatami et al. 2021). These polymers are synthesized by both gram-positive and

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gram-negative bacteria as an intracellular energy and carbon storage material. Some anaerobic bacteria are also reported to produce of these biopolymers (Valentino et al. 2019). Wild type, as well as engineered bacterial strains, are used to produce PHAs. Engineered bacteria seem to be more efficient in terms of contamination resistance, controlling PHA synthase activity, morphology, and polymer yield. Hence, these characteristics make the genetically modified bacteria more valuable (Chen and Jiang 2017). Various organic waste streams provide an opportunity to utilize as renewable carbon source for PHA accumulating bacteria. This class of natural polymers can be synthesized by sugarcane and corn; however, these carbon sources are competent food materials. Hence organic waste streams could be an alternative substrate. Various classes of waste, including vegetable fat, waste oils, straw, sawdust, and food waste, could be utilized for sustainable production of polyhydroxyalkanoates (PHAs). Different conversion technologies like pretreatment, hydrolysis, fermentation, and extraction are optimized in order to obtain to increase the efficiency and cost-effectiveness of production of PHA on the industrial scale.

This review gives an insight of biopolymer PHA and its biosynthesis in microorganism. Latest strategies for PHA production from different types of organic waste stream, including lignocellulosic waste, municipal waste, whey waste, and kitchen/food industrial waste, are discussed (Fig. 1). Depending upon type of waste stream, the possible conversation pathways are outlined in detail for PHA accumulation. The variable composition of substrate affects the sustainable production of bioplastics. Improvement of PHA accumulation in microbial strain and advancement in PHA extraction methods are also discussed, and in later section, the potential application of this biopolymer is reviewed. There are several bottlenecks for industrialization of this product. A few practical explanations are also provided to overcome the challenges for microbial accumulation of PHA as only a small number of industries utilizing waste for bioplastic production. Hence, it is a great requirement for commercializing of waste to PHA synthesis-based industries by improving the process design.

PHA, structure and composition

PHAs are polymers that can be easily degraded. It is accumulated in various bacteria intracellularly in the form of inclusion bodies as a carbon storage material under the stress environment. During the unbalanced growth condition, this granular PHA is advantageous for the survival of the bacteria. The properties of PHA are similar to petroleum-based plastic but produced by microbes in shortage of nutrients with excessive sugar concentration. The common structure of PHAs is composed of 3-hydroxy fatty acid subunits. The carboxyl group monomer is joined with the hydroxy group of nearby monomers. Various classes of the bioplastic family are known to depend upon the alkyl side chain present in the structure of PHAs. At the carbon-3 position, it may be aromatic, unsaturated, halogenated, epoxidized, or branched alkyl group present (Naik et al. 2008) (Fig. 2). Carbon numbers in monomeric units classified the PHAs. Different polymers include poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyvalerate) (PHV), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly(3-hydroxyoctanoate) (PHO), poly(3-hydroxynonanoate) (PHN), 3-hydroxyhexanoate

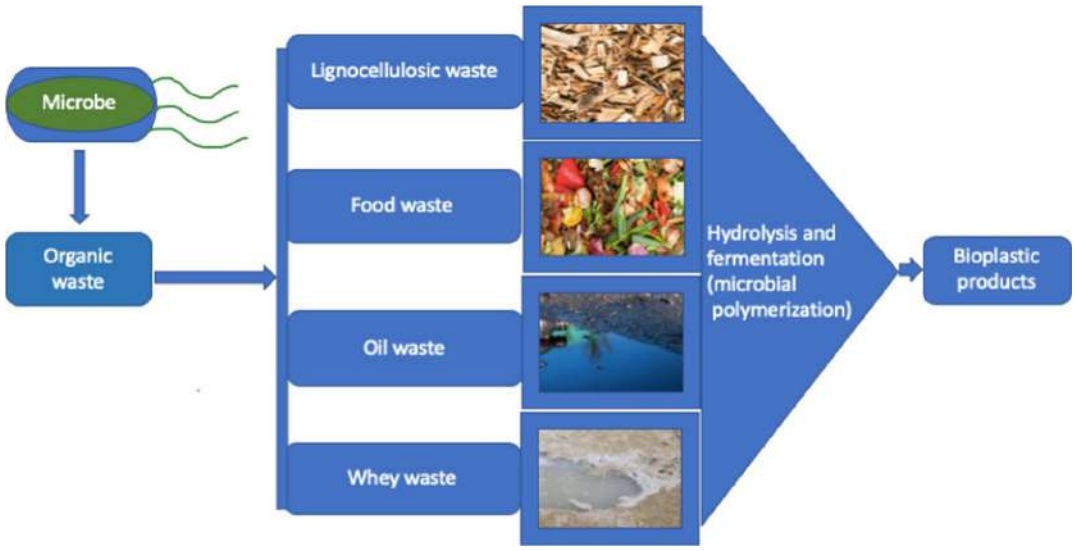

Fig. 1 Various type of organic waste for PHA production

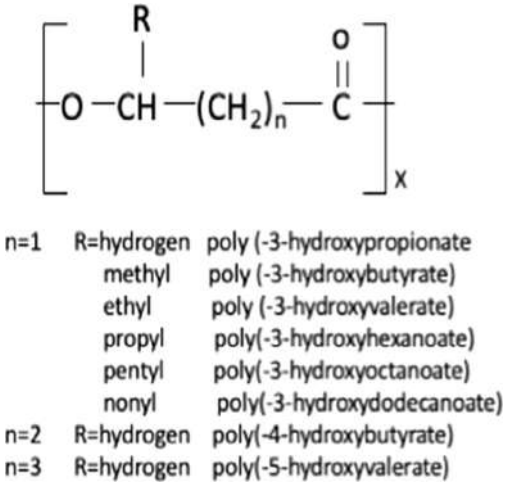

Fig. 2 Molecular structure of polyhydroxyalkanoates (PHA)

(HHx), 3-hydroxyheptanoate (HH), and 3-hydroxydecanoate (HD) are the main form of PHAs. Mainly two types of PHAs are accumulated by bacteria, short-chain length PHAs (scl-PHAs) and medium-chain length PHAs (mcl-PHAs). A scl-PHAs contain 3–5 carbon monomers; however, mcl-PHAs are composed of 6–14 carbon monomers. PHB, PHV, and PHBV are scl-PHAs, and PHO, PHN, HHx, HH, and HD are mcl class of PHAs (Li et al. 2016).

Biosynthesis pathways for the synthesis of PHAs

Variation in carbon source gives a way to synthesize different PHAs in a bacterial cell. Diversification of PHAs depends on the type of monomer, polymer chain structure, functional groups, molecular weight, and carbon source available for its production (Zheng et al. 2020). The monomers present in the complete chain characterize the type of polymer formed. There are four types of polymers homopolymers, random polymers, block polymers, and graft polymers which shows different properties. Homopolymers are made up of the same monomeric units, whereas the other three are made of different monomeric units with a different pattern of combination (Fig. 3). The exact structure of PHA depends on the type of monomer of carbon utilized in PHA synthase in the bacterial cell. There are 12 different pathways for synthesizing the PHAs. However, most three common pathways are found in the bacterial cell (Fig. 4). These include fatty acid synthesis pathway, acetoacyl pathway, and beta-oxidation pathway (Chen et al. 2015). Acetate and formate are highly toxic compounds generated during the metabolic pathway of bacteria *E. coli*, and these toxic compounds are used in the anaerobic metabolic cycle of bacteria for the production of PHB and other polymers (Chen et al. 2016).

In the first pathway, acetyl-CoA is converted into acetoacetyl-CoA and further proceeds into 3 hydroxy butyryl CoA. With the help of scl-PHA synthase, it forms PHB. On the other hand, in the second pathway in situ fatty acid synthesis occurs in a bacterial cell, and phaG converts R-3-hydroxyaceyl-ACP into R-3-hydroxyacyl-CoA, and it is further

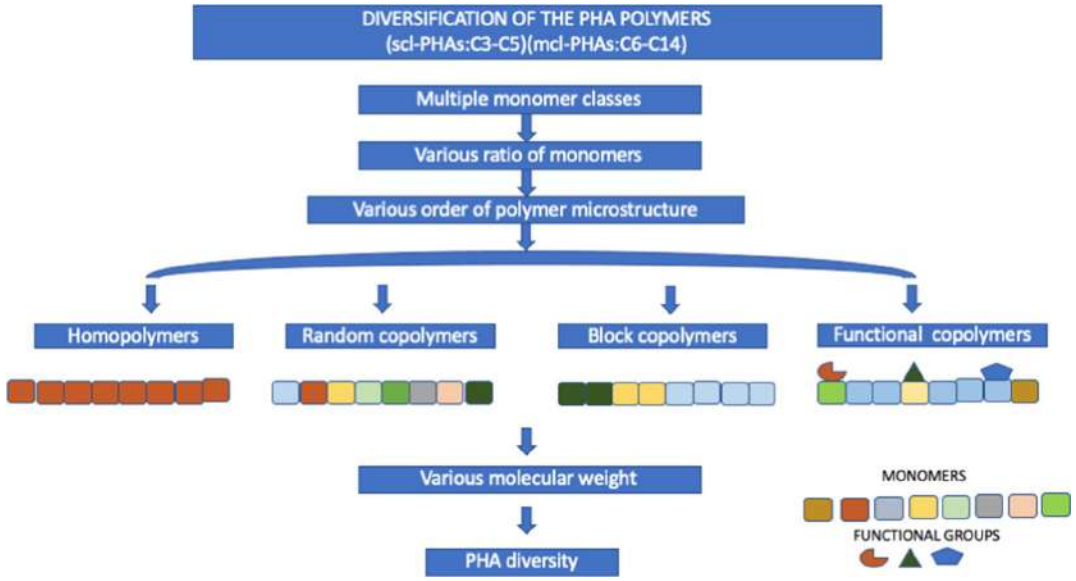

Fig. 3 Various ratios of monomers were arranged into four different copolymers (homopolymers, random polymers, block polymers and functional copolymers). These copolymers have different characteristic properties and molecular weight

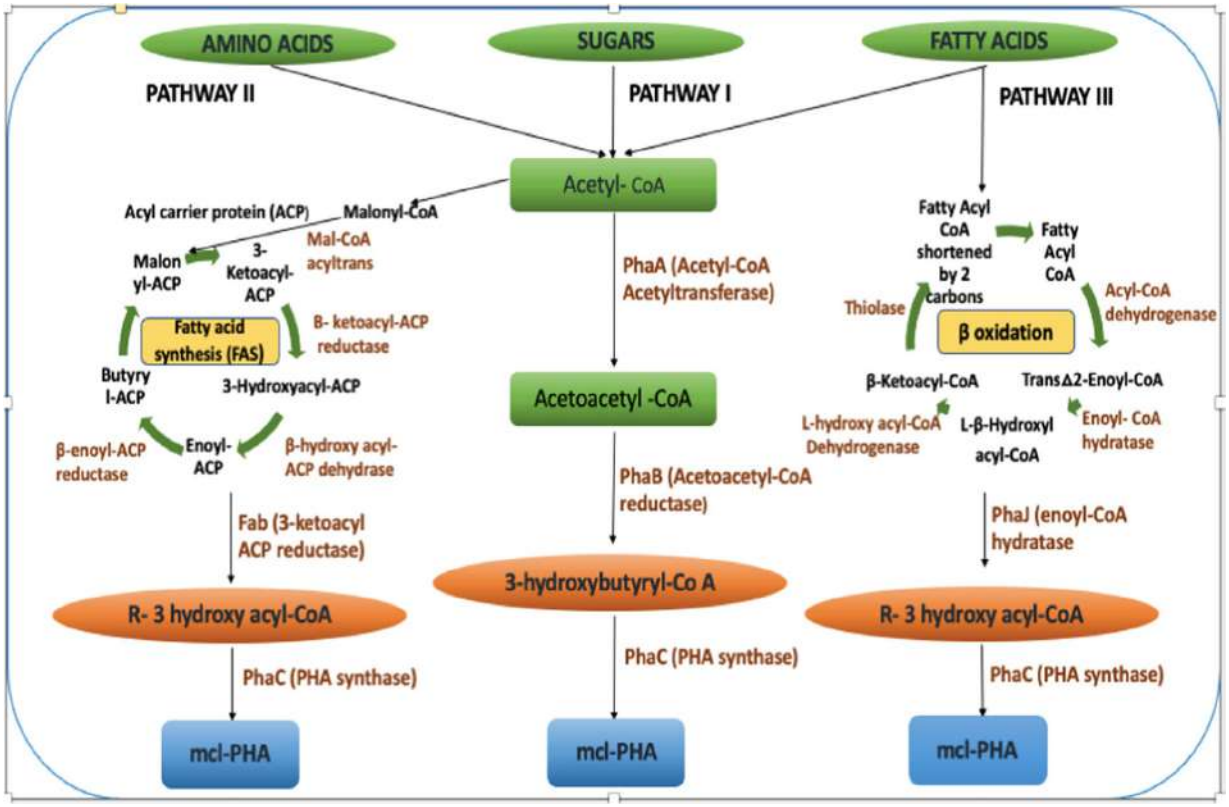

Fig. 4 Common pathways for PHA production in bacterial cell

converted into mcl PHA with the help of mcl-PHA synthase. In the third pathway, β oxidation of fatty acid synthesizes mcl-PHA by mcl PHA synthase (Chen et al. 2016). Various classes of enzymes include PhaA, PhaB, PhaC, FabG, PhaJ, β ketothiolase, acetoacetyl-CoA reductase, and PHA synthase, 3 ketoacyl-acyl carrier protein (ACP) reductase, and eoyl-coenzyme participate in the whole process (Numata et al. 2013).

Gram-positive and gram-negative bacteria can produce PHA and copolymer of PHA. Homopolymers of PHA are brittle and have low strength. *Clostridium, Streptomyces*, *Rhodococcus, Staphylococcus,* and *Bacillus* are some common PHA-producing bacteria. *Bacillus* can produce homopolymer and copolymer of PHA. Glucose, glycerol, and acetate are used as the main carbon sources for the synthesis. *Methylobacterium rhodesianum*, *E.coli* JM109, and *Azohydromonas australica* are common gram-negative bacteria that can produce PHA (Ray and Kalia 2017). *E.coli* is the most promising bacteria, which shows binary fission cell division, and it is beneficial for the accumulation of a high amount of PHA in inclusion body of the bacterial cell. However, the main problem is the reduction in the size of a cell during cell division. Recombinant technologies target the genes involved in cell division; hence, the growth pattern of a cell can be changed from binary division into multiple fission. *FtsZ* gene is over-expressed to speed up the cell division, and *mreB* gene increases the size of a cell for PHA production (Wu et al. 2016b).

In bacteria, PHA is produced intracellularly in the form of cytosolic granules. The amount of PHA depends upon the size of the granules. In the *C. necator*, the size of granules varies from 0.2 to 0.5 µm (Sen and Baidurah 2021). Bacteria experience various biotic and abiotic stress. During this stress, bacteria synthesized PHA in the form of intracellular granules which works as a carbon source to cope with the stress conditions and provide nutrients and energy for cellular function. However, under higher stress conditions amount of PHA may decrease (Müller-Santos et al. 2021).

Waste glycerol contains glycerin, and this sugar is converted into volatile fatty acids (VFAs). These VFAs are further utilized by bacteria to produce PHAs. Acetic acid is converted into PHB in the bacterial cell. The product of acetic acid can be homopolymer PHB and co-polymer (3HV) poly hydroxyvalerate. These are synthesized by a combination of acetate and propionate (Ciesielski et al. 2015). When oil is consumed as a carbon source by the bacteria, triglycerides

break down into the fatty acids by lipase enzyme, and this enzyme breaks the ester bond in triglycerides. All of this breakdown occurs in bacterial cells (Tanikkul et al. 2020). The ß oxidation occurs in the cell to break down fatty acids into the acetyl-CoA for providing the nutrients for further growth of the cell. This process of turning the fatty acid into acetyl CoA is costly. Engineering this process could be a target and helps to increase the PHA productivity (Chen et al. 2020).

Conversion technologies involved in PHA synthesis

Pretreatment

Pretreatment is an essential step in the liberation of sugars from plant-based biomass. Lignocellulosic biomass is mainly composed of complex polymers including, cellulose, hemicellulose, and lignin. Lignin is the main obstacle to hydrolysis of carbohydrate complex; it is tightly bound with cellulose and hemicellulose and restricts the saccharification process. After the pretreatment step, lignin is removed, the free cellulose or hemicellulose could be enzymatically hydrolyzed into monomer sugars, and these sugars are further used by microbes for PHA accumulation. Depending on the biomass composition, different types of pretreatment methods are used in the process of production of bioplastic from different types of biomass. The various class of pretreatment methods includes physical, chemical, physicochemical, and biological.

Physical pretreatment

Mechanical extrusion is a technique by which reduction in particle size of the organic waste is performed, which increases the surface area of the substrates and efficiently hydrolyzes (Hjorth et al. 2011). It is a promising process of treatment, high pressure is applied to the substrate, and the liquid fraction is formed after this pretreatment (Cesaro et al. 2021). Extruders are of two types (1) single-screw extruders and (2) twin-screw extruders. A single-screw extruder is made up of a single solid screw while a twin-screw extruder is made up of two screws that are connected to the motor, hopper, and temperature regulators (Duque et al. 2017).

Milling is process in which the particle size of the substrate is decreased by the grinding instrument. The milling instrument is made up of an electromotor (1.1 kW) and a grinder to decrease the particle size (Liu et al. 2016). Generally, this pretreatment is performed with a combination of other pretreatment methods. The grinder of electromotor grinds the substrate and revolves at a particular rpm. The substrate should be chemical-free during this process. After this pretreatment, centrifuge the slurry for further enzymatic hydrolysis (Zhang et al. 2021). Water in the substrate affects this process, and a low moisture content in the substrate speeds up the milling process. A fast grinding and high glucose content form when there is no water content during the milling process (Gu et al. 2018).

Microwave pretreatment is done for the breakdown the lignin. Heat is provided to the sample to disrupt the lignin. The range of heat may be 60–140 °C with different time ranges (Yan et al. 2021a, b). This technique is majorly used for lignin extraction; however, the sugar content can decrease under harsh operating conditions (Sun et al. 2021). This is a potential technique to extract highly purified lignin extraction (Zhong et al. 2022). The maximum recovery of sugar by this process obtained is 0.512 g/g with corn cob under the optimized conditions which also enhanced the productivity of bioethanol (Ocreto et al. 2021).

Ultrasonic pretreatment is performed by ultrasonic bath reactor with different frequencies. Single, dual, and multiple frequencies are used in the generator to perform the pretreatment. Samples in the tube are placed 4 cm deep in this water bath, with different frequencies such as 20, 40, and 60 kHz. Different pretreatment time settings are used with fixed temperatures (Yan et al. 2021a, b). The combination of alkaline in ultrasonic pretreatment could increase the saccharification of the lignocellulosic biomass, and a sugar recovery of 90% is obtained (Saratale et al. 2020).

Pulse electric field is another pretreatment technology, in which a high electric field is applied on the substrate for milli-seconds at a short pulse. Pulse electric field pretreatment gives better performance for enzymatic accessibility of the substrate (Kovačić et al. 2021). Lipid extraction is essential for industrially important chemical synthesis. Wastewater *Chlorella pyrenoidosa* (microalgae) could be a good carbon source for biodiesel and other metabolites including PHA production. For the delignification step pulse, the electric field is an efficient pretreatment method (Han et al. 2019). Lignocellulosic biomass is treated with a short burst of high intensity of electric field for fraction of seconds, and after that, the polymers of the cell wall breakdown into monomers of sugar for further use in the industry (Haldar and Purkait 2021).

Chemical pretreatment

Alkali and acidic pretreatments are efficient pretreatment methods for the accessibility of enzymes and breakdown of complex material in the plant cells into the simple sugars for further use in the PHA accumulation. Different acids, such as sulfuric acid, hydrochloric acid, acetic acid, boric acids, are used for acid pretreatment. Here, mostly hemicellulosic sugars are recovered. However, for alkali pretreatment sodium hydroxide, ammonia, and calcium hydroxide

are used, which result in the solubilization of lignin components. These chemicals are used with a combination of temperature and various time duration and concentrations, depending upon the biomass type. As compared to the acidic pretreatment, alkali pretreatment is most beneficial for the enzymatic hydrolysis (Murciano Martínez et al. 2015).

Organosolv pretreatment is used to extract lignin from the lignocellulosic biomass. Many organic solvents are used with a combination of acid or alkali in this pretreatment. Ethanol, methanol, acetone, butanol, and diethylene glycol are used as a solvent. Glycerol and methanol are commonly used for efficient sugar liberation (Joy and Krishnan 2022). The organosolv pretreatment method is also used to obtain the highly purified lignin from the lignocellulosic biomass. About 97% of pure lignin was recovered by using ethanol as a solvent (Parot et al. 2022).

Physiochemical pretreatment

Steam explosion pretreatment process is performed at high temperature and high pressure on the biomass. High pressure is generated by a steam generator in the steam explosion equipment. The biomass is treated with this steam for a short duration of time, which can be from a few seconds to several minutes, and the pressure is released to atmospheric pressure instantly. The sudden change in pressure cause disruption of the substrate by the release of sugars and lignin disruption. This pretreatment could be performed alone or in combination with another pretreatment. In a study, sequential pretreatment is performed; after steam explosion pretreatment, acidic pretreatment with 1.5% sulfuric acid is done. Optimized conditions for banana pretreatment found to be 219.31 °C temperature with 10 min of residence time for PHB production (Anna Mabazza et al. 2020). Acid-catalyzed reaction during steam explosion gives higher xylose recovery which is further used for pentose utilizing microbe for production of value-added chemicals (Cavalaglio et al. 2021).

Liquid hot water pretreatment reduces the recalcitrance property of lignocellulosic material. It is also called hydrothermal pretreatment and this pretreatment does not release any toxic materials, hence enhancing enzymatic activity for hydrolysis of cellulose (Martín-Lara et al. 2020). This treatment is beneficial for the fermentation process because in the fermentation step microbes utilize sugars free from any toxic material. However, the sugar yield is lower in comparison with other pretreatment processes (Zhuang et al. 2016).

Carbon dioxide explosion is a technique in which biomass is placed in a reactor that contains pressurized carbon dioxide. Anaerobic digestion is an essential step in the waste management process and the pretreatment process for sustainable valuable chemical production (Ampese et al. 2022). Carbon dioxide works as a solvent in this process, and it mixes with the substrate in the reactor with high pressure by which the breakdown of hemicellulose and cellulose into simple forms of sugars (Das et al. 2021). Benefits of this pretreatment include low cost; the release of a low amount of hazardous molecules and this process needs low temperature (Agbor et al. 2011).

Biological pretreatment

Chemical pretreatments release toxic bioproducts in the hydrolysate which become hazardous to the environment as well as fermenting bacteria. Biological pretreatment is an alternative to chemical pretreatments. Bacteria and fungi are the main microbes involved in this process. During biological pretreatment, some controlled factors like pH, inoculum, temperature, moisture content, and time duration are used to optimize the process. Breakdown of lignin is the first step for rapid enzymatic saccharification for the production of biodegradable products (Naik et al. 2021). Microbes such as *Cellulomonas fimi, Paenibacillus compinasensis*, *Zymomonas mobilis, Azospirillum lipoferum*, *Pseudomonas*, *Rahnella* used as cellulolytic in nature. Some fungus strains such as *Byssochlamys nivea* can grow on the chemical compounds and decompose them (Zanellati et al. 2021). Other than microbes, some insect species like termites, beetles, earthworms, and crickets carry the ability of enzymatic degradation of lignin. Various enzymes present in the guts of these organisms are effective for all this enzymatic degradation process (Sun et al. 2014).

Detoxification

Detoxification is an essential step after the chemical pretreatment, especially acid pretreatment. As many toxic compounds are generated due to the reaction between substrate and acid. Different types of material are used for the detoxification of hydrolysates like activated carbon, membrane nanofiltration, and calcium carbonate. Charcoal is activated by mixing with NaOH, and then this activated charcoal is added to the hydrolysate slowly for 1 h. After that, it is removed from hydrolysate by vacuum filtration. GE-Sepa CF cross-flow module with pressure pump used for the membrane filtration. Complete removal of toxic material is done by this filtration unit (Tavares et al. 2022). Calcium

carbonate is another chemical that is used during the neutralization of hydrolysate after the acidic pretreatment which can remove the toxic materials from the hydrolysate (Ahmed et al. 2019).

Hydrolysis

Hydrolysis is the main step in between pretreatment and fermentation. It enhances the accessibility of sugars for the microbes and enzymes for the production of PHAs. Chemical hydrolysis and enzymatic hydrolysis are mainly performed during the production of valuable products. Enzymes like cellulase and hemicellulase are generally used for the carbohydrate breakdown into simple forms of monomers. The substrate is mixed with the sodium acetate buffer, enzyme and incubated at 50 °C for a different duration of up to 3–4 days. The detection of monomeric sugars is performed by HPLC (Thuoc et al. 2017). Hydrolysis is also done by dilute acid, and it can be sulfuric acid or hydrochloric acid. Toxic chemical generation after pretreatment is the main disadvantage of acid hydrolysis. For the removal of these toxic materials, another detoxification step is to be necessary for further steps (Kucera et al. 2017).

Fermentation

Fermentation is an important step to convert sugars into valuable compounds. The sugars obtained after different pretreatment and hydrolysis are utilized by various microbes to generate renewable chemicals such as PHA. In fermentation step, substrate is added in the initial time in the bioreactor under the controlled condition until the maximum production achieved (Simona et al. 2022). One-step fermentation is done by the recombinant *E.coli* for the production of the aromatic polymer from a renewable source. (Yang et al. 2018). Mixed microbial culture (MMC) is enriched with the PHAs producing bacteria. (Colombo et al. 2017).

Transesterification

The transesterification process is used to convert the frying oil into ester content with the help of a catalyst which is further used for PHAs production (Almeida da Silva et al. 2022). Sodium hydroxide, methoxide, and potassium hydroxide are used as catalysts of which sodium hydroxide promises to have the highest potential for transesterification (Leung et al. 2006; Vastano et al. 2019). Transesterification of the oil depends upon the fatty acids, catalysts, water in oils, reaction time, and reaction temperature. Glycerol found after this process is further used for the PHAs production (Belkhanchi et al. 2021).

PHA extraction and characterization techniques

Extraction is an essential step in the production of PHA. Extracted PHAs from the bacterial cells are further characterized. Different methods are used for the analysis and quantification (Table 1). Various techniques are being used for the extraction process including solvent extraction, digestion by chemicals, enzymatic digestion method, mechanical disruption method, supercritical fluids, and cell fragility (Jacquel et al. 2008). Bioextraction method is also suitable at present scenario.

Solvent extraction method

In the solvent extraction method, different chemical solvents are used; however, acetone is the most preferred solvent. PHAs production by the palm oil mill effluent is extracted by the acetone, and 93% maximum recovery is reported which is higher in comparison with methyl isobutyl ketone and ethyl acetate (Shakirah et al. 2020). A simple chloroform extraction can be performed in the Soxhlet apparatus. A high-pressure extraction is performed for a longer time of up to 12 h at high pressure (HPE) with acetone as solvent. A HPE apparatus is composed of an extraction chamber, filtration, and precipitation chamber. After the extraction, the extract is dissolved in acetone, filtered, and followed by methanol precipitation, and PHAs are recovered (Koller et al. 2013). The extraction from mixed microbial culture is done by chloroform and dichloromethane (Samorì et al. 2015). A rotary evaporator separates the solvent and polymer (Jiang et al. 2018).

Digestion by chemicals

Digestion by chemicals is performed by dilute ammonia, and it extracts highly purified PHAs. Temperature plays an essential role during the digestion for extraction, a temperature > 75 °C is appropriate for efficient digestion, and a temperature range 75–140 °C is used for extraction and recovery of polymer (Burniol-Figols et al. 2020). Sodium hydroxide (0.1 M) is important for the recovery of P(3HB-co-HHx) from the recombinant *C. necator* pBBR1MCS-C2. Polishing of the extracted polymer is done with methanol, ethanol, and acetone (Anis et al. 2012).

Table 1 Quantification techniques for PHAs

Technique	Sample preparation	Quantification	Temperature	References
GC–MS (Gas chromatography–mass spectroscopy)	Lyophilized cells reacted with acid catalyzed methanolysis	Methyl ester quantified by GC–MS	–	(Yang et al. 2018)
Pyrolysis	Direct sample	Pyrolyzed sample further quantified by GC–MS	500 °C for 100 s	(Torri et al. 2014)
TGA (Thermogravimetric analysis)	Powder form	Analysis conducted in nitrogen flow (20 ml/min) for decomposition temperature and thermal stability	30–500 °C at 10 °C/min	(Lorini et al. 2021)
DSC (differential scanning calorimetry)	Powder form	Detection of melting temperature and melting enthalpy	From room temperature to 190 °C at 10 °C/min with instant cooling by 30 °C/min from 190 to − 70 °C	(Lorini et al. 2021)
FTIR (fourier transform-infrared spectroscopy)	Powder form	Transmittance and wave shows different stretches in the groups	Extracted polymer shows a particular peak which characterize the polymer	(Muneer et al. 2022)
Crotonic acid test	Extracted polyester	Change in colour from yellow to brown shows the presence of PHA s. Qualitative confirmation is done by that	Acid hydrolyzed polyester reacted with chloroform then the colour changed proved the presence of polymer	(Muneer et al. 2022)
HPLC (high performance liquid chromatography)	P(3HB) accumulated cells reacted with hot sulfuric acid converted into crotonic acid	Detected at UV detector at 210 nm	–	(Samrot et al. 2021)

Extraction by detergent

To avoid harsh solvents for PHA extraction, detergents are used as environmentally friendly chemicals. The recovery rate depends upon the microbe's capability of production. Alkylbenzene sulfonic acid (LAS-99) and sodium dodecyl sulfate (SDS) were used with microbial cells for extraction at 60 °C. LAS-99 is known to give maximum purity of bioplastic as compared to the other detergents (Yang et al. 2011).

Extraction by enzymes

Another alternative to the solvent is enzymes. The enzyme extraction and ultra-filtration give a purity of 92.6% for medium chain length (mcl-PHAs) polymer (Kathiraser et al. 2007). However, enzyme cost could be a limiting factor.

Biological methods for extraction

The recovery of the polymer by the biological method is sustainable and eco-friendly. Mealworm digestion system works significantly to recover PHB. *Cupriviadus necatorH16* is common bacteria that can store PHB in the cell. These bacteria are consumed by many insects, and recovery of PHAs is done in their fecal material. Mealworms, small cockroaches, and crickets are some insects, eat bacterial cells, and excrete the polymer (Ong et al. 2018). Mealworms can give 97% pure PHB. Fecal matter is further washed with water and 1% sodium dodecyl sulfate to increase the purity (Murugan et al. 2016.).

Bacteriophage-mediated lysis: Intracellularly produced PHB is extracted by cell lysis method. This cell lysis occur in the cell by lytic phase infection. It is ecofriendly method of extraction. *Pseudomonas putida* BXHL strain is bioengineered for main two property: First genetically programmed apoptosis, it extracts out the PHB which is regulated by the exogenous protein holin. Secondly, it is cheap and harmless for the environment as compared to the detergent or chemical recovery (Haddadi et al. 2019).

Predatory system: *Bdellovibrio bacteriovorus* is a bacterium which act as a predator/ectoparasite for the gram-negative and gram-positive bacteria for essential biomolecules so that it is used in isolation of PHB. However, it has PHA depolymerase activity which hamper the maximum recovery of PHB. Engineered *B. bacteriovorus* (Bd3709) used for extraction in which PHA depolymerase is not encoded. The extraction by recombinant stain is higher than the wild stain, and extraction formula is in Eq. 1. Martínez et al. 2016; Haddadi et al. 2019; Vu et al. 2021).

PHA extraction formula:

$$\text{PHA}\ (\%) = \frac{\text{PHAs weight}}{\text{Cell dry weight}} \times 100 \quad (1)$$

Microbial conversion of the organic waste stream to PHA

Different organic waste streams can be utilized as feedstock in the industrial process of production of PHAs. (Table 2). This organic waste can be kitchen/industrial food, lignocellulosic, waste, and dairy waste. PHA is the most common byproduct which is formed by the microbes by consuming the waste materials (Povolo and Casella 2003; Silva et al. 2021; Argiz et al. 2021) as microbes store the PHA granules in their cytoplasm under stressed conditions and with a suitable amount of carbon source increase the amount of PHA in bacterial cells (Tyagi et al. 2021). In some cases, two different bacteria would be compatible with each other and increase the production of PHA, such as *Ralstonia eutropha* and *Bacillus subtilis*, which are compatible and produce poly(3-hydroxybutyrate-co-hydrovalerate) copolymer by use of sucrose as a carbon source (Bhatia et al. 2018). During the acid pretreatment of the substrate, toxic bioproducts are generated, and these chemicals could be microbial inhibitors and cause obstacles in PHA production. For the removal of these inhibitors, detoxification is performed to remove these toxic chemicals (Pradhan et al. 2017; Kovalcik et al. 2018). The double limitation condition can increase its production when imposed at the C/P ratio in the reactor (Korkakaki et al. 2017). Under the stressed condition like gas exchange limitation with the increase in carbon source, boost the PHB accumulation in the bacteria (Samantaray and Mallick 2015). Four different types of waste are converted into PHAs by different methods which are shown in Fig. (5). Different waste contains different composition. Cellulase, hemicellulose, lignin, and glycerol are main component of the organic waste. All these components are breakdown in the simpler form by the pretreatment, and then these are used by the enzymes for the PHA production (Table 3).

Production of PHA from kitchen waste

Food waste and kitchen waste are the daily wastes generated in households and food industries. The most common

Table 2 Waste converted into bioplastic by microbes with technical specifications

Type of waste	Microbes involved	Technical specification with economic details	PHA accumulation	References
Waste cooking oils	–	For optimized substrate biocatalyst hydration done. Polycondensation reaction in three stage melt method at 180 °C with addition of ester 2c (1.91 mmol, 600 mg) and catalyst dibutyltin oxide (0.011 mmol, 2.85 mg) with three vacuum argon cycle for 15 min with continuous flushing of argon. Effective method for polymers	Polycondensation is a very effective process for polymer production from the triglycerides	(Vasilakis et al. 2023)
Wood waste and sewage	Mixed microbial consortia	Wood hydrolysis-enzymatic hydrolysate used for fermentation, temp. 35 °C, chemical oxygen demand (COD) wsa about 6000 mg/L, 12 h cycle with 5 min influent, 680 min aerobic period, 30 min sedimentation and 5 min effluent with 300 rpm	This co fermentation of wood waste and sewage gives 50.3% PHA accumulation which can be performed on the industrial scale	(Li et al. 2020)
Whey	*Haloferax medoterranei*	Acidic hydrolysis with Fermenter, Batch. this microbe have no need of sterilized conditions because this culture require high salinity	53% PHA content 7.45 g/l of DCW PHBV formed	(Pais et al. 2016)
Sludge contain non-volatile fatty acid (non-VFAs) organic fraction	Mixed microbial culture used	Thermal hydrolyzed sludge (THS) fermentation used as substrate for the PHA production. Reducing the non VFAs solution by addition of volatile fatty acid is useful for the culture selection for PHA production by this strategies gives highest PHA accumulation microbes for fermented THS	Non VFAs with enriched nutrients used for fermentation process for production of PHA 61.4 wt % obtained	(Tu et al. 2020)
Waste activated sludge	*Bacillus aryabhattai*	Phase-separated pretreatment via mild sonication followed by thermos-Fenton disintegration. Cheap because it is done by shake flask method. High amount of accumulation occurs	93.1% was accumulated at optimum 42 h	(Banu et al. 2023)
Lignocellulosic waste (soybean hull)	*C.necator*	Alkali pretreatment (2% NaOH, at 121 °C for 1 h) non-commercial cellulase enzyme used. 20% cellulose conversion achieved. Non-commercial cocktail enzyme used which is cheap as compared to commercially used enzymes	39% of PHB with in 96 h	(Sarmiento-Vásquez et al. 2022)

Table 2 (continued)

Type of waste	Microbes involved	Technical specification with economic details	PHA accumulation	References
Corn waste & rice straw(Agro-waste) and crude vegetables oils	*Alcaligenes faecalis* RZS4 & *pseudomonas* spp. RZS1	Cost effective production of PHA by flask method	From corn-79.90% DCM from rice straw-66.22% of DCM PHB formed	(Sayyed et al. 2021)
Grape residue	*Bacillus cereus* ATCC-14,579	Pretreatment performed by diluted sulfuric acid and then hydrolysis done which give 52.9% (w/w) glucose. Fermentation by flask method. Substrate is very cheap	0.53 g/l PHB formed	(Andler et al. 2021)
Pineapple residue and sugarcane	*Bacillus* spp. SV13	PHA production in Batch fermentation. It can be done on the industrial scale	1.86 g/l PHA formed	(Suwannasing et al. 2015)
Untreated paper industry effluents	*Ancyclobacter* spp. ART_41	UUtilization of this effluent is cheap carbon source and there is no need of pretreatment for the PHA production	41.7% of PHA	(Tyagi and Sharma 2021)
Industrial waste (food processing industry)	*Bacillus thuringensis* & *Bacillus safensis*	Sedimentation tank used and it can perform economically at the industrial scale	*Bacillus thuringensis*-0.96 g/l PHB *Bacillus safensis*-0.330 g/l	(Giraldo-Montoya et al. 2020)
Cheese whey mother liquor (Dairy waste)	*Paracoccus homiensis*	Positive aspect of extremophiles in industrial biotechnology	3.3 g/L of cell dry mass scl-PHA with 1.1 g/L of P(3HB-co-3HV) copolymer (29% OF CDM) at 72 h	(Mozejko-Ciesielska et al. 2022)
Sewage sludge and vegetable waste	*C. necator*	Hydrodynamic cavitation pretreatment. It increase the volatile fatty acids. Hydrodynamic cavitation is a promising pretreatment for vegetable waste by which high amount of volatile fatty acid found	0.37 g/g PHAs formed	(Lanfranchi et al. 2022)
Waste paper to volatile fatty acids	*Cupriviadus necator*	Waste paper is converted into the VFAs and this is a cheap substrate for production of PHA with promising approach in which there is no need of pretreatment,hydrolysis and detoxification not required	56.98% of PHA & 0.31 g/g yield	(Al Battashi et al. 2021)
Molasses	*Parapedobacter* sp. ISTM3	There is pretreatment and hydrolysis not required and done by Shake flask method which is cheap	PHB 55.62% PHA as CDW	(Tyagi et al. 2021)
Kitchen waste	*C.necator* by shake flak method, organic acid from this waste incubated with bacteria at 30 °C, 200 rpm for 24 h	Fermentation by shake flask method, organic acid from this waste incubated with bacteria at 30 °C, 200 rpm for 24 h. Direct fermentation is done without pretreatment and hydrolysis. So that the cost of pretreatment and hydrolysis is removed from this	Accumulation of PHB high 84.54%w/w by fed-batch fermentation as compared to the batch fermentation	(Yee and hassan 2011)

Table 2 (continued)

Type of waste	Microbes involved	Technical specification with economic details	PHA accumulation	References
Rubber wood waste	Mixed microbial culture	Batch experimental reactor. Mixed microbial condition does not require sterilized conditions	0.31 g PHA/g CDW (cell dry weight)	(Li et al. 2022)
Waste cooking oil and waste fish oil	*Cupriavidus necator* H16	PHA production done by the batch fermentation and shake flask. These waste are inexpensive substrate for PHA production industrially	PHB content of from waste cooking oil are 76.9 wt % with productivity of 1.73 g/L/h with yield 0.92 g/g, by the waste fish oil the PHA content. 1.73 g/L/h an yield is 0.92 g/g	(Loan et al. 2022)

waste released from the kitchen and industries is cooked food, vegetables, meat, baked foods, and beverages (Tsang et al. 2019). Many value-added products that can be synthesized from food waste are bioethanol, enzymes, bioplastics, and biohydrogen with the help of the fermentation process (Ravindran and Jaiswal 2016). Bioplastic is an attractive molecule as an alternative plastic for the environment. Most bacterial cells produce PHAs by metabolizing food waste. In nutrient-deficient conditions, bacteria cannot reproduce but the size of bacterial cells increases rapidly because of the accumulation of PHAs (Albuquerque and Malafaia 2018; Jõgi and Bhat 2020). For the breakdown of complex substrate materials into a simpler form, pretreatment of feedstock is a necessary step to increase production. Different pretreatment technologies including biological, chemical, and physical generally are used for the accessibility of microbes (Chong et al. 2021). Various microbes are used in the fermentation process to convert the nutrient, mainly carbon source of food waste to PHA (Pan et al. 2021). Higher production of PHAs reported in the mixed microbial culture at 29.2–33 °C in the sequence batch reactor of the food waste (Valentino et al. 2020). During covid-19, biowaste in the form of personal protective equipment (PPE) kits was a big issue to solve, and researchers took initiative to make the PHBV from the kitchen waste. This PHBV is efficient to make PPE which is helpful to reduce the medical waste (Hathi et al. 2022).

Production of PHA from oils and fatty acids

For the production of PHAs from the oils, beta-oxidation process is performed in which triacylglycerol converts into fatty acid, and it further used by the microbes for the production of the bioplastic (Talan et al. 2020). Different types of oils such as coconut oil, date seed oil, olive oil, palm oil, and jatropha oil are used (Ganesh Saratale et al. 2021). Crude and emulsified oils contain fatty acids with different compositions, and these are utilized by the microbes for the production of valuable products including PHBV. Lauric, myristic, palmitic, stearic, oleic, linoleic are some common fatty acid found in the plant-based oil. Different surfactants are used for emulsification to cultivate the bacterial strain on these oils for the PHA production. (Ingram and Winterburn 2022). Both pure culture and recombinant microbes have been used to produce the PHA_S from oil waste. Different microbes including *Cupriavidus necator* H16, *Pseudomonas mendocina*, *H. mediterranei, P. putida* KT2440, and recombinant *Cupriavidus nectaor* H16 are used for PHAs accumulation (Ganesh Saratale et al. 2021; Pernicova et al. 2019). Native *Pseudomonas resnovorans* and recombinant *E.coli* accumulated medium chain length PHAs (more than 1.5 g/L) in waste food oils. Chilled methanol is used for precipitation (Vastano et al. 2019). Food waste oils are converted into the

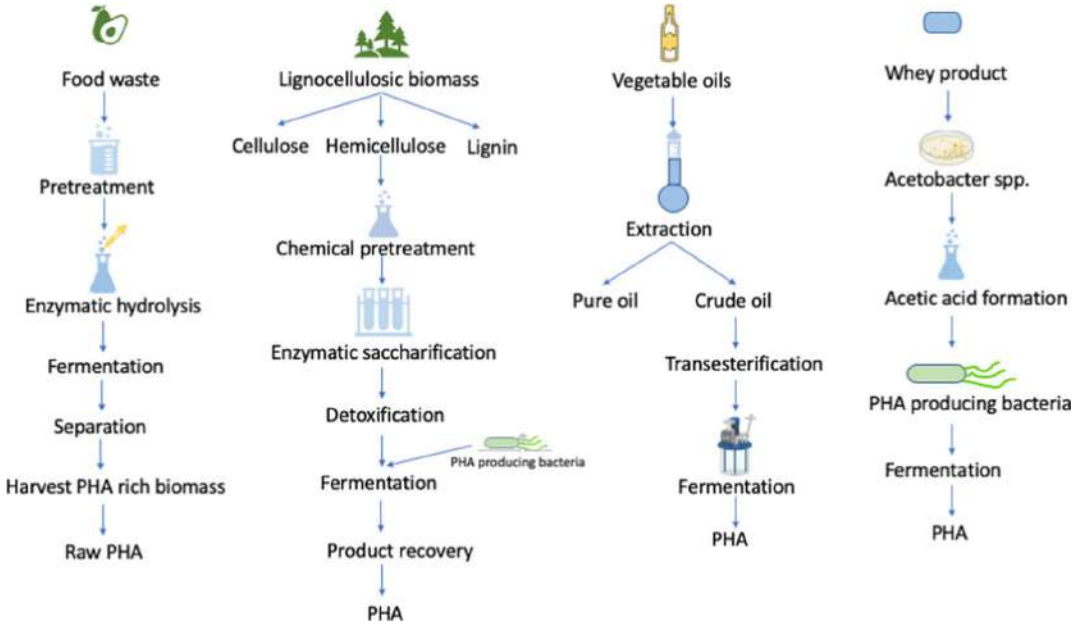

Fig. 5 Conversion of four different type of waste into PHA

Table 3 Composition of different organic waste

S.No	Waste	Composition	Product formed	References
1	Kitchen waste	Carbohydrates-141.64 g/kg wet weight	Biogas	(Hu et al. 2022)
2	Lignocellulosic waste	Cellulose, hemicellulose and lignin	114.16 mg/L mcl-PHA by use of lignin	(Sohn et al. 2022)
3	Lignocellulosic waste	Cellulose and hemicellulose rich waste	PHAs	(Sohn et al. 2022)
4	Oils waste	Fatty acids,oil parameters like acid value, saponification, ester value and free fatty acids	Biopolymer by *Cupriavidus necator* DSM545	(Ingram and Winterburn 2022)
5	Waste whey	Cheese whey mother liquor: total solid-18.44% Carbohydrates-13%, fat- < 0.05, lactose-12.6, cheese whey: total soild-13.15%, protein-2.46%, lactose-15.10%	Scl-PHAs formed by *Paracoccus homiensis*	(Mozejko-Ciesielska et al. 2022)
6	Apple industrial waste	Apple pomace: cellulose-47.49%, hemicellulose-27.77%, lignin-24.72% and pectin, Sugars: fructose-3.025%, glucose-0.76, sucrose-1.26%	*Pseudomaonas sp.* and *Cupriavidus necator* are used for PHA production	(Liu et al. 2021; Muneer et al. 2020)
7	Waste cooking oil (WCO) and waste fish oil (WFO)	WCO: linoleic acid-55%, oleic acid-22%,palmitic acid-12%, linolenic acid-8% and stearic acid-4% WFO: oleic acid-38.6%,palmitic acid-30.6%, linoleic acid-9%, stearic acid-8.2%, myristic acid-4.2% and palmitoleic acid-2.5%	*C.necator H16* bacteria used consume waste oil for production of PHB	(Loan et al. 2022)

PHAs by the fermentation process by *pseudomonas alcaligenes* with optimization of temperature and inoculum size (Pan et al. 2021).

PHA production from waste whey

Cheese whey is generated during the production of the cheese production process. Several bacteria such as *Bacillus spp*., *Streptomyces spp*., and *Pseudomonas spp* accumulate PHA_S, using waste whey stream (Bugnicourt et al. 2016; Zotta et al. 2020). Cheese whey is converted into a two-step process: Firstly, it is transformed into the acetic acid by *Acetobactor pasteurianus* C1, and then further acetic acid is metabolized into the PHAs by the *Bacillus sp.* (Chang et al. 2021). Under optimized production conditions, 11.5 folds of increased production of PHA_S by *Bacillus megaterium is* reported (Israni et al. 2020). *Bacillus flexusAzu-A2* strain isolated from the aquatic environment and used in conversion of cheese whey waste into 0.95 g/l PHA (Khattab et al. 2021). Mixed microbial culture is another technique by which PHAs can be formed by providing a carbon source to these microbes. Cheese waste whey contains sugars that can be converted into the organic acid and further converted into the PHAs by MMCs (mixed microbial culture) (Asunis et al. 2021). Mixed microbial culture found popularity in last decade because there is no need of pure culture, processed substrate, and sterilized conditions which make overall process costly (Tu et al. 2019)**.** Other third-generation technology for PHA production is by used of CO_2 as feedstock. It is also called photoautotrophic conversion. It is beneficial to reduce the impact on the environment during downstream process as compared to the other production methods (Koch et al. 2022). Cyanobacteria with some archaeal genera accumulate CO_2 as carbon source for this purpose (Khatami et al. 2021).

Production of PHA from lignocellulosic biomass

Plants-based wastes such as agricultural residues, forestry waste, and waste streams from paper and pulp industries are categorized under lignocellulosic waste. Lignocellulosic biomass comprises cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%) (Sawant et al. 2016). Cellulose and hemicellulose derived PHAs are formed by lignocellulose biomass-derived sugars, and these sugars are utilized by the microbes by different pathways. (Sohn et al. 2022). All of the lignocellulosic components are very complex, and the breakdown of complex structures into simpler forms needs to be done for the accessibility of enzymes (Allegue et al. 2021). Pretreatment technology is used for the accessibility of enzymes, on the substrate for hydrolysis. Further, in the fermentation process sugars are converted to PHAs (Al-Battashi et al. 2019). For reducing the cost of production, many techniques have been applied, and hot water pretreatment is one of the commonly used pretreatment methods by which improvement in the saccharification process and yield of the PHAs increased (Yan et al. 2021a, b). The extracted sugar utilized by microbes and the inclusion body in the bacterial cell accumulates polymer. Other than this carbon source nutrition deficiency (nitrogen and oxygen) is also an important factor to achieve high PHA production. *Bacillus megaterium* Ti_3 produces 57.8% PHA from the corn husk (de Souza et al. 2020).

Bacillus subtilis is efficient microbe for conversion of agro-industrial waste (dairy effluent, paper mill effluent and the sugarcane molasses) into PHA production (Rathika et al. 2019; Hassan et al. 2019). Industrially production of PHBHHx by the waste is done by a China-based company Bluepha Co. Ltd. in which alternative carbon source (crop and kitchen waste) is used with recombinant *C. necator* bacteria (Alves et al., 2022).

Recombinant strains and their impact on PHA accumulation

Microbial strains are capable of the production of PHA by utilization of carbon sources. Production of PHA can be maximized by the addition of recombinant or genetically modified strains of bacteria (Table 4). Several recombinant strains are used for high PHA accumulation, such as recombinant *Ralstonia eutropha* H16 (Kahar et al. 2004). It can utilize xylose for PHB production (Kim et al. 2016). Recombinant *E.coli* (Balakrishna Pillai et al. 2018; Saranya and Shenbagarathai 2011), *Alcaligenes eutrophus* (Salamanca-Cardona et al. 2013)(Lee et al. 1998), p*seudomonas* strains (Huisman et al. 1992), and *Halomonas campaniensis* LS21 (Yue et al. 2014) impacted on the accumulation of PHA. By changing genes involved in the cell division pattern in *E.coli* JM109, it can divide in multiple fission rather than binary fission so that the accumulation of PHA rises in the bacterial cells (Wu et al. 2016a). phbCAB Operon from *Ralstonia eutropha* was introduced by genetic engineering in the *E. coli* JM109SG, which resulted in an increase in cell size and higher PHA accumulated in modified bacterial cells (Jiang et al. 2015). Recombinant *E.coli* strain (K24K) produces PHB which has a high glass transition temperature of approximately 20 °C than the naturally PHB producer bacterial cells (Nikel et al. 2006). Recombinant *Ralstonia eutropha* PHB_4 composed of $phaC_2$PsQKST produced scl-mcl-PHA with high no. of PHB (Shen et al. 2011). Recombinant *E.coli* produced mcl-PHA from the non-fatty acid carbon source with a new synthesis pathway, and the production of this polymer also rises 400 mg/l and 11.6% per DCW (Wang

et al. 2012). Ferulic acid is a phenolic compound present in the lignocellulosic material, and it is cross-linked with hemicellulose and lignin. Metabolically engineered *pseudomonas putida* converted it into the mcl-PHA. Genome editing in the *pseudomonas putida* is done with integrated CRISPR/Cas9n. The mutation in the genome increased the utilization of ferulic acid for mcl-PHA production (Zhou et al. 2020). Hence, recombinant bacteria can produce a high amount of PHA in the form of an inclusion body in the cytoplasm.

Applications of PHAs in biotechnological prospects

PHAs have advantageous properties in the biomedical, agricultural, and industrial fields. Polymers and copolymers could be favorable materials for bone tissue engineering (YJ et al. 2009) as PHA is thermoplastic, tensile in strength, and elastomeric in nature. Due to these specific properties, PHA is used in the medical implantation, construction of artificial organs (Abitha et al. 2020), implantation and regeneration (Jiang et al. 2021), vascular graft and tissue engineering (Wang and Chen 2019), advanced proteolytic detector polyHydroxyAlkanoates(AL-PHA) beads, which is bioplastic based protease biosensors and in drug delivery sectors (Kelwick et al. 2021).

Drug delivery metrics system

The efficiency of polymeric material as a matrix for drug delivery depends on the diffusion, dissolving ability, and degradation of the matrix in which the drug is embedded. To achieve the therapeutic demands, the carrier matrix must have the potential for the bioavailability of the drug and estimated release kinetics (Macha et al. 2019). PHAs are compatible with blood cells, and these polymers do not activate the hemostasis process in the blood cells but also activate the coagulation. PHB and PHBV are used in the biomedical process after the purification of these polymers. An injury in the human body can be classified based on chronic and acute wounds. For the cure of these injuries, a drug should be provided for the healing of wounds (Demidova-Rice et al. 2012). Drug delivery vehicles should be cheap, biocompatible, sustainable, rapid to synthesize, and non-toxic to the body. Synthesis of the agarose bioplastic with glycerol as plasticizers is used to create a drug delivery carrier (Awadhiya et al. 2017).PHB-carboxymethyl chitosan(CMCh) makes a complex and product forms as nanoparticles, which are further used for cancer treatment (Akbal et al. 2016). Poly(lactide-co-glycolide)-block-poly(ethylene glycol)-block-poly(lactide-co-glycolide)(PLGA-PEG-PLGA) copolymer prepared in situ which injected and able of carried high amount of drug sustainably released on tumor (Zhang et al. 2018). A copolymer such as poly (ethylene glycol) (PEG) and poly (3-hydroxyoctanotate-co-3-hydroxyhexonate) (PHOHHx) is self-assembled and form a micelle in hydrated media. This copolymer is promising for drug delivery (Babinot et al. 2012). Recently, a new research finding is reported about the treatment of protein aggregation disease (PAD). This disease can harm the nervous system. PHA-767491 inhibits cell checkpoint kinase CDC7/CDK9 and also degenerates the TDP-43 phosphorylation by which the neural disability is cured. It has also anti-inflammatory and antitumor activity (Chung et al. 2020). Poly(hydroxybutyrate-co-hydroxyhexonate) (PHBHHx) is hydrophobic, it encapsulates the insulin which is hydrophilic, and it makes insulin phospholipid complex encapsulated with the polymer (PHBHHx) nanoparticles (INS-PLC-NPs). This complex increased insulin release in diabetic conditions (Peng et al. 2012).

Tissue engineering and implantation

In biomedical advancement research, it is possible to get engineered tissue, drugs, implant organs, and artificial organs. For this purpose, ceramic and metallic implants are used but they are not much compatible. Synthetic/natural polymers are known to be efficient in tissue formation and implantation (Lutolf and Hubbell 2005; Malafaya et al. 2007). PHA copolymer PHBHHx contains hydroxybutyrate and hydroxyhexanoate which have a high capability of peripheral nerve regeneration (Evans et al. 1999). PHB, PHBV, P3HB4HB, and PHBHHx films are used in the preparation of scaffolds. After a spinal cord injury, neural treatment and axon repair are crucial for the normal functioning of the body. Here, implantation of alginate hydrogel with neonatal Schwann cells is done. (Novikov et al. 2002). Neural stem cells are capable of regeneration of the central nervous system and peripheral nervous system. Nanopolymer is used for repairing neural cell because of its biocompatible property and promote cell–cell adhesion, migration, and proliferation characteristics (Xu et al. 2010).

Different types of PHAs such as PHB, PHBV, and PHBHHx are tested for the scaffolding efficiency (Sadat-Shojai et al. 2016). 3D fabrication is most common technology for polymer scaffolding and for making the scaffolds. electrospinning, melt moldings, extrusion leaching, solvent caste leaching, paraffin template emulsion freeze-drying, freeze-drying, gas foaming, and 3D printing fabrication technologies are used (Yuan Li et al. 2012).

Table 4 Production of PHAs by genetically modified microbes

Microbial strain	Gene/genetic manipulation	Enhancement of PHA accumulation	Substrate	References
Cupriviadus necator H16	Variant V6C6	19% more than wild strain	Gluconate and glycerol	(González-Villanueva et al. 2019)
Recombinant *E.coli* JM 109 with (pCold1-pha CAB_{A-04}) & *E.coli* JM109 (pCold TF-pha CAB_{A-04})	Pha CAM promoter from *cupriviadus necator*	PHB content (%) increased 8 folds & productivity increased 16 folds	Glucose	(Boontip et al. 2021)
Engineered *E.coli*	Plasmids-pMCSH5 &p68orfZ in *E.coli* JM109	PHB content(%)4HB content enhanced 20% more in (P(3HB-co-4HB)	Glucose	(Li et al. 2010)
Recombinant *E.coli* JM109	Plasmid pGEM- pha CABCo of *Comamonas* sp.EB172	PHA content (%) 37.7% CDW (g/l) is 0.2 in shake flask and 0.3 in batch. High as compared to wild strain P(3HB-co-4HB)	Mixed organic acid	(Hassan and Osman 2012)
Recombinant *Ralstonia eutropha*	Harboring pJRDEE32d13 plasmid contain $phaC_{AC}$	Increase 10 g/l DCW, PHA yield 0.76 g/g	Soyabean oil	(Kahar et al. 2004)
Recombinant *E.coli* XL-1-blue WL3110 BL 21(DE3) *E.coli* XLdh	Plasmid p619C1437-pet540 pKA32Ci-mALeuBCD pKM22PanE	P(2HB-co-LA)	Glucose	(Kim et al. 2016)
E.coli XL1-Blue	–	–	Organic acids	(Eshtaya, Rahman, and Hassan 2013)
Metabolically engineered *E.coli* strain XL1 Blue strain	Pha C variant	High production of aromatic polyesters	Glucose	Yang et al. 2018)
Recombinant *Cupriavidus necator* DSM 545 JR11	*lipC* and *lipH* of *P. stutzeri* BT3 cloned in *C. necator*	PHA 65% of DCM	Slaughterhouse waste (lipids)	(Rodríguez et al. 2021)
Engineered Putida KT 2440	Inactivate tricarboxylate transport	mcl-PHA increase from 1.01 to 1.91 g/L2-fold increment	Waste vegetable oil	(Borrero-de Acuña et al. 2019)
Metabolic engineered Pseudomonas putida	PHA Depolymerase gene knocked out	mcl-PHA 20% and 100% increase in yield (g mcl-PHA/g cell dry weight)	lignin	(Salvachúa et al. 2020)
Engineered pathway in E.coli	Beta oxidation pathway	mcl-PHA contain even and odd numbered monomers	Glucose	(Zhuang and Qi 2019)
Modified gene expression in R. palustris CGA009	Phap1 phasin (phaP1) gene add in R. palustris	PHBV production increase from 0.41 g/L to 0.7 g/L	Lignocellulosic biomass	(Brown et al. 2022)

Artificial organ

PHAs have flexible and mechanical strong properties which make them potential molecules in the biomedical field. The most common form of PHA is PHB and other copolymers of PHB which have been used for the manufacture of artificial organs or tissues for treatment (He et al. 2017) repair patches (Narancic et al. 2020) cardiovascular patches (Chaudhuri et al. 2017), orthopedic pins (Singh et al. 2015), cartilage of arteries (Ye et al. 2009), nerve suture, bone tendon treatment, bone marrow regeneration (Wang et al. 2005), and wound dressing (Shishatskaya et al. 2016). Biopolymer-like poly(3-hydroxyoctanoate) P(3HO) is a form of mcl-PHA and from this polymer. Cardiac patches are manufactured which are compatible with the cardiomyocytes so they can be implanted without any deleterious effect on the body (Bagdadi et al. 2018). Poly(3-hydroxybutyrate) P(-3HB) and poly(3-hydroxyoctanoate) P(3-HO) are blended, and the mixture is used in the coronary stents to reduce the blockage of arteries (Basnett et al. 2013). Synthetic biomaterials mimic the natural extracellular matrices. The application of synthetic biomaterials in different therapeutic areas is as follows: bone repairing, neuron regeneration, and induction of angiogenesis (Malafaya et al. 2007).

PHA as antimicrobial agents

A re-establishment of the tissue or organ in the body, by implantation, is carried out by tissue engineering. The main problem arises that the bacterial biofilm surrounds the implanted tissue, and it can create a chronic infection in the body. For resolving this issue, some antimicrobial properties should be there in the implanted material. *Staphylococcus epidermidis* is a pathogen that can infect the tissue; for this amylin and despersin B, both are used for the formation of activated PHA to prevent microbial activity (Piarali et al. 2020). Fungal infection is also a more common infection, and its target includes mucosa, skin surface area, nails, and hairs (Katharina Kainz et al. 2020). *Candida* spp. can cause chronic infection in the human body, and it can resist antimicrobial drugs (Roilides et al. 2015). mcl-PHA is used to make the drugs in the form of biofilms. The properties and the structure of the mcl-PHA are capable of resisting the growth of the various fungus (*C.albicans, C. parapsilosis, Trichophyton mentagrophytes, Microsporum gypseum,* and *A. fumigatus*) (Pekmezovic et al. 2021). PHB, PHBV, and PHB4HB are used for the coating of tantalum (Ta). It is a bio-metal that is used for implantation after bone fracture. The coating by PHB4HB gives the best results in comparison with the other two forms of PHAs, against *Staphylococcus aureus* and *E. coli* bacteria (Rodríguez-Contreras et al. 2019). Albumin and wheat-based protein are used for making the bioplastic with the addition of oregano essential oil to introduce antimicrobial properties in the bioplastic (Martínez et al. 2013). The bacterial PHA is a second-generation polymer that can be implanted in the human body because of its antibacterial properties. It has thioester groups on the side chain that's why it has this unique property. It shows antimicrobial activity against *S. aureus* (Dinjaski et al. 2014).

Packaging for industrial applications

The application of PHA in the packaging industry is beneficial as it is, biodegradable, renewable, and non-toxic in comparison with petroleum-based packaging material. PHA is hydrophobic so it can be used for coating. To increase the utilization of PHA in the food packing industries, some modifications are necessary. In biopolymers like PHBV, clay loading improved the gas and vapor barrier and UV blockage for packing material (Sanchez-Garcia and Lagaron 2010). PHA is highly fragile, to increase the mechanical strength of PHB, and it is blended with the PCL (polycaprolactone). PHB-PCL blended bioplastic becomes flexible and ductile and improves the degradation temperature which can be used for packaging (Garcia-Garcia et al. 2016). PHA is used in the manufacturing of bottles, films, containers, laminates, and sheets. PHB and its copolymers have tensile strength, oil resistance capability, temperature stability, and are easy to dye (Mangaraj et al. 2018). PHA produced from the peanut oil as a carbon source increased elongation and permeability which makes it suitable for the packaging material (Pérez-Arauz et al. 2019).

Agricultural applications

Bioplastic plays an important role in the agricultural field, and different products used in the agricultural field are packed in the bioplastic envelopes. Bioplastics are used in the mulching of the field which persists the land wet and avoids the land to direct sunlight which increases the earthworm number in the agricultural field and improves the field quality. Polylactic acid (PLA) and PLA blended with PHA provide mulching (Hablot et al. 2014). Sprayable bioplastic can control the microbial activity in crops. This bioplastic forms a film on the seeds of crops (Accinelli et al. 2016). The bioplastic coating is done by mixing the insecticide (imidacloprid) and fungicide (pyraclostrobin) (Accinelli et al. 2018).

Application for the construction industry

Bioplastic is highly valuable in the construction industry to reduce non-biodegradable plastic waste. Some bioplastic

products such as bioplastic foams, construction slits, and dust fences are generated. However, there is an issue with the strength of the material, so the blending overcomes this problem. PLA is blended with polytetrafluoroethylene (PTFE) and PHA to increase the strength of foam (Lee et al. 2020). Apart from this, PHBV foam is modified by the addition of nanocellulose (Nc) to increase the cell density and porosity of the foams. This foam works as an insulator in the walls of the building construction (Panaitescu et al. 2020). Bioplastic is biodegradable, so it would not be of any use in the construction industry in long run. The products used during the construction made of bioplastic reduce the area for disposal of construction waste which is environmentally friendly. Bioplastic sheets, foams, liners, and cement bioplastic bags can be left in the soil for degradation (Ikada and Tsuji 2000).

Current scenario and future aspects for sustainability

PHA is better alternative than other conventional plastic in many aspects due to its biodegradable property which is considered to solve the main problem of waste management. It is biocompatible in nature by which it can be used in the medical field like in tissue engineering, drug delivery, drug coating, for making the artificial organ and implantation; on the other hand, conventional plastic is toxic in nature which is not suitable in the medical field. It is sustainable in environment when mixed in agricultural fields for mulching because of its biodegradability. Poly (lactic acid) is another biodegradable plastic, and it has lesser biodegradability than PHA, so that PHA is better in terms of biodegradability than PLA (Othman et al. 2022). PLA has high permeability for moisture and oxygen so that it cannot be used in the food packaging material because it can spoil the food material, on the other hand PHA is a good option for packaging material. PHA has high glass transition temperature as compared to the PLA by which PHA is better than the PLA in use of high-temperature applications. PHA is applied in the implantation on the other hand polybutylene succinate (PBS) which is also an alternative of PHA and has limited applications in this area. PHA is better than PLA in the mulching properties because PLA increases the acid concentration in the soil which is not beneficial for the agriculture.

Merits of PHAs

- Biodegradable property of PHA makes it sustainable to the environment. The degradation of the polymer is performed in the water, soil, and sewage where microorganisms have capability to degrade it into the methane (Basnett et al. 2017). The enzyme PHA depolymerase, excreted by the fungi and bacteria, degrades PHA extracellularly. Petroleum-based plastic takes many years for degradation so it is considered a good alternative to traditional plastic. (Kaniuk and Stachewicz 2021).
- Biocompatibility property makes it suitable in the medical field; on the other hand, conventional plastic is harmful (Lim et al. 2017).
- Bioplastic is considered to be a beneficial option compared to the conventional plastic used for food packaging under the safety guidelines (Zhang et al. 2022).
- It is a sustainable material because it does not require non-renewable material for its production and provide equilibrium in natural resources and environment compared to the conventional plastic which is produced by nonrenewable sources (Mohapatra et al. 2021).
- It enhances agricultural productivity by mulching process and does not affect the ecosystem as it is eco-friendly material compared conventional used plastic (Mohapatra et al. 2021).
- It is non immunogenic and non-carcinogenic in nature by which it can be used as nanocarrier in drug delivery because of nontoxic nature as compared to the conventional plastic. (Prakash et al. 2022)

Limitations

- At present scenario, PHA gains popularity because of its biodegradable and biocompatible nature; however, a high production cost is a major limitation (Sharma et al. 2021). Also, for industrially scale production an establishment of process is required. Processes like pretreatment and sugar extraction methods need to be optimized with individual feedstock/substrate. (Sharma et al. 2021).
- PHA create obstacles in their application part of drug delivery. PHA acts as carrier in the drug delivery but it hamper the solubility of drug because it is hydrophobic in nature. To overcome this problem, PHB copolymer is blended with polyethylene glycol (PEG) by transesterification process. Then it will become suitable as carrier (Prakash et al. 2022).
- PHB has poor thermal stability so that it is a challenge to use in the medical field. Copolymerization is best way to resolve this. PHB is copolymerized with 3-hydroxyvalerate (3HV) increasing the thermal stability of polymer which is further used in drug delivery (Prakash et al. 2022).
- In the agriculture field mulching is essential. For mulching, PHA does not contain high biodegradability. So that for increase the biodegradability PHA and polycaprolac-

tone (PCL) are used as mulch in the dual layered film by the process of hot press method (Othman et al. 2022).

- In order to produce a pure forms of bioplastic various chemicals are used at downstream processing. These chemicals are not eco-friendly. However, alternatives of these chemical during extraction process and this limitation can be overcome.
- Landfills of this bioplastic release methane emission which cause negative impact on environment, and this problem can be resolved by chemical recycling like pyrolysis and solvolysis into alternative feedstock. (Fredi et al. 2021).

Economical aspects for PHA production

PHA from the second-generation biowaste is economically and environmentally performed better as compared to other polymers and PHA from the first generation (Andreasi Bassi et al. 2021). Crude glycerol is the main carbon source for *C. necator* for the optimal production of PHA economically and environmentally (Leong et al. 2016). The economic improvement during the PHA production depends upon the raw material from which PHA is produced. The carbon source should be cheap and easily available. The unwanted waste streams are a potential source for the production of bioplastic, which is economically and environmentally sustainable (Leong et al. 2016). Biogas valorization from waste treatment plant produces the PHA efficiently. The PHA-producing bacteria use the methane from biogas and accumulate PHA (Pérez et al. 2020). In the present scenario, the main focus goes on the production of bioplastic with minimum cost. Several industries are involved in the production of bioplastic by using low-cost carbon sources (Table 5). Biogas is used as a cheap source for PHA in the waste treatment plants by which its production cost will be reduced (Pérez et al. 2020). Mixed microbial culture is an advantageous platform to produce PHA in the wastewater treatment plant (de Souza Reis et al. 2020). The market of PHA is calculated as USD 62 million in 2020 but in the projection, it is estimated that it will be USD 121 million by 2025 (*Polyhydroxyalkanoate (PHA) Market Global Forecast to 2025 | MarketsandMarkets*) (Palmeiro-Sánchez et al. 2022). There is a requirement for PHA production with economic and environmental suitability.

Challenges in production of PHA and possible overcome strategies

PHA production from food crops such as sugarcane or corn is not a sustainable process. The replacement of waste stream as substrate for microbial PHA accumulation would be sustainable, economical, non-competitive to food crops. During the production of PHA at industrial scale, there are many challenges which resist scaling up of the production. The utilization of expensive carbon source, low substrate conversion for industrialization, costly extraction step for production (Khatami et al. 2021) are some of the challenges need to be addressed.

The production cost could be reduced by replacement of synthetic sugars by cost-effective ceap substrates. For industrial process, the establishment of process is to be done adjacent to the sources of organic waste or wastewater treatment plant, by which investment on the transportation reduces and it works in the field of zero waste biorefinery. Conversion of substrate to PHA can be increased by metabolic engineering and synthetic biology. Extraction by biological method is efficient and non-hazardous to environment. It maintains a contamination-free environment. Hybrid biological system such as mixotrophic cultivation could increase the PHA yield by providing the substrate to autotrophic and heterotrophic

Table 5 Global industries for microbes mediated PHA producers

Industry	Country and year of establishment	Substrate	Polymer	Production	References
Dupont	–	Corn	Fiber plastic	–	(Iles and Martin 2013)
Banimer scientific	USA	Canola oil	Bioplastic	13,600 tonnes/year	(Dietrich et al. 2017)
bruksem	–	Sugarcane	polyethylene	–	(Iles and Martin 2013)
Bio-on	Italy since 2017	Sugar beet and sugarcane	PHB, PHBV	> 10,000 tonnes/year	(Vandi et al. 2018)
Metabolix	USA since 2002	Corn sugar	PHA	55 Kilo tonne/year	(Rosenboom, Langer, and Traverso 2022)
Nature works	USA	Plant resources	PLA	70Kt/year	(Rosenboom, Langer, and Traverso 2022)
Chemi linz	Austria	Carbohydrates	PHB	< 50 tonne/year	(Koller et al. 2017)
Polyferm	Canada since 2013	Vegetable oil and sugar	mcl PHA	–	(Tan et al. 2014)
Tianan biologic	China since 2000	Corn sugar	PHBV	2000 tonnes/year	(Pakalapati et al. 2018)

bacteria like cyanobacteria. It synthesizes PHA during day time by use of CO_2 as a substrate, and during night time, heterotrophic condition follows because of sodium and phosphorous limitation in night (Afreen et al. 2021). Other than production part, more research required toward the enhancement of mechanical strength of poly (3-hydroxybutyric acid) (PHB) with use of natural cross-linker incorporation in the blending of bioplastic.

For commercialization of PHA production, one of the main challenges exist is expensive infrastructure, high energy requirement which elevates the cost of the process. Hence improved and economical infrastructure is needed to establish for industrial process. In conversion pathways, different thermal and chemical process could be replace by mild conditions and low chemical concentration. These process could be optimized for processing time, temperature, and concentrations of chemicals. These technologies are environmental friendly and reduce the overall process cost. Several modeling and prediction tools such as artificial intelligence (AI) and artificial neural network (ANN) are also applied for an efficient practices for the thermal and biochemical conversion pathways. The socioeconomical aspect could be evaluated by life cycle assessment (LCA) for industrial production of PHA.

Conclusion

Polyhydroxyalkanoates are potential replacement of conventional plastic due to its durability as well as biodegradation property. The waste stream is composed of significant quantity of carbohydrate, which could be uitilzed for economical bacterial PHA acculation. Depending upon the waste stream type, different conversion technologies and pathways are important for high yield of polymers accumolation. It is still challenging to produce economical PHA in industrial scale by using waste stream. To overcome this, bioconversion of cheap waste streams, utilization of less toxic chemicals, optimization of pretreatment and extration processes, to explore high production yield microorganisms and establishment of mathamatical tools including AI, ML, and LCA, needed to be explore for industrial scale setup. These natural polymers have wide range of applications in medical, agriculture, industries sectors because of its biocompatibility and non-toxicity. Hence, bioplastics from waste streams give a sustainable opportunity for waste management and maintain a clean environment.

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References

Abitha H, Kavitha V, Gomathi B, Ramachandran B (2020) A recent investigation on shape memory alloys and polymers based materials on bio artificial implants-hip and knee joint. Mater Today: Proc 33:4458–4466. https://doi.org/10.1016/J.MATPR.2020.07.711

Accinelli C, Abbas HK, Little NS, Kotowicz JK, Mencarelli M, Shier WT (2016) A liquid bioplastic formulation for film coating of agronomic seeds. Crop Prot 89:123–128. https://doi.org/10.1016/J.CROPRO.2016.07.010

Accinelli C, Abbas HK, Shier WT (2018) A bioplastic-based seed coating improves seedling growth and reduces production of coated seed dust. J Crop Improv 32(3):318–330. https://doi.org/10.1080/15427528.2018.1425792

Afreen R, Tyagi S, Singh GP, Singh M (2021) Challenges and perspectives of polyhydroxyalkanoate production from microalgae/cyanobacteria and bacteria as microbial factories: an assessment of hybrid biological system. Front Bioeng Biotechnol. https://doi.org/10.3389/FBIOE.2021.624885/FULL

Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB (2011) Biomass pretreatment: fundamentals toward application. Biotechnol Adv 29(6):675–685. https://doi.org/10.1016/J.BIOTECHADV.2011.05.005

Ahmed F, Yan Z, Bao J (2019) Dry biodetoxification of acid pretreated wheat straw for cellulosic ethanol fermentation. Bioresour Bioprocess. https://doi.org/10.1186/s40643-019-0260-x

Akbal Ö, Erdal E, Vural T, Kavaz D, Denkbaş EB (2016) Comparison of protein-and polysaccharide-based nanoparticles for cancer therapy: synthesis, characterization, drug release, and interaction with a breast cancer cell line. Artif Cells, Nanomed, Biotechnol 45(2):193–203. https://doi.org/10.3109/21691401.2016.1170694

Al-Battashi HS, Annamalai N, Sivakumar N, Al-Bahry S, Tripathi BN, Nguyen QD, Gupta VK (2019) Lignocellulosic biomass (LCB): a potential alternative biorefinery feedstock for polyhydroxyalkanoates production. Rev Environ Sci Biotechnol 18(1):183–205. https://doi.org/10.1007/S11157-018-09488-4

Al Battashi H, Al-Kindi S, Gupta VK, Sivakumar N (2021) Polyhydroxyalkanoate (PHA) production using volatile fatty acids derived from the anaerobic digestion of waste paper. J Polym Environ 29:250–259. https://doi.org/10.1007/s10924-020-01870-0

Albuquerque PBS, Malafaia CB (2018) Perspectives on the production, structural characteristics and potential applications of bioplastics

derived from polyhydroxyalkanoates. Int J Biol Macromol 107:615–625. https://doi.org/10.1016/J.IJBIOMAC.2017.09.026

Allegue LD, Ventura M, Melero JA, Puyol D (2021) Integrated sustainable process for polyhydroxyalkanoates production from lignocellulosic waste by purple phototrophic bacteria. GCB Bioenergy 13(5):862–875. https://doi.org/10.1111/GCBB.12807

Almeida da Silva C, Nunes dos Santos R, Gabriel Oliveira G, de Souza P, Ferreira T, Dias LG, de Souza N, Souza Soares A, Ferreira de Melo J, Jovania Gomes C, de José Borges Souza U, de Neponuceno AraújoFilho R, de Wagner Souza Aguiar R, Rodrigues dos SantosEvgeniev Gabev GE, Souza Campos F (2022) Biodiesel and bioplastic production from waste-cooking-oil transesterification: an environmentally friendly approach. Energies. https://doi.org/10.3390/en15031073

Alves AA, Siqueira EC, Barros MPS, Silva PEC, Houllou LM (2022) Polyhydroxyalkanoates: a review of microbial production and technology application. Int J Environ Sci Technol. https://doi.org/10.1007/S13762-022-04213-9/TABLES/4

Ampese LC, Sganzerla WG, Di Domenico Ziero H, Mudhoo A, Martins G, Forster-Carneiro T (2022) Research progress, trends, and updates on anaerobic digestion technology: a bibliometric analysis. J Clean Prod 331:130004. https://doi.org/10.1016/J.JCLEPRO.2021.130004

Andler R, Pino V, Moya F, Soto E, Valdés C, Andreeßen C (2021) Synthesis of poly-3-hydroxybutyrate (PHB) by *Bacillus cereus* using grape residues as sole carbon source. Int J Biobased Plast 3(1):98–111. https://doi.org/10.1080/24759651.2021.1882049

Andreasi Bassi S, Boldrin A, Frenna G, Astrup TF (2021) An environmental and economic assessment of bioplastic from urban biowaste. The example of polyhydroxyalkanoate. Bioresour Technol 327:124813. https://doi.org/10.1016/J.BIORTECH.2021.124813

Anis SNS, Nurhezreen MI, Sudesh K, Amirul AA (2012) Enhanced recovery and purification of P(3HB-co-3HHx) from recombinant cupriavidus necator using alkaline digestion method. Appl Biochem Biotechnol 167(3):524–535. https://doi.org/10.1007/S12010-012-9677-9/FIGURES/9

Anna Mabazza KA, Requiso PJ, Alfafara CG, Rey Nayve FP, Ventura JRS (2020) Steam Explosion and Sequential Steam Explosion-Dilute Acid Pretreatment Optimization of Banana Pseudostem for Polyhydroxybutyrate (PHB) Production. Philipp J Sci 149(2):285–297

Argiz L, Gonzalez-Cabaleiro R, Correa-Galeote D, Val del Rio A, Mosquera-Corral A (2021) Open-culture biotechnological process for triacylglycerides and polyhydroxyalkanoates recovery from industrial waste fish oil under saline conditions. Sep Purif Technol 270:118805. https://doi.org/10.1016/J.SEPPUR.2021.118805

Asunis F, De Gioannis G, Francini G, Lombardi L, Muntoni A, Polettini A, Pomi R, Rossi A, Spiga D (2021) Environmental life cycle assessment of polyhydroxyalkanoates production from cheese whey. Waste Manag 132:31–43. https://doi.org/10.1016/J.WASMAN.2021.07.010

Awadhiya A, Tyeb S, Rathore K, Verma V (2017) Agarose bioplastic-based drug delivery system for surgical and wound dressings. Eng Life Sci 17(2):204–214. https://doi.org/10.1002/ELSC.201500116

Babinot J, Guigner JM, Renard E, Langlois V (2012) A micellization study of medium chain length poly(3-hydroxyalkanoate)-based amphiphilic diblock copolymers. J Colloid Interface Sci 375(1):88–93. https://doi.org/10.1016/J.JCIS.2012.02.042

Bagdadi AV, Safari M, Dubey P, Basnett P, Sofokleous P, Humphrey E, Locke I, Edirisinghe M, Terracciano C, Boccaccini AR, Knowles JC, Harding SE, Roy I (2018) Poly(3-hydroxyoctanoate), a promising new material for cardiac tissue engineering. J Tissue Eng Regen Med 12(1):e495–e512. https://doi.org/10.1002/TERM.2318

Balakrishna Pillai A, Jaya Kumar A, Kumarapillai H (2018) Enhanced production of poly(3-hydroxybutyrate) in recombinant *Escherichia coli* and EDTA–microwave-assisted cell lysis for polymer recovery. AMB Express 8(1):1–15. https://doi.org/10.1186/S13568-018-0672-6

Banu JR, Gunasekaran M (2023) Augmentation in polyhydroxybutyrate and biogas production from waste activated sludge through mild sonication induced thermo-fenton disintegration. Bioresour Technol 369:128376. https://doi.org/10.1016/j.biortech.2022.128376

Basnett P, Ching KY, Stolz M, Knowles JC, Boccaccini AR, Smith C, Locke IC, Keshavarz T, Roy I (2013) Novel Poly(3-hydroxyoctanoate)/Poly(3-hydroxybutyrate) blends for medical applications. React Funct Polym 73(10):1340–1348. https://doi.org/10.1016/J.REACTFUNCTPOLYM.2013.03.019

Basnett P, Ravi S, Roy I (2017) Natural bacterial biodegradable medical polymers: polyhydroxyalkanoates. Science and principles of biodegradable and bioresorbable medical polymers: materials and properties. Woodhead Publishing, Cambridge, pp 257–277. https://doi.org/10.1016/B978-0-08-100372-5.00008-8

Belkhanchi H, Rouan M, Hammi M, Ziat Y, Chigr M (2021) Synthesis of biodiesel by transesterification of used frying oils (UFO) through basic homogeneous catalysts (NaOH and KOH). Biointerface Res Appl Chem 11(5):12858–12868. https://doi.org/10.33263/BRIAC115.1285812868

Bhatia SK, Yoon JJ, Kim HJ, Hong JW, Gi Hong Y, Song HS, Moon YM, Jeon JM, Kim YG, Yang YH (2018) Engineering of artificial microbial consortia of *Ralstonia eutropha* and *Bacillus subtilis* for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer production from sugarcane sugar without precursor feeding. Biores Technol 257:92–101. https://doi.org/10.1016/J.BIORTECH.2018.02.056

Boontip T, Waditee-Sirisattha R, Honda K, Napathorn SC (2021) Strategies for poly (3-hydroxybutyrate) production using a cold-shock promoter in *Escherichia coli*. Front Bioeng Biotechnol 9:666036. https://doi.org/10.3389/fbioe.2021.666036

Borrero-de Acuña JM, Aravena-Carrasco C, Gutierrez-Urrutia I, Duchens D, Poblete-Castro I (2019) Enhanced synthesis of medium-chain-length poly (3-hydroxyalkanoates) by inactivating the tricarboxylate transport system of Pseudomonas putida KT2440 and process development using waste vegetable oil. Process Biochem 77:23–30. https://doi.org/10.1016/j.procbio.2018.10.012

Brown B, Immethun C, Alsiyabi A, Long D, Wilkins M, Saha R (2022) Heterologous phasin expression in Rhodopseudomonas palustris CGA009 for bioplastic production from lignocellulosic biomass. Metab Eng Commun 14:e00191. https://doi.org/10.1016/j.mec.2021.e00191

Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V (2016) Polyhydroxyalkanoate (PHA): review of synthesis, characteristics, processing and potential applications in packaging. Express Polym Lett 8(11):791–808. https://doi.org/10.3144/EXPRESSPOLYMLETT.2014.82

Burniol-Figols A, Skiadas IV, Daugaard AE, Gavala HN (2020) Polyhydroxyalkanoate (PHA) purification through dilute aqueous ammonia digestion at elevated temperatures. J Chem Technol Biotechnol 95(5):1519–1532. https://doi.org/10.1002/JCTB.6345

Cavalaglio G, Gelosia M, Giannoni T, Temporim BRL, Nicolini A, Cotana F, Bertini A (2021) Acid-catalyzed steam explosion for high enzymatic saccharification and low inhibitor release from lignocellulosic cardoon stalks. Biochem Eng J 174:108121. https://doi.org/10.1016/J.BEJ.2021.108121

Cesaro A, Cieri V, Belgiorno V (2021) Press-extrusion pretreatment of the organic fraction of municipal solid waste for enhanced

methane production. J Mater Cycles Waste Manage 23(1):130–138. https://doi.org/10.1007/S10163-020-01105-3/FIGURES/6

Chang Y-C, Reddy MV, Imura K, Onodera R, Kamada N, Sano Y (2021) Two-stage polyhydroxyalkanoates (pha) production from cheese whey using acetobacter pasteurianus C1 and *Bacillus* sp. CYR1. Bioengineering 8(11):157. https://doi.org/10.3390/BIOENGINEERING8110157

Chaudhuri R, Ramachandran M, Moharil P, Harumalani M, Jaiswal AK (2017) Biomaterials and cells for cardiac tissue engineering: current choices. Mater Sci Eng, C 79:950–957. https://doi.org/10.1016/J.MSEC.2017.05.121

Chen GQ, Jiang XR (2017) Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis. Synth Syst Biotechnol 2(3):192–197. https://doi.org/10.1016/J.SYNBIO.2017.09.001

Chen GQ, Hajnal I, Wu H, Lv L, Ye J (2015) Engineering biosynthesis mechanisms for diversifying polyhydroxyalkanoates. Trends Biotechnol 33(10):565–574. https://doi.org/10.1016/J.TIBTECH.2015.07.007

Chen GQ, Jiang XR, Guo Y (2016) Synthetic biology of microbes synthesizing polyhydroxyalkanoates (PHA). Synth Syst Biotechnol 1(4):236–242. https://doi.org/10.1016/J.SYNBIO.2016.09.006

Chen GQ, Chen XY, Wu FQ, Chen JC (2020) Polyhydroxyalkanoates (PHA) toward cost competitiveness and functionality. Adv Ind Eng Polym Res 3(1):1–7. https://doi.org/10.1016/J.AIEPR.2019.11.001

Chong JWR, Yew GY, Khoo KS, Ho SH, Show PL (2021) Recent advances on food waste pretreatment technology via microalgae for source of polyhydroxyalkanoates. J Environ Manag 293:112782. https://doi.org/10.1016/J.JENVMAN.2021.112782

Chung Y-H, Lin C-W, Huang H-Y, Chen S-L, Huang H-J, Sun Y-C, Lee G-C, Lee-Chen G-J, Chang Y-C, Hsieh-Li HM (2020) Targeting inflammation, PHA-767491 shows a broad spectrum in protein aggregation diseases. J Mol Neurosci 70(7):1140–1152. https://doi.org/10.1007/S12031-020-01521-Y

Ciesielski S, Mozejko J, Pisutpaisal N (2015) Plant oils as promising substrates for polyhydroxyalkanoates production. J Clean Prod 106:408–421. https://doi.org/10.1016/J.JCLEPRO.2014.09.040

Colombo B, Favini F, Scaglia B, Sciarria TP, D'Imporzano G, Pognani M, Alekseeva A, Eisele G, Cosentino C, Adani F (2017) Enhanced polyhydroxyalkanoate (PHA) production from the organic fraction of municipal solid waste by using mixed microbial culture. Biotechnol Biofuels. https://doi.org/10.1186/S13068-017-0888-8

Das N, Jena PK, Padhi D, Kumar Mohanty M, Sahoo G (2021) A comprehensive review of characterization, pretreatment and its applications on different lignocellulosic biomass for bioethanol production. Biomass Convers Biorefinery 2021:1–25. https://doi.org/10.1007/S13399-021-01294-3

de Souza L, Manasa Y, Shivakumar S (2020) Bioconversion of lignocellulosic substrates for the production of polyhydroxyalkanoates. Biocatal Agric Biotechnol 28:101754. https://doi.org/10.1016/J.BCAB.2020.101754

de Souza Reis GA, Michels MHA, Fajardo GL, Lamot I, de Best JH (2020) Optimization of green extraction and purification of pha produced by mixed microbial cultures from sludge. Water 12(4):1185. https://doi.org/10.3390/W12041185

Demidova-Rice TN, Hamblin MR, Herman IM (2012) Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. Adv Skin Wound Care 25(7):304. https://doi.org/10.1097/01.ASW.0000416006.55218.D0

Dietrich K, Dumont MJ, Del Rio LF, Orsat V (2017) Producing PHAs in the bioeconomy—towards a sustainable bioplastic. Sustain Prod Consum 9:58–70. https://doi.org/10.1016/j.spc.2016.09.001

Dinjaski N, Fernández-Gutiérrez M, Selvam S, Parra-Ruiz FJ, Lehman SM, Román JS, García E, García JL, García AJ, Prieto MA (2014) PHACOS, a functionalized bacterial polyester with bactericidal activity against methicillin-resistant *Staphylococcus aureus*. Biomaterials 35(1):14. https://doi.org/10.1016/J.BIOMATERIALS.2013.09.059

Duque A, Manzanares P, Ballesteros M (2017) Extrusion as a pretreatment for lignocellulosic biomass: fundamentals and applications. Renew Energy 114:1427–1441. https://doi.org/10.1016/J.RENENE.2017.06.050

Eshtaya MK, NorAini AR, Hassan MA (2013) Bioconversion of restaurant waste into Polyhydroxybutyrate (PHB) by recombinant *E. coli* through anaerobic digestion. Int J Environ Waste Manage 11(1):27–37. https://doi.org/10.1504/IJEWM.2013.050521

Evans GRD, Brandt K, Widmer MS, Lu L, Meszlenyi RK, Gupta PK, Mikos AG, Hodges J, Williams J, Gürlek A, Nabawi A, Lohman R, Patrick CW (1999) In vivo evaluation of poly(l-lactic acid) porous conduits for peripheral nerve regeneration. Biomaterials 20(12):1109–1115. https://doi.org/10.1016/S0142-9612(99)00010-1

Fredi G, Dorigato A (2021) Recycling of bioplastic waste: a review. Elsevier. Retrieved Apr 26 2023. From https://www.sciencedirect.com/science/article/pii/S2542504821000373

Ganesh Saratale R, Cho SK, Dattatraya Saratale G, Kadam AA, Ghodake GS, Kumar M, Naresh Bharagava R, Kumar G, Su Kim D, Mulla SI, Seung Shin H (2021) A comprehensive overview and recent advances on polyhydroxyalkanoates (PHA) production using various organic waste streams. Bioresour Technol 325:124685. https://doi.org/10.1016/J.BIORTECH.2021.124685

Garcia-Garcia D, Ferri JM, Boronat T, Lopez-Martinez J, Balart R (2016) Processing and characterization of binary poly(hydroxybutyrate) (PHB) and poly(caprolactone) (PCL) blends with improved impact properties. Polym Bull 73(12):3333–3350. https://doi.org/10.1007/S00289-016-1659-6

Giraldo-Montoya JM, Castaño-Villa GJ, Rivera-Páez FA (2020) Bacteria from industrial waste: potential producers of polyhydroxyalkanoates (PHAs) in Manizales, Colombia. Environ Monit Assess 192:1–8. https://doi.org/10.1007/s10661-020-08461-5

Gu YM, Kim H, Sang B-I, Lee JH (2018) Effects of water content on ball milling pretreatment and the enzymatic digestibility of corn stover. Water-Energy Nexus 1(1):61–65. https://doi.org/10.1016/J.WEN.2018.07.002

Hablot E, Dharmalingam S, Hayes DG, Wadsworth LC, Blazy C, Narayan R, Hablot E, Blazy ÁC, Narayan ÁR, Dharmalingam S, Hayes ÁDG, Wadsworth LC (2014) Effect of simulated weathering on physicochemical properties and inherent biodegradation of PLA/PHA nonwoven mulches. J Polym Environ 22:417–429. https://doi.org/10.1007/s10924-014-0697-0

Haddadi MH, Asadolahi R, Negahdari B (2019) The bioextraction of bioplastics with focus on polyhydroxybutyrate: a review. Int J Environ Sci Technol 16(3):3935–3948. https://doi.org/10.1007/s13762-019-02352-0

Haldar D, Purkait MK (2021) A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: mechanistic insight and advancements. Chemosphere. https://doi.org/10.1016/J.CHEMOSPHERE.2020.128523

Han SF, Jin W, Yang Q, El-Fatah Abomohra A, Zhou X, Tu R, Chen C, Xie GJ, Wang Q (2019) Application of pulse electric field pretreatment for enhancing lipid extraction from *Chlorella pyrenoidosa* grown in wastewater. Renew Energy 133:233–239. https://doi.org/10.1016/J.RENENE.2018.10.034

Hassan MA, Bakhiet EK, Hussein HR, Ali SG (2019) Statistical optimization studies for polyhydroxybutyrate (PHB) production by novel *Bacillus subtilis* using agricultural and industrial wastes.

Int J Environ Sci Technol 16(7):3497–3512. https://doi.org/10.1007/S13762-018-1900-Y/FULLTEXT.HTML

Hathi ZJ, Haque MA, Priya A, Qin ZH, Huang S, Lam CH, Ladakis D, Pateraki C, Mettu S, Koutinas A, Du C (2022) Fermentative bioconversion of food waste into biopolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) using cupriavidus necator. Environ Res. https://doi.org/10.1016/j.envres.2022.114323

He M, Wang X, Wang Z, Chen L, Lu Y, Zhang X, Li M, Liu Z, Zhang Y, Xia H, Zhang L (2017) Biocompatible and biodegradable bioplastics constructed from chitin via a "Green" pathway for bone repair. ACS Sustainable Chemistry and Engineering 5(10):9126–9135. https://doi.org/10.1021/ACSSUSCHEMENG.7B02051

Hjorth M, Gränitz K, Adamsen AP, Møller HB (2011) Extrusion as a pretreatment to increase biogas production. Elsevier. Retrieved Nov 27 2021. https://www.sciencedirect.com/science/article/pii/S0960852411001362?casa_token=yyGrZPVaGRIAAAAA:sX1aFujz_iKJce3EBrjksi9rAMq603a9iJ9Iwgwym_Yed3_9RIohQcmeQefKGeOwXvWBOj-LXg

Hu F, Zhang S, Wang X, Wang C, Wu J, Xu L, Hu Y (2022) Investigating the role of different materials supplementation in anaerobic digestion of kitchen waste: performance and microbial community dynamics. Biochem Eng J 184:108490. https://doi.org/10.1016/j.bej.2022.108490

Huisman GW, Wonink E, de Koning G, Preusting H, Witholt B (1992) Synthesis of poly(3-hydroxyalkanoates) by mutant and recombinant *Pseudomonas* strains. Appl Microbiol Biotechnol 38(1):1–5. https://doi.org/10.1007/BF00169409

Ikada Y, Tsuji H (2000) Biodegradable polyesters for medical and ecological applications. Macromol Rapid Commun 21:117–132. https://doi.org/10.1002/(SICI)1521-3927(20000201)21:3

Iles A, Martin AN (2013) Expanding bioplastics production: sustainable business innovation in the chemical industry. J Clean Prod 45:38–49. https://doi.org/10.1016/j.jclepro.2012.05.008

Ingram HR, Winterburn JB (2022) Influence of emulsified plant oil composition on growth and biopolymer production of Cupriavidus necator DSM 545. Food Bioprod Process 132:23–34. https://doi.org/10.1016/J.FBP.2021.12.005

Israni N, Venkatachalam P, Gajaraj B, Varalakshmi KN, Shivakumar S (2020) Whey valorization for sustainable polyhydroxyalkanoate production by *Bacillus megaterium*: production, characterization and in vitro biocompatibility evaluation. J Environ Manag 255:109884. https://doi.org/10.1016/J.JENVMAN.2019.109884

Jacquel N, Lo CW, Wei YH, Wu HS, Wang SS (2008) Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochem Eng J 39(1):15–27. https://doi.org/10.1016/J.BEJ.2007.11.029

Jadhav HS, Fulke AB, Giripunje MD (2022) Recent global insight into mitigation of plastic pollutants, sustainable biodegradable alternatives, and recycling strategies. Int J Environ Sci Technol. https://doi.org/10.1007/S13762-022-04363-W/FULLTEXT.HTML

Jiang XR, Wang H, Shen R, Chen GQ (2015) Engineering the bacterial shapes for enhanced inclusion bodies accumulation. Metab Eng 29:227–237. https://doi.org/10.1016/J.YMBEN.2015.03.017

Jiang G, Johnston B, Townrow DE, Radecka I, Koller M, Chaber P, Adamus G, Kowalczuk M (2018) Biomass extraction using non-chlorinated solvents for biocompatibility improvement of polyhydroxyalkanoates. Polymers 10(7):731. https://doi.org/10.3390/POLYM10070731

Jiang Y, Pan X, Yao M, Han L, Zhang X, Jia Z, Weng J, Chen W, Fang L, Wang X, Zhang Y, Duan R, Ren F, Wang K, Chen X, Lu X (2021) Bioinspired adhesive and tumor microenvironment responsive nanoMOFs assembled 3D-printed scaffold for antitumor therapy and bone regeneration. Nano Today 39:101182. https://doi.org/10.1016/J.NANTOD.2021.101182

Jõgi K, Bhat R (2020) Valorization of food processing wastes and by-products for bioplastic production. Sustain Chem Pharm 18:100326. https://doi.org/10.1016/J.SCP.2020.100326

Joy SP, Krishnan C (2022) Modified organosolv pretreatment for improved cellulosic ethanol production from sorghum biomass. Ind Crops Prod 177:114409. https://doi.org/10.1016/J.INDCROP.2021.114409

Kahar P, Tsuge T, Taguchi K, Doi Y (2004) High yield production of polyhydroxyalkanoates from soybean oil by *Ralstonia eutropha* and its recombinant strain. Polym Degrad Stab 83(1):79–86. https://doi.org/10.1016/S0141-3910(03)00227-1

Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D (2020) Fungal infections in humans: the silent crisis. Microb Cell. https://doi.org/10.15698/mic2020.06.718

Kaniuk Ł, Stachewicz U (2021) Development and advantages of biodegradable PHA polymers based on electrospun PHBV fibers for tissue engineering and other biomedical applications. ACS Biomater Sci Eng 7:5339–5362. https://doi.org/10.1021/acsbiomaterials.1c00757

Kathiraser Y, Aroua MK, Ramachandran KB, Tan IKP (2007) Chemical characterization of medium-chain-length polyhydroxyalkanoates (PHAs) recovered by enzymatic treatment and ultrafiltration. J Chem Technol Biotechnol 82(9):847–855. https://doi.org/10.1002/JCTB.1751

Kelwick RJR, Webb AJ, Wang Y, Heliot A, Allan F, Emery AM, Templeton MR, Freemont PS (2021) AL-PHA beads: Bioplastic-based protease biosensors for global health applications. Mater Today. https://doi.org/10.1016/J.MATTOD.2021.02.018

Khatami K, Perez-Zabaleta M, Owusu-Agyeman I, Cetecioglu Z (2021) Waste to bioplastics: How close are we to sustainable polyhydroxyalkanoates production? Waste Manag 119:374–388. https://doi.org/10.1016/J.WASMAN.2020.10.008

Khattab AM, Esmael ME, Farrag AA, Ibrahim MIA (2021) Structural assessment of the bioplastic (poly-3-hydroxybutyrate) produced by *Bacillus flexus* Azu-A2 through cheese whey valorization. Int J Biol Macromol 190:319–332. https://doi.org/10.1016/J.IJBIOMAC.2021.08.090

Kim HS, Oh YH, Jang Y-A, Kang KH, David Y, Yu JH, Song BK, Choi J, Chang YK, Joo JC, Park SJ (2016) Recombinant Ralstonia eutropha engineered to utilize xylose and its use for the production of poly(3-hydroxybutyrate) from sunflower stalk hydrolysate solution. Microb Cell Fact 15(1):1–13. https://doi.org/10.1186/S12934-016-0495-6

Kim YJ, Chae CG, Kang KH, Oh YH, Joo JC, Song BK, Park SJ (2016) Biosynthesis of lactate-containing polyhydroxyalkanoates in recombinant *Escherichia coli* by employing new CoA transferases. Korean Soc Biotechnol Bioeng. https://doi.org/10.7841/ksbbj.2016.31.1.27

Koch M, Spierling S, Venkatachalam V, Endres H-J, Owsianiak M, Vea EB, Daffert C, Neureiter M, Fritz I (2023) Comparative assessment of environmental impacts of 1st generation (corn feedstock) and 3rd generation (carbon dioxide feedstock) PHA production pathways using life cycle assessment. Scie Total Environ 863:160991. https://doi.org/10.1016/j.scitotenv.2022.160991

Koller M, Maršálek L, de Sousa Dias MM, Braunegg G (2017) Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. New Biotechnol 37:24–38. https://doi.org/10.1016/j.nbt.2016.05.001

Koller M, Bona R, Chiellini E, Braunegg G (2013) Extraction of short-chain-length poly-[(R)-hydroxyalkanoates] (scl-PHA) by the "anti-solvent" acetone under elevated temperature and pressure. Biotech Lett 35(7):1023–1028. https://doi.org/10.1007/S10529-013-1185-7/TABLES/1

Korkakaki E, van Loosdrecht MCM, Kleerebezem R (2017) Impact of phosphate limitation on PHA production in a feast-famine process. Water Res 126:472–480. https://doi.org/10.1016/J.WATRES.2017.09.031

Kovačić Đ, Rupčić S, Kralik D, Jovičić D, Spajić R, Tišma M (2021) Pulsed electric field: an emerging pretreatment technology in a biogas production. Waste Manag 120:467–483. https://doi.org/10.1016/J.WASMAN.2020.10.009

Kovalcik A, Kucera D, Matouskova P, Pernicova I, Obruca S, Kalina M, Enev V, Marova I (2018) Influence of removal of microbial inhibitors on PHA production from spent coffee grounds employing *Halomonas halophila*. J Environ Chem Eng 6(2):3495–3501. https://doi.org/10.1016/J.JECE.2018.05.028

Kucera D, Benesova P, Ladicky P, Pekar M, Sedlacek P, Obruca S (2017) Production of polyhydroxyalkanoates using hydrolyzates of spruce sawdust: comparison of hydrolyzates detoxification by application of overliming, active carbon, and lignite. Bioengineering 4(2):53. https://doi.org/10.3390/BIOENGINEERING4020053

Lanfranchi A, Tassinato G, Valentino F, Martinez GA, Jones E, Gioia C, Cavinato C (2022) Hydrodynamic cavitation pre-treatment of urban waste: integration with acidogenic fermentation, PHAs synthesis and anaerobic digestion processes. Chemosphere 301:134624

Lee SY, Choi J, Wang F (1998) Production of poly(3-hydroxybutyrate) by recombinant bacteria. Sci Technol Polym Adv Mater. https://doi.org/10.1007/978-1-4899-0112-5_40

Lee RE, Azdast T, Wang G, Wang X, Lee PC, Park CB (2020) Highly expanded fine-cell foam of polylactide/polyhydroxyalkanoate/nano-fibrillated polytetrafluoroethylene composites blown with mold-opening injection molding. Int J Biol Macromol 155:286–292. https://doi.org/10.1016/J.IJBIOMAC.2020.03.212

Leong YK, Show PL, Lin HC, Chang CK, Loh H-S, Lan JC-W, Ling TC (2016) Preliminary integrated economic and environmental analysis of polyhydroxyalkanoates (PHAs) biosynthesis. Bioresour Bioprocess 3(1):1–9. https://doi.org/10.1186/S40643-016-0120-X

Leung DYG (2006) Transesterification of neat and used frying oil: optimization for biodiesel production. Elsevier. Retrieved June 28 2022. From https://www.sciencedirect.com/science/article/pii/S0378382006000762?casa_token=kf-6N3LS810AAAAA:gnv2-nnuB2OOu3isTPNQ04GyulqBxrdst4lPiGZU9exLFXJkHSGebfz9xOuwf19lausxIuMnbQ

Li D, Yin F, Ma X (2020) Towards biodegradable polyhydroxyalkanoate production from wood waste: using volatile fatty acids as conversion medium. Bioresour Technol 299:122629. https://doi.org/10.1016/j.biortech.2019.122629

Li J, Li D, Su Y, Yan X, Wang F, Yu L, Ma X (2022) Efficient and economical production of polyhydroxyalkanoate from sustainable rubber wood hydrolysate and xylose as co-substrate by mixed microbial cultures. Bioresource Technol 355:127238. https://doi.org/10.1016/j.biortech.2022.127238

Li ZJ, Shi ZY, Jian J, Guo YY, Wu Q, Chen GQ (2010) Production of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) from unrelated carbon sources by metabolically engineered *Escherichia coli*. Metab Eng 12(4):352–359. https://doi.org/10.1016/j.ymben.2010.03.003

Li Z, Yang J, Loh XJ (2016) Polyhydroxyalkanoates: opening doors for a sustainable future. NPG Asia Mater 8(4):e265–e265. https://doi.org/10.1038/am.2016.48

Lim J, You M, Li J, Li CZLMSE (2017) Emerging bone tissue engineering via Polyhydroxyalkanoate (PHA)-based scaffolds. Elsevier. Retrieved Jan 2 2023. From https://www.sciencedirect.com/science/article/pii/S0928493117315357?casa_token=drC7ZdKx0coAAAAA:eLO4bnEgmzx9IRmYZpLK1CZKOzMnC_eFQh1-9dEGT3hh7W_uPQbZ6KkiZnQ2UbqoMI9o2jqb0w

Liu H, Kumar V, Jia L, Sarsaiya S, Kumar D, Juneja A, Awasthi MK (2021) Biopolymer poly-hydroxyalkanoates (PHA) production from apple industrial waste residues: a review. Chemosphere 284:131427. https://doi.org/10.1016/j.chemosphere.2021.131427

Liu Q, Li W, Ma Q, An S, Li M, Jameel H, Chang HM (2016) Pretreatment of corn stover for sugar production using a two-stage dilute acid followed by wet-milling pretreatment process. Biores Technol 211:435–442. https://doi.org/10.1016/J.BIORTECH.2016.03.131

Loan TT, Trang DTQ, Huy PQ, Ninh PX, Van Thuoc D (2022) A fermentation process for the production of poly (3-hydroxybutyrate) using waste cooking oil or waste fish oil as inexpensive carbon substrate. Biotechnol Rep 33:e00700. https://doi.org/10.1016/j.btre.2022.e00700

Lorini L, Martinelli A, Pavan P, Majone M, Valentino F (2021) Downstream processing and characterization of polyhydroxyalkanoates (PHAs) produced by mixed microbial culture (MMC) and organic urban waste as substrate. Biomass Convers Biorefinery 11:693–703. https://doi.org/10.1007/s13399-020-00788-w

Lutolf MP, Hubbell JA (2005) Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nature Biotechnol 23(1):47–55. https://doi.org/10.1038/nbt1055

Macha IJ, Ben-Nissan B, Vilchevskaya EN, Morozova AS, Abali BE, Müller WH, Rickert W (2019) Drug delivery from polymer-based nanopharmaceuticals—an experimental study complemented by simulations of selected diffusion processes. Front Bioeng Biotechnol. https://doi.org/10.3389/FBIOE.2019.00037

Malafaya PB, Silva GA, Reis RL (2007) Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev 59(4–5):207–233. https://doi.org/10.1016/J.ADDR.2007.03.012

Mangaraj S, Yadav A, Bal LM, Dash SK, Mahanti NK (2018) Application of biodegradable polymers in food packaging industry: a comprehensive review. J Package Technol Res 3(1):77–96. https://doi.org/10.1007/S41783-018-0049-Y

Martínez I, Partal P, García-Morales M, Guerrero A, Gallegos C (2013) Development of protein-based bioplastics with antimicrobial activity by thermo-mechanical processing. J Food Eng 117(2):247–254. https://doi.org/10.1016/J.JFOODENG.2013.02.014

Martínez V, Herencias C, Edouard Jurkevitch M, Prieto A (2016) Engineering a predatory bacterium as a proficient killer agent for intracellular bio-products recovery: the case of the polyhydroxyalkanoates. Sci Rep. https://doi.org/10.1038/srep24381

Martín-Lara MA, Chica-Redecillas L, Pérez A, Blázquez G, Garcia-Garcia G, Calero M (2020) Liquid hot water pretreatment and enzymatic hydrolysis as a valorization route of italian green pepper waste to delivery free sugars. Foods 9(11):1640. https://doi.org/10.3390/FOODS9111640

Mohapatra S, Vishwakarma K, Joshi NC, Maity S, Kumar R, Ramchander M, Pattnaik S, Samantaray DP (2021) A review on PHAs: the future biopolymer. Environ Agric Microbiol: Adv Appl. https://doi.org/10.1002/9781119525899.CH5

Mozejko-Ciesielska J, Marciniak P, Moraczewski K, Rytlewski P, Czaplicki S, Zadernowska A (2022) Cheese whey mother liquor as dairy waste with potential value for polyhydroxyalkanoate production by extremophilic *Paracoccus homiensis*. Sustain Mater Technol 33:e00449. https://doi.org/10.1016/j.susmat.2022.e00449

Müller-Santos M, Koskimäki JJ, Alves LPS, De Souza EM, Jendrossek D, Pirttilä AM (2021) The protective role of PHB and its

degradation products against stress situations in bacteria. FEMS Microb Rev. https://doi.org/10.1093/FEMSRE/FUAA058

Muneer F, Rasul I, Azeem F, Siddique MH, Zubair M, Nadeem H (2020) Microbial polyhydroxyalkanoates (PHAs): efficient replacement of synthetic polymers. J Polym Environ 28:2301–2323. https://doi.org/10.1007/s10924-020-01772-1

Muneer F, Rasul I, Qasim M, Sajid A, Nadeem H (2022) Optimization, production and characterization of polyhydroxyalkanoate (PHA) from indigenously isolated novel bacteria. J Polym Environ 30(8):3523–3533. https://doi.org/10.1007/s10924-022-02444-y

Murciano Martínez P, Bakker R, Harmsen P, Gruppen H, Kabel M (2015) Importance of acid or alkali concentration on the removal of xylan and lignin for enzymatic cellulose hydrolysis. Ind Crops Prod 64:88–96. https://doi.org/10.1016/J.INDCROP.2014.10.031

Murugan P, Han L, Gan CY, Maurer FH, Sudesh K 2016 A new biological recovery approach for PHA using mealworm, tenebrio molitor. Elsevier. Retrieved Nov 28 2021. From https://www.sciencedirect.com/science/article/pii/S0168165616315681?casa_token=J4UFA3VO27MAAAAA:1REH93F8j3ZIKgRCRww1RLn4CWuIzp7fyFqgpbrfpyD-aoOlL01DiHbkJcrTpN0iM0YhGYQyjA

Naik S, Venu Gopal SK, Somal P (2008) Bioproduction of polyhydroxyalkanoates from bacteria: a metabolic approach. World J Microbiol Biotechnol 24(10):2307–2314. https://doi.org/10.1007/S11274-008-9745-Z

Naik GP, Poonia AK, Chaudhari PK (2021) Pretreatment of lignocellulosic agricultural waste for delignification, rapid hydrolysis, and enhanced biogas production: a review. J Indian Chem Soc 98(10):100147. https://doi.org/10.1016/J.JICS.2021.100147

Narancic T, Cerrone F, Beagan N, O'Connor KE (2020) Recent advances in bioplastics: application and biodegradation. Polymers. https://doi.org/10.3390/POLYM12040920

Ng JS, Kiew PL, Lam MK, Yeoh WM, Ho MY (2022) Preliminary evaluation of the properties and biodegradability of glycerol- and sorbitol-plasticized potato-based bioplastics. Int J Environ Sci Technol 19(3):1545–1554. https://doi.org/10.1007/S13762-021-03213-5

Nikel PI, De Almeida A, Melillo EC, Galvagno MA, Pettinari MJ (2006) New recombinant Escherichia coli strain tailored for the production of poly(3-hydroxybutyrate) from agroindustrial by-products. Appl Environ Microbiol 72(6):3949–3954. https://doi.org/10.1128/AEM.00044-06

Novikov LN, Novikova LN, Mosahebi A, Wiberg M, Terenghi G, Kellerth JO (2002) A novel biodegradable implant for neuronal rescue and regeneration after spinal cord injury. Biomaterials 23(16):3369–3376. https://doi.org/10.1016/S0142-9612(02)00037-6

Numata K, Morisaki K, Tomizawa S, Ohtani M, Demura T, Miyazaki M, Nogi Y, Deguchi S, Doi Y (2013) Synthesis of poly- and oligo(hydroxyalkanoate)s by deep-sea bacteria, *Colwellia* spp., *Moritella* spp., and *Shewanella* spp. Polym J 45(10):1094–1100. https://doi.org/10.1038/pj.2013.25

Ocreto JB, Chen W-H, Ubando AT, Park Y-K, Sharma AK, Ashokkumar V, Ok YS, Kwon EE, Rollon AP, De Luna MDG (2021) A critical review on second-and third-generation bioethanol production using microwaved-assisted heating (MAH) pretreatment. Renew Sustain Energy Rev 152:111679. https://doi.org/10.1016/J.RSER.2021.111679

Ong SY, Zainab-L I, Pyary S, Sudesh K (2018) A novel biological recovery approach for PHA employing selective digestion of bacterial biomass in animals. Appl Microb Biotechnol 102(5):2117–2127. https://doi.org/10.1007/S00253-018-8788-9/TABLES/1

Othman N, Selambakkannu S, Nexus NSE (2022) Biodegradable dual-layer polyhydroxyalkanoate (pha)/polycaprolactone (pcl) mulch film for agriculture: preparation and characterization. Elsevier. Retrieved Jan 6 2023. From https://www.sciencedirect.com/science/article/pii/S2772427122000924

Pais J, Serafim LS, Freitas F, Reis MA (2016) Conversion of cheese whey into poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. New Biotechnol 33(1):224–230. https://doi.org/10.1016/j.nbt.2015.06.001

Pakalapati H, Chang CK, Show PL, Arumugasamy SK, Lan JCW (2018) Development of polyhydroxyalkanoates production from waste feedstocks and applications. J Biosci Bioeng 126(3):282–292. https://doi.org/10.1016/j.jbiosc.2018.03.016

Palmeiro-Sánchez T, O'Flaherty V, Lens PNL (2022) Polyhydroxyalkanoate bio-production and its rise as biomaterial of the future. J Biotechnol 348:10–25. https://doi.org/10.1016/J.JBIOTEC.2022.03.001

Pan L, Li J, Wang R, Wang Y, Lin Q, Li C, Wang Y (2021) Biosynthesis of polyhydroxyalkanoate from food waste oil by Pseudomonas alcaligenes with simultaneous energy recovery from fermentation wastewater. Waste Manag 131:268–276. https://doi.org/10.1016/J.WASMAN.2021.06.008

Panaitescu DM, Trusca R, Gabor AR, Nicolae CA, Casarica A (2020) Biocomposite foams based on polyhydroxyalkanoate and nanocellulose: morphological and thermo-mechanical characterization. Int J Biol Macromol 164:1867–1878. https://doi.org/10.1016/J.IJBIOMAC.2020.07.273

Parot M, Rodrigue D, Stevanovic T (2022) High purity softwood lignin obtained by an eco-friendly organosolv process. Bioresour Technol Rep 17:100880. https://doi.org/10.1016/J.BITEB.2021.100880

Pekmezovic M, Krusic MK, Malagurski I, Milovanovic J, Stępień K, Guzik M, Charifou R, Babu R, O'Connor K, Nikodinovic-Runic J (2021) Polyhydroxyalkanoate/antifungal polyene formulations with monomeric hydroxyalkanoic acids for improved antifungal efficiency. Antibiotics. https://doi.org/10.3390/antibiotics10060737

Peng Q, Zhang ZR, Gong T, Chen GQ, Sun X (2012) A rapid-acting, long-acting insulin formulation based on a phospholipid complex loaded PHBHHx nanoparticles. Biomaterials 33(5):1583–1588. https://doi.org/10.1016/J.BIOMATERIALS.2011.10.072

Pérez V, Mota CR, Muñoz R, Lebrero R (2020) Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: assessing the potential impacts on economy, environment and society. Chemosphere 255:126929. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126929

Pérez-Arauz AO, Aguilar-Rabiela AE, Vargas-Torres A, Rodríguez-Hernández AI, Chavarría-Hernández N, Vergara-Porras B, López-Cuellar MR (2019) Production and characterization of biodegradable films of a novel polyhydroxyalkanoate (PHA) synthesized from peanut oil. Food Packag Shelf Life 20:100297. https://doi.org/10.1016/J.FPSL.2019.01.001

Pernicova I, Kucera D, Nebesarova J, Kalina M, Novackova I, Koller M, Obruca S (2019) Production of polyhydroxyalkanoates on waste frying oil employing selected halomonas strains. Bioresour Technol 292:122028. https://doi.org/10.1016/J.BIORTECH.2019.122028

Piarali S, Marlinghaus L, Viebahn R, Lewis H, Ryadnov MG, Groll J, Salber J, Roy I (2020) Activated polyhydroxyalkanoate meshes prevent bacterial adhesion and biofilm development in regenerative medicine applications. Front Bioeng Biotechnol. https://doi.org/10.3389/FBIOE.2020.00442

Povolo S, Casella S (2003) Bacterial production of PHA from lactose and cheese whey permeate. Macromol Symp 197(1):1–10. https://doi.org/10.1002/MASY.200350701

Pradhan S, Borah AJ, Poddar MK, Dikshit PK, Rohidas L, Moholkar VS (2017) Microbial production, ultrasound-assisted extraction and characterization of biopolymer polyhydroxybutyrate (PHB)

from terrestrial (P. *hysterophorus*) and aquatic (E. *crassipes*) invasive weeds. Bioresour Technol 242:304–310. https://doi.org/10.1016/J.BIORTECH.2017.03.117

Prakash P, Lee W-H, Loo C-Y, Seung H, Wong J, Parumasivam T (2022) Advances in polyhydroxyalkanoate nanocarriers for effective drug delivery: an overview and challenges. Nanomaterials. https://doi.org/10.3390/nano12010175

Rathika R, Janaki V, Shanthi K, Kamala-Kannan S (2019) Bioconversion of agro-industrial effluents for polyhydroxyalkanoates production using *Bacillus subtilis* RS1. Int J Environ Sci Technol 16(10):5725–5734. https://doi.org/10.1007/S13762-018-2155-3/FULLTEXT.HTML

Ravindran R, Jaiswal AK (2016) Exploitation of food industry waste for high-value products. Trends Biotechnol 34(1):58–69. https://doi.org/10.1016/J.TIBTECH.2015.10.008

Ray S, Kalia VC (2017) Microbial cometabolism and polyhydroxyalkanoate co-polymers. Indian J Microbiol 57(1):39. https://doi.org/10.1007/S12088-016-0622-4

Rodríguez-Contreras A, Guillem-Marti J, Lopez O, Manero JM, Ruperez E (2019) Antimicrobial PHAs coatings for solid and porous tantalum implants. Colloids Surf b: Biointerfaces 182:110317. https://doi.org/10.1016/J.COLSURFB.2019.06.047

Rodríguez G, Brojanigo S, Basaglia M, Favaro L, Casella S (2021) Efficient production of polyhydroxybutyrate from slaughterhouse waste using a recombinant strain of *Cupriavidus* necator DSM 545. Sci Total Environ 794:148754. https://doi.org/10.1016/j.scitotenv.2021.148754

Rohadi TNT, Ridzuan MJM, Abdul Majid MS, Sulaiman MH (2022) Biodegradability of bioplastic film using different regions of pennisetum purpureum incorporated with gelatine and chitosan. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-022-04614-w

Roilides E, Simitsopoulou M, Katragkou A, Walsh TJ (2015) How biofilms evade host defenses. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.mb-0012-2014

Rosenboom JG, Langer R, Traverso G (2022) Bioplastics for a circular economy. Nature Rev Mater 7(2):117–137. https://doi.org/10.1038/s41578-021-00407-8

Sadat-Shojai M, Khorasani MT, Jamshidi A (2016) A new strategy for fabrication of bone scaffolds using electrospun nano-HAp/PHB fibers and protein hydrogels. Chem Eng J 289:38–47. https://doi.org/10.1016/J.CEJ.2015.12.079

Salamanca-Cardona L, Ashe CS, Stipanovic AJ, Nomura CT (2013) Enhanced production of polyhydroxyalkanoates (PHAs) from beechwood xylan by recombinant Escherichia coli. Appl Microbiol Biotechnol 98(2):831–842. https://doi.org/10.1007/S00253-013-5398-4

Salvachúa D, Rydzak T, Auwae R, De Capite A, Black BA, Bouvier JT, Guss AM (2020) Metabolic engineering of *Pseudomonas putida* for increased polyhydroxyalkanoate production from lignin. Microb Biotechnol 13(1):290–298. https://doi.org/10.1111/1751-7915.13481

Samantaray S, Mallick N (2015) Impact of various stress conditions on poly-β-hydroxybutyrate (PHB) accumulation in aulosira fertilissima ccc 444. Curr Biotechnol 4(3):366–372. https://doi.org/10.2174/2211550104666150806000642

Samorì C, Abbondanzi F, Galletti P, Giorgini L, Mazzocchetti L, Torri C, Tagliavini E (2015) Extraction of polyhydroxyalkanoates from mixed microbial cultures: impact on polymer quality and recovery. Biores Technol 189:195–202. https://doi.org/10.1016/J.BIORTECH.2015.03.062

Samrot AV, Samanvitha SK, Shobana N, Renitta ER, Senthilkumar P, Kumar SS, Thirumurugan R (2021) The synthesis, characterization and applications of polyhydroxyalkanoates (PHAs) and PHA-based nanoparticles. Polymers 13(19):3302. https://doi.org/10.3390/polym13193302

Sanchez-Garcia MD, Lagaron JM (2010) Novel clay-based nanobiocomposites of biopolyesters with synergistic barrier to UV light, gas, and vapour. J Appl Polym Sci 118(1):188–199. https://doi.org/10.1002/APP.31986

Saranya V, Shenbagarathai R (2011) Production and characterization of PHA from recombinant *E. coli* harbouring phaC1 gene of indigenous *Pseudomonas* sp. LDC-5 using molasses. Braz J Microbiol 42(3):1109–1118. https://doi.org/10.1590/S1517-83822011000300032

Saratale GD, Saratale RG, Varjani S, Cho SK, Ghodake GS, Kadam A, Mulla SI, Bharagava RN, Kim DS, Shin HS (2020) Development of ultrasound aided chemical pretreatment methods to enrich saccharification of wheat waste biomass for polyhydroxybutyrate production and its characterization. Ind Crops and Prod 150:112425. https://doi.org/10.1016/J.INDCROP.2020.112425

Saravanan K, Umesh M, Kathirvel P (2022) Microbial polyhydroxyalkanoates (PHAs): a review on biosynthesis, properties, fermentation strategies and its prospective applications for sustainable future. J Polym Environ. https://doi.org/10.1007/S10924-022-02562-7

Sarmiento-Vásquez Z, Vandenberghe LPDS, Karp SG, Soccol CR (2022) Production of polyhydroxyalkanoates through soybean hull and waste glycerol valorization: subsequent alkaline pretreatment and enzymatic hydrolysis. Fermentation 8(9):433. https://doi.org/10.3390/fermentation8090433

Sawant SS, Salunke BK, Tran TK, Kim BS (2016) Lignocellulosic and marine biomass as resource for production of polyhydroxyalkanoates. Korean J Chem Eng 33(5):1505–1513. https://doi.org/10.1007/s11814-016-0019-4

Sayyed RZ, Shaikh SS, Wani SJ, Rehman MT, Al Ajmi MF, Haque S, El Enshasy HA (2021) Production of biodegradable polymer from agro-wastes in *Alcaligenes* sp. and *Pseudomonas* sp. Molecules 26(9):2443. https://doi.org/10.3390/molecules26092443

Sen KY, Baidurah S (2021) Renewable biomass feedstocks for production of sustainable biodegradable polymer. Curr Opin Green Sustain Chem 27:100412. https://doi.org/10.1016/J.COGSC.2020.100412

Shakirah HL, Zulilah ZNA, Aniyyah MSN, Nabihah A, Radhiah GS (2020) Extraction of polyhydroxyakanoate (PHA) from palm oil mill effluent (POME) using chemical solvent extraction. J Phys: Conf Ser 1532(1):012015. https://doi.org/10.1088/1742-6596/1532/1/012015

Sharma V, Sehgal R, Gupta R (2021) Polyhydroxyalkanoate (PHA): properties and modifications. Polymer 212:123161. https://doi.org/10.1016/J.POLYMER.2020.123161

Shen X-W, Shi Z-Y, Song G, Li Z-J, Chen G-Q (2011) Engineering of polyhydroxyalkanoate (PHA) synthase PhaC2Ps of Pseudomonas stutzeri via site-specific mutation for efficient production of PHA copolymers. Appl Microbiol Biotechnol 91(3):655–665. https://doi.org/10.1007/S00253-011-3274-7

Shishatskaya EI, Nikolaeva ED, Vinogradova ON, Volova TG (2016) Experimental wound dressings of degradable PHA for skin defect repair. J Mater Sci: Mater Med 27(11):1–16. https://doi.org/10.1007/S10856-016-5776-4

Silva F, Matos M, Pereira B, Ralo C, Pequito D, Marques N, Carvalho G, Reis AM (2021) An integrated process for mixed culture production of 3-hydroxyhexanoate-rich polyhydroxyalkanoates from fruit waste. Chem Eng J. https://doi.org/10.1016/J.CEJ.2021.131908

Simona C, Laura L, Francesco V, Marianna V, Cristina MG, Barbara T, Mauro M, Simona R (2022) Effect of the organic loading rate on the PHA-storing microbiome in sequencing batch reactors

operated with uncoupled carbon and nitrogen feeding. Sci Total Environ 825:153995. https://doi.org/10.1016/J.SCITOTENV.2022.153995

Singh LK, Dhasmana N, Kamble SS, Sharma AK, Singh Y (2015) Frontiers in Biomedical Engineering: PHA-fabricated implants. Microb Fact: Biodivers, Biopolym, Bioact Mol 2:91–102. https://doi.org/10.1007/978-81-322-2595-9_6

Sohn YJ, Son J, Lim HJ, Lim SH, Park SJ (2022) Valorization of lignocellulosic biomass for polyhydroxyalkanoate production: status and perspectives. Bioresour Technol 360:127575. https://doi.org/10.1016/J.BIORTECH.2022.127575

Sun SF, Yang HY, Yang J, Shi ZJ (2021) Structural characterization of poplar lignin based on the microwave-assisted hydrothermal pretreatment. Int J Biol Macromol 190:360–367. https://doi.org/10.1016/J.IJBIOMAC.2021.08.230

Sun J, Ding S, JDPBC (2014) Biomass and its biorefinery: novel approaches from nature-inspired strategies and technology. Books.Google.Com. Retrieved Jan 18 2022. From https://books.google.com/books?hl=en&lr=&id=D9IVAgAAQBAJ&oi=fnd&pg=PA1&ots=i92CMPWh5g&sig=djtxDB_Iq5-mBSaHcmylXWmKOSU

Suwannasing W, Imai T, Kaewkannetra P (2015) Cost-effective defined medium for the production of polyhydroxyalkanoates using agricultural raw materials. Bioresour Technol 194:67–74. https://doi.org/10.1016/j.biortech.2015.06.087

Talan A, Kaur R, Tyagi RD, Drogui P (2020) Bioconversion of oily waste to polyhydroxyalkanoates: sustainable technology with circular bioeconomy approach and multidimensional impacts. Bioresour Technol Rep 11:100496. https://doi.org/10.1016/J.BITEB.2020.100496

Tan GYA, Chen CL, Li L, Ge L, Wang L, Razaad IMN, Wang JY (2014) Start a research on biopolymer polyhydroxyalkanoate (PHA): a review. Polymers 6(3):706–754. https://doi.org/10.3390/polym6030706

Tanikkul P, Sullivan GL, Sarp S, Pisutpaisal N (2020) Biosynthesis of medium chain length polyhydroxyalkanoates (mcl-PHAs) from palm oil. Case Stud Chem Environ Eng 2:100045. https://doi.org/10.1016/J.CSCEE.2020.100045

Tavares APM, Gonçalves MJA, Brás T, Pesce GR, Xavier AMRB, Fernandes MC (2022) Cardoon hydrolysate detoxification by activated carbon or membranes system for bioethanol production. Energies. https://doi.org/10.3390/en15061993

Torri C, Fabbri D (2014) Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass. Bioresour Technol 172:335–341. https://doi.org/10.1016/j.biortech.2014.09.021

Tsang YF, Kumar V, Samadar P, Yang Y, Lee J, Ok YS, Song H, Kim KH, Kwon EE, Jeon YJ (2019) Production of bioplastic through food waste valorization. Environ Int 127:625–644. https://doi.org/10.1016/J.ENVINT.2019.03.076

Tu W, Zhang D, Wang H (2019) Polyhydroxyalkanoates (PHA) production from fermented thermal-hydrolyzed sludge by mixed microbial cultures: the link between phosphorus and PHA yields. Waste Manage 96:149–157. https://doi.org/10.1016/j.wasman.2019.07.021

Tu W, Zou Y, Wu M, Wang H (2020) Reducing the effect of non-volatile fatty acids (non-VFAs) on polyhydroxyalkanoates (PHA) production from fermented thermal-hydrolyzed sludge. Int J Biol Macromol 155:1317–1324. https://doi.org/10.1016/j.ijbiomac.2019.11.103

Tyagi B, Takkar S, Meena R, Thakur IS (2021) Production of polyhydroxybutyrate (PHB) by *Parapedobacter* sp. ISTM3 isolated from Mawsmai cave utilizing molasses as carbon source. Environ Technol Innov 24:101854. https://doi.org/10.1016/J.ETI.2021.101854

Tyagi P, Sharma A (2021) Utilization of crude paper industry effluent for polyhydroxyalkanoate (PHA) production. Environ Technol Innov 23:101692. https://doi.org/10.1016/j.eti.2021.101692

Valentino F, Moretto G, Lorini L, Bolzonella D, Pavan P, Majone M (2019) Pilot-scale polyhydroxyalkanoate production from combined treatment of organic fraction of municipal solid waste and sewage sludge. Ind Eng Chem Res 58(27):12149–12158. https://doi.org/10.1021/ACS.IECR.9B01831

Valentino F, Lorini L, Gottardo M, Pavan P, Majone M (2020) Effect of the temperature in a mixed culture pilot scale aerobic process for food waste and sewage sludge conversion into polyhydroxyalkanoates. J Biotechnol 323:54–61. https://doi.org/10.1016/J.JBIOTEC.2020.07.022

Van Thuoc D, Chung NT, Hatti-Kaul R (2021) Polyhydroxyalkanoate production from rice straw hydrolysate obtained by alkaline pretreatment and enzymatic hydrolysis using *Bacillus* strains isolated from decomposing straw. Bioresour Bioprocess. https://doi.org/10.1186/s40643-021-00454-7

Vandi LJ, Chan CM, Werker A, Richardson D, Laycock B, Pratt S (2018) Wood-PHA composites: mapping opportunities. Polymers 10(7):751. https://doi.org/10.3390/polym10070751

Vasilakis P, Pyrgakis K, Psycha M, Biundo, A, Kokossis A (2023) A novel industrial biotechnology approach to valorize fatty acids to bioplastics: scope for scale-up and process efficiency using an integrated approach. In: Computer Aided Chemical Engineering, vol 52, p. 2563-2568. Elsevier. https://doi.org/10.1016/B978-0-443-15274-0.50407-8

Vastano M, Corrado I, Sannia G, Solaiman DKY, Pezzella C (2019) Conversion of no/low value waste frying oils into biodiesel and polyhydroxyalkanoates. Sci Rep 9(1):1–8. https://doi.org/10.1038/s41598-019-50278-x

Vu DH, Wainaina S, Taherzadeh MJ, Åkesson D, Ferreira JA (2021) Production of polyhydroxyalkanoates (PHAs) by *Bacillus megaterium* using food waste acidogenic fermentation-derived volatile fatty acids. Bioengineered. https://doi.org/10.1080/21655979.2021.1935524

Wang C, Chen S (2019) Biodegradable and water-responsive shape memory PHA-based polyurethane for tissue engineering. Mater Today: Proc 16:1475–1479. https://doi.org/10.1016/J.MATPR.2019.05.326

Wang YW, Wu Q, Chen J, Chen GQ (2005) Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) for bone reconstruction. Biomaterials 26(8):899–904. https://doi.org/10.1016/J.BIOMATERIALS.2004.03.035

Wang Q, Tappel RC, Zhu C, Nomura CT (2012) Development of a new strategy for production of medium-chain-length polyhydroxyalkanoates by recombinant Escherichia coli via inexpensive non-fatty acid feedstocks. Appl Environ Microbiol 78(2):519–527. https://doi.org/10.1128/AEM.07020-11

Wu H, Fan Z, Jiang X, Chen J, Chen G-Q (2016a) Enhanced production of polyhydroxybutyrate by multiple dividing *E. coli*. Microb Cell Fact 15(1):1–13. https://doi.org/10.1186/S12934-016-0531-6

Xia Z, Zhao H, Li Y, Ma Y, Tian F, Chen W (2021) Stress-Induced Crystallization of the Metastable β-Form of Poly((R)-3-hydroxybutyrate-co-4-hydroxybutyrate). ACS Appl Poly Mater 3(8):4109–4117. https://doi.org/10.1021/ACSAPM.1C00590

Xu XY, Li XT, Peng SW, Xiao JF, Liu C, Fang G, Chen KC, Chen GQ (2010) The behaviour of neural stem cells on

polyhydroxyalkanoate nanofiber scaffolds. Biomaterials 31(14):3967–3975. https://doi.org/10.1016/J.BIOMATERIALS.2010.01.132

Ya-Jun H, Wei X, Zhao W, Liu Y-S, Chen G-Q (2009) Biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) with bone marrow mesenchymal stem cells. Acta Biomater 5(4):1115–1125. https://doi.org/10.1016/J.ACTBIO.2008.09.021

Yan D, Ji Q, Yu X, Li M, Abiola Fakayode O, Yagoub AEGA, Chen L, Zhou C (2021a) Multimode-ultrasound and microwave assisted natural ternary deep eutectic solvent sequential pretreatments for corn straw biomass deconstruction under mild conditions. Ultrason Sonochem 72:105414. https://doi.org/10.1016/J.ULTSONCH.2020.105414

Yan X, Li D, Ma X, Li J (2021b) Bioconversion of renewable lignocellulosic biomass into multicomponent substrate via pressurized hot water pretreatment for bioplastic polyhydroxyalkanoate accumulation. Bioresour Technol 339:125667. https://doi.org/10.1016/J.BIORTECH.2021.125667

Yang YH, Brigham C, Willis L, Rha CK, Sinskey A (2011) Improved detergent-based recovery of polyhydroxyalkanoates (PHAs). Biotech Lett 33(5):937–942. https://doi.org/10.1007/S10529-010-0513-4/FIGURES/4

Yang JE, Park SJ, Kim WJ, Kim HJ, Kim BJ, Lee H, Shin J, Lee SY (2018) One-step fermentative production of aromatic polyesters from glucose by metabolically engineered Escherichia coli strains. Nature Commun 9(1):1–10. https://doi.org/10.1038/s41467-017-02498-w

Ye C, Hu P, Ma MX, Xiang Y, Liu RG, Shang XW (2009) PHB/PHBHHx scaffolds and human adipose-derived stem cells for cartilage tissue engineering. Biomaterials 30(26):4401–4406. https://doi.org/10.1016/J.BIOMATERIALS.2009.05.001

Yee LN, Mumtaz T, Mohammadi M, Phang LY, Ando Y, Rahim Raha A, Zakaria MR (2012) Polyhydroxyalkanoate synthesis by recombinant Escherichia coli JM109 expressing PHA biosynthesis genes from *Comamonas* sp. EB172. J Microb Biochem Technol 4(4):103–110. https://doi.org/10.4172/1948-5948.1000079

Yee PL, Hassan MA (2011) Utilization of kitchen waste for the production of green thermoplastic polyhydroxybutyrate (PHB) by Cupriavidus necator CCGUG 52238. Afr J Microbiol Res 5:2873–2879. https://doi.org/10.5897/AJMR11.156

Yuan Li A, Thouas G, Chen Q-Z (2012) Biodegradable soft elastomers: synthesis/properties of materials and fabrication of scaffolds. RSC Adv 2(22):8229–8242. https://doi.org/10.1039/C2RA20736B

Yue H, Ling C, Yang T, Chen X, Chen Y, Deng H, Wu Q, Chen J, Chen G-Q (2014) A seawater-based open and continuous process for polyhydroxyalkanoates production by recombinant halomonas campaniensis LS21 grown in mixed substrates. Biotechnol Biofuels 7(1):1–12. https://doi.org/10.1186/1754-6834-7-108

Zanellati A, Spina F, Bonaterra M, Dinuccio E, Varese GC, Scarpeci TE (2021) Screening and evaluation of phenols and furans degrading fungi for the biological pretreatment of lignocellulosic biomass. Int Biodeterior Biodegrad 161:105246. https://doi.org/10.1016/J.IBIOD.2021.105246

Zhang Y, Zhang J, Xu W, Xiao G, Ding J, Chen X (2018) Tumor microenvironment-labile polymer–doxorubicin conjugate thermogel combined with docetaxel for in situ synergistic chemotherapy of hepatoma. Acta Biomater 77:63–73. https://doi.org/10.1016/J.ACTBIO.2018.07.021

Zhang F, Lan W, Li Z, Zhang A, Tang B, Wang H, Wang X, Ren J, Liu C (2021) Co-production of functional xylo-oligosaccharides and fermentable sugars from corn stover through fast and facile ball mill-assisted alkaline peroxide pretreatment. Bioresour Technol 337:125327. https://doi.org/10.1016/J.BIORTECH.2021.125327

Zhang M, Biesold GM, Choi W, Yu J, Deng Y, Silvestre C, Lin Z (2022) Recent advances in polymers and polymer composites for food packaging. Mater Today 53:134–161. https://doi.org/10.1016/J.MATTOD.2022.01.022

Zheng Y, Chen JC, Ma YM, Chen GQ (2020) Engineering biosynthesis of polyhydroxyalkanoates (PHA) for diversity and cost reduction. Metab Eng 58:82–93. https://doi.org/10.1016/J.YMBEN.2019.07.004

Zhong L, Wang C, Yang G, Chen J, Xu F, Geun Yoo C, Lyu G (2022) Rapid and efficient microwave-assisted guanidine hydrochloride deep eutectic solvent pretreatment for biological conversion of castor stalk. Bioresour Technol 343:126022. https://doi.org/10.1016/J.BIORTECH.2021.126022

Zhou Y, Lin L, Wang H, Zhang Z, Zhou J, Jiao N (2020) Development of a CRISPR/Cas9n-based tool for metabolic engineering of pseudomonas putida for ferulic acid-to-polyhydroxyalkanoate bioconversion. Commun Biol 3(1):1–13. https://doi.org/10.1038/s42003-020-0824-5

Zhuang Q, Qi Q (2019) Engineering the pathway in Escherichia coli for the synthesis of medium-chain-length polyhydroxyalkanoates consisting of both even-and odd-chain monomers. Microb Cell Fact 18:1–13. https://doi.org/10.1186/s12934-019-1186-x

Zhuang X, Wang W, Yu Q, Qi W, Wang Q, Tan X, Zhou G, Yuan Z (2016) Liquid hot water pretreatment of lignocellulosic biomass for bioethanol production accompanying with high valuable products. Biores Technol 199:68–75. https://doi.org/10.1016/J.BIORTECH.2015.08.051

Zotta T, Solieri L, Iacumin L, Picozzi C, Gullo M (2020) Valorization of cheese whey using microbial fermentations. Appl Microbiol Biotechnol 104(7):2749–2764. https://doi.org/10.1007/S00253-020-10408-2

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ORIGINAL ARTICLE

Optimization of acidic pre-treatment conditions using response surface methodology for ethanol production from *Pistia stratiotes* using *Saccharomyces cerevisiae* and *Pichia stipitis*

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Abstract
Bioethanol is a renewable chemical that can replace fossil fuels. *Pistia stratiotes*, an aquatic weed, was used as the substrate for the synthesis of ethanol in the current investigation. The presence of high amount of cellulose (25.90%) and hemicellulose content (18.44%) makes it suitable to produce bioethanol. Optimization of acidic pre-treatment conditions was performed using response surface methodology where independent variables acid concentration (0.15–3.1% H_2SO_4), time (12.96–97.0 min) and temperature (93.18–126.82 °C) were taken. The responses were recorded as sugar and cellulose concentrations. Subsequently, optimized condition was subjected to fermentation for the production of ethanol using *Saccharomyces cerevisiae* and *Pichia stipitis* strains. The maximum amount of sugar was produced under optimal conditions (2.5% acid concentration) at 120 °C for 15 min. Initial sugar was taken as 10 g/L in the hydrolysate, which is consumed by the strains with maximum sugar conversion rate (0.24 g/L/h). Maximum sugar consumption was observed by *S. cerevisiae* and *P. stipitis* and was 85.9% and 87.9%, respectively. Thus, we present that the optimized acidic pre-treatment conditions for maximum yield of ethanol from *Pistia stratiotes* using *S. cerevisiae* and *P. stipitis* were observed to be 0.37 and 0.39 respectively. Hence, this study showed an effective conversion of plant biomass into ethanol.

Keywords Bioethanol · Fermentation · Independent variables · Pre-treatment · Optimization · Sugar

1 Introduction

In the present scenario, energy demands are fulfilled by fossil fuels, which are rapidly depleted with the release of an excess number of greenhouse gases like CO_2 and CO during combustion. To overcome the problem for future perspective, searching for fossil fuel alternatives, such as biobutanol, bioethanol and biodiesel, that work in a sustainable manner for a clean environment will fulfil the energy demand [1]. Renewable and sustainable energy resources gained focus as an alternative to fossil fuels due to their lesser impact on the environment. Biomass from renewable feedstock attains interest in the research and development field due to its composition that contains a high amount of carbohydrates, proteins and lipids and cost-effectiveness [2]. Ecofriendly substrate obtained from plants is considered a renewable energy source with carbon-free emission [3]. At the industrial level, bioethanol has been produced by corn, wheat, sugar beet and sugarcane which are first-generation biomass. However, these feedstocks may have an unfortunate effect on the farmland, causing conflict in food and fuel crop generation. Lignocellulosic biomass falls under the second-generation feedstock, such as certain invasive plants, weeds and other lignocellulosic biomass from terrestrial and aquatic ecosystems. This material could be used as a carbon source in an economic way for energy production. USA, Russia, China, Germany, Brazil and India have taken action to generate bioenergy products from agriculture and industrial waste [4]. The Indian National Policy on Biofuels

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in 2018 provides a suggestive goal of attaining 20% ethanol blending with petrol by 2030 [5].

Bioethanol production is challenging due to high enzyme cost and technological barriers, such as biomass recalcitrance, diligent pre-treatment and inhibitory product formed during pre-treatment, which decreases the fermentation efficiency. The polysaccharides, cellulose and hemicellulose, present in lignocellulosic biomass, constitute 60% of its total mass and are reluctant to break it into simple sugar naturally [6]. The complex structure of plant cell walls prohibits the degradation of plant biomass into simple sugar. Different steps such as retreatment, hydrolysis and fermentation are required to convert the plant biomass into valuable products [7].

In recent decades, a lot of research is conducted on the optimization of processes for maximizing the production of valuable products from lignocellulosic biomass. Animal dung, agricultural waste and organic waste are examples of lignocellulosic biomass, which is pre-treated before being transformed into biogas. Due to the complex material's pre-treatment degradation into simple sugars, the results were incredibly effective [8]. Various pre-treatment methods are used for aquatic weeds, but 1% NaOH (alkali) pre-treatment is used for *Pistia stratiotes*, which increases biogas production [9]. Effective pre-treatment methods are required to increase the accessibility of substrate to the enzymes by changing the complex lignocellulosic structure into a simpler form [10]. The development of systematic pre-treatment methods resolves the initial obstacle in cellulosic ethanol production [11]. Many pre-treatment methods, viz. physical, mechanical and chemical (acidic and alkaline etc.) pre-treatment, have been applied to different types of lignocellulosic materials. The main motive of these pre-treatment methods is to solubilize the cell wall components (cellulose, hemicellulose and lignin). Acidic pre-treatment is much more effective compared to other pre-treatment methods as it does not require hydrolysis of the sample to solubilization of sugar reducing the energy consumption for the process [12]. H_2SO_4 is commonly used in pre-treatment due to its effectiveness in the removal of hemicellulose and lignin [13]. During acidic pre-treatment, lignocellulosic biomass is heated by an autoclave with a range of 100–120 °C, and during this procedure, some toxic compounds were generated, which can be minimized by adding the different chemicals [14].

Aquatic weeds present in freshwater bodies cause complications in irrigation and aquaculture projects. These weeds grow rapidly in the presence of proper nutrients and restrict the penetration of light on the lower surface of water bodies, which impairs the growth of aquatic biota. So it has become a topic of research to use it as a renewable carbon source for the production of valuable products in a sustainable manner [15]. The main advantages of these aquatic weeds are (1) they are an economical carbon source, (2) they have a higher CO_2 diminution impact as compared to the terrestrial plants and (3) they possess lower lignin content as compared to the terrestrial plants [16]. Extensive studies are conducted for the production of bioethanol from aquatic weeds due to these advantages.

Pre-treatment optimization gives a good direction in the field of bioethanol production. Besides pre-treatment, other barriers in biofuel processing are the commercialization of lignocellulosic biorefinery, feedstock organization and ample water consumption for the cultivation of weeds [17]. The aquatic weed *Pistia stratiotes* is composed of high carbohydrate content and can grow rapidly in the water. Therefore, it was selected as a substrate for ethanol production in our study. It was established in the earlier research on *Pistia stratiotes* that this weed's dry biomass produces the best results following pre-treatment. Following acid treatment, alkaline treatment was administered [18]. In our study, we have performed the optimization of acidic pre-treatment on this weed to get the maximum sugar, which was not done previously by any researcher. The parameters used for acidic pre-treatment can be optimized with the help of response surface methodology (RSM) design by Minitab software. The main objective of this model is to find out the main response in a particular area of interest, optimize the response, achieve the specific condition using a minimum number of experiments and observe the interaction between the parameters [19]. Combination of mathematical and statistical methods in RSM is used for making the model formation by which optimization of factors is done. The RSM method takes quantitative data from different experiments to estimate the regression model for optimization of responses (dependent variable) affected by process variables (independent variables) [20]. Central composite design (CCD) is a fractional factorial design consisting of 2^n factorial runs with $2n$ axial runs and centre runs that decide the experimental error. Independent variables determine the number of runs in the model. If the number of variables increases, the number of runs also increases in the replicates of the model. The RSM method for optimization consists of three steps. First step is designing of experiment statistically, the second step is to calculate the coefficients in the model and the last is to determine the response and examine the competency of the model within the design of the experiment [21].

The sugar obtained from the optimized condition was used for ethanol production. *Saccharomyces cerevisiae* and *Pichia stipitis* both strains were used for production. *Pichia stipitis* can use both C5 (e.g. xylose) and C6 (e.g. glucose) carbon sources present in the acid hydrolysate. This research makes a genuine contribution to the advancement of the technology used in the production of ethanol and may help in the development of an economical and sustainable approach for the production of ethanol by acidic pre-treatment.

2 Materials and methods

2.1 Collection and preparation of biomass

Pistia stratiotes was collected from a pond located at Hauz Rani City Forest, Delhi, India (28.5159° N, 77.2111° E). Average plant size was 7.57 cm, and after the collection of a sample, it is washed with tap water 4 times and then air dried in shadow at room temperature for 80 h. Air dried sample was put in the oven for 3 h at 40 °C and further ground using an electric grinder. Grounded samples were sieved with particle size 425 µm and stored in an air-tight jar for further use at room temperature [22].

2.2 Reagents

All the reagents used in this study were of analytical gradient grade. Sulphuric acid used for the acidic pre-treatment was purchased from Fisher Scientific, India. For the ethanol analysis, HPLC grade ethanol, purchased from Sigma-Aldrich, USA, was used in GC analysis.

2.3 Composition analysis of the biomass

Composition of biomass is essential to determine its capability for bioethanol production. Physicochemical analysis was performed on the raw sample through proximate analysis. The characterization of the sample was done by Fourier-transform infrared (FTIR) spectroscopy, TGA (thermo-galvanometric analysis) and scanning electron microscopy (SEM). The FTIR analysis and TGA were done at the Delhi Technological University, Delhi (India), while SEM was carried out at AIIMS, Delhi (India). The details for these analyses are mentioned below.

2.3.1 Determination of ash, total solids and moisture content

All of these compositions were done by the NREL protocols. Ash content of oven dried sample was estimated by the muffle furnace. In a pre-heated crucible, 1 g sample was burned at 575 °C for 6 h. Then, the crucible was put into a desiccator until it cooled down, and weight was recorded [23, 24]. The moisture content and total solid content were estimated by oven-dry procedure. The crucible was pre-heated at 105 °C for 4 h, and its weight was recorded. One gramme sample was put in the crucible at 105 °C for 4 h, then the crucible was transferred to the desiccator for cooling and the final weight was recorded [24].

$$\text{Total solids } (\%) = \frac{(\text{weight}_{\text{dry pan+dry sample}}) - \text{Weight dry pan}}{\text{Weight sample as received}} \times 100$$

$$\text{Total moisture}(\%) = 100 - \text{total solids}$$

2.3.2 Determination of cellulose content

Cellulose content was estimated by the Updegraff method [25]. In the first step, alcohol insoluble residue (AIR) was prepared using ethanol. This AIR was further mixed with the acid nitric reagent and then put in a water bath at boiling temperature for 30 min. It was washed with Milli-Q and then ethanol until the cellulose appears. This residue was then kept in the oven at 37 °C overnight. The next day, 67% H_2SO_4 was added to it and put on a shaker for the complete dissolution of cellulose. The cellulose estimation of the sample was done by anthrone reagent [26].

2.3.3 Determination of hemicellulose

Estimation of the hemicellulose content was done by fibre analysis method [27]. Difference between neutral detergent fibre (NDF) and acid detergent fibre (ADF) gives the total hemicellulose in a given sample. All the chemicals used in this experiment were procured from Fisher Scientific Ltd. One gramme sample is treated with 100 mL Neutral detergent solution with the addition of 2 mL decahydronaphthalene and 0.5 g sodium sulfite in refluxing flask for 1 h. The sample was then transferred to the pre-heated crucible. The sample was filtered and washed two times with Milli-Q and acetone consecutively. The sample was transferred to the hot air oven at 100 °C for 8 h and then put in a desiccator. On the other hand, for the determination of ADF, a 1 g sample is treated with an acid detergent solution in a refluxing flask for 1 h and the same procedure was followed as for NDF [28].

$$\text{Hemicellulose } (\%) = \text{NDF} - \text{ADF}$$

2.3.4 Determination of lignin content

This was performed by the NREL protocols. Before the estimation of the lignin, the extraction of the sample was performed using Milli-Q in 1:20 ratio [29]. Three hundred milligrammes extracted sample with 72% H_2SO_4 in a pressure tube was put in a water bath at 30 °C for 1 h with periodic stirring. Four percent dilution was done using Milli-Q, and the tube was autoclaved for 1 h. Pre-heated and labelled crucible was weighted, and the sample was filtered by a filtration unit in the crucible and then washed twice. After that, the sample was dried at 105 °C for 4 h and cooled in a desiccator, and acid insoluble residue (AIR) was calculated. Later, the crucible was transferred to a muffle furnace at 575 °C for 24 h to calculate the percentage of ash in the filter [30]. The lignin content (%) was

calculated using AIR (%)and ash in the filter (%). Acid-soluble lignin was calculated by measuring absorbance at 280 nm [31]. All the calculations were performed using the formulas mentioned below.

$$\%\ \text{AIR} = \frac{(\text{crucible plus AIR weight} - \text{crucible weight})}{\text{sample loading in crucible}} \times 100$$

$$\%\ \text{Ash in filter} = \frac{(\text{crucible plus ash weight} - \text{crucible weight})}{\text{sample weight}} \times 100$$

$$\%\ \text{Acid insoluble lignin} = \%\ \text{AIR} - \%\ \text{ash in filter}$$

$$\%\ \text{Acid} - \text{soluble lignin} = \frac{\text{UV}_{\text{absorbance (280nm)}} \times \text{volume of filtrate} \times \text{dilution}}{\text{absorptivity} \times \text{dry weight} \times \text{pathlength}}$$

2.3.5 Determination of starch

Starch was estimated by anthrone reagent. Chemical used in this composition was anthrone reagent which was prepared in 95% sulphuric acid, 52% perchloric acid and 80% ethanol. Initial step of this was done by washing the sample with 80% ethanol repeatedly till the colour of the residue was removed. Then, the residue was dried in a hot air oven, mixed with perchloric acid and incubated for 20 min. The tubes were centrifuged at 10,000 rpm for 10 min, and the supernatant was taken out. The glucose standard was prepared using 1 mg/mL stock solution. Anthrone is added to the supernatant of the sample and glucose standard and heated at boiling temperature. After heating, the reading was taken on a spectrophotometer at 630 nm for colour detection, and starch concentration was calculated [32].

2.3.6 Fourier-transform infrared (FTIR) spectroscopy

The changes in functional groups after providing the acidic pre-treatment were analysed by FTIR spectroscopy (PerkinElmer 400 FTIR/FTIR) control with a frequency range of 4000–400 cm^{-1} with the control sample. The pellet preparation was done with potassium bromide (KBr), and scanning was performed at 4 cm^{-1} resolution [33].

2.3.7 Thermogravimetric analysis (TGA)

The TGA of raw and pre-treated samples was performed by PerkinElmer TGA 4000. Samples were heated from room temperature to 600 °C at a heating range of 10 °C min^{-1} with 10 mL/min nitrogen flow rate [33, 34].

2.3.8 Scanning electron microscopy (SEM)

The changes in the morphology of the pre-treated sample were investigated with SEM (Model: EV018 Zeiss, Germany) with 5 kV voltage at × 10,000 magnifications. The breakdown of the cell wall was identified after SEM analysis [34].

2.4 Response surface methodology (RSM) experimental design

In this study, three independent variables (acid concentration (X_1, vol.%), time (X_2, min) and temperature (X_3, °C) with two dependent variables (reduced sugar (mg/mL) and cellulose (%))) as response were chosen for experiment design. These three independent variables along with their respective ranges were found to be critical parameters for maximum sugar production. It is expected in the design that the independent variable is managed by experiments with minor errors. The main aim of this model was to optimize the response variables (Y). This statistical model gives the approximate correlation between independent variables and dependent variables (responses) [35]. Experiments were run in random order for negligible error in the model. This empirical model is formed by the responses which show the correlation of independent variables with the help of a polynomial equation:

$$\text{Y} = \beta_o + \sum_{i=1}^{k} \beta_i \text{X}_i + \sum_{i=1}^{k} \beta_{ii} \text{X}_i^2 + \sum_{i<j}^{k} \beta_{ij} \text{X}_i \text{X}_j + e$$

where Y is the variable of response, β_o is the constant coefficient,$\beta_)$ is the linear constant, $\beta_{))}$ is the quadratic coefficients, $\beta_{)|}$ is the interaction effect of coefficients, $\text{X}_)$ and $\text{X}_|$ are the coded values used for variable parameters and ⎤ is the random error [36].

2.4.1 Design of pre-treatment experiments

Acidic pre-treatment was performed with sulphuric acid. The experiment design was done to optimize the conditions and determine the maximum reduced sugar liberation that was further used in ethanol production. Biomass sample was mixed with acid with a 10% biomass loading. Pre-treatment was done in an autoclave and neutralization of the sample is done by the $CaCO_3$ for the detoxification. After pre-treatment, the estimation of reduced sugar is done by the DNSA method, and cellulose is determined by the Updegraff method. Experiment design for pre-treatment was done by the central composition design (CCD), and the response surface methodology (RSM) approach was used to

Table 1 Composition analysis of *Pistia stratiotes*

Pistia stratiotes		
S. No	Biochemical composition	(%) of component
1	Total solid	92.85 ± 0.11
2	Ash content	18.36 ± 1.31
3	Moisture content	7.15 ± 0.11
4	Cellulose	25.90 ± 0.017
5	Hemicellulose	18.44 ± 0.71
6	Lignin	25.25 ± 1.15
7	Starch	0.6 ± 0.03

analyse the data [22]. Three variables, including temperature (93.18 °C, 100 °C, 110 °C, 120 °C and 126.82 °C), time (12.96, 30, 55, 80 and 97.04 min) and acid concentration (0.15%, 0.75%, 1.63%, 2.5% and 3.1%), were selected to find out the optimized condition. Reducing sugar and cellulose were recorded as a response for this design. A total of 20 runs in design with three levels (− 1, 0, + 1) with a 1.68 alpha value were performed.

After the pre-treatment, the detoxification of liquid residue was done by calcium carbonate ($CaCO_3$) powder. Toxic compounds may be retained in the hydrolysate, which can inhibit the microorganism's growth. After pre-treatment in an autoclave, the residue was centrifuged, and the liquid hydrolysate was separated from the solid fraction. This liquid fraction was detoxified by adding calcium carbonate to liquid hydrolysate, and pH was monitored by the amount of calcium carbonate addition [37].

2.5 Total reducing sugar analysis

Total reducing sugars in the liquid hydrolysate were determined by the 3,5-dinitrosalicylic acid (DNSA) method [38]. DNSA reagent was prepared and stored in an amber bottle for further use. Glucose stock solution was prepared for standard curve determination. This DNSA reagent was added to the standards as well as a sugar sample, boiled for 15 min and cooled down at room temperature. The samples' optical density (OD) was measured at 540 nm for the estimation of reducing sugar with the help of a standard curve [38]. The total reducing sugar of raw and pre-treated samples was quantified.

2.6 Fermentative process

2.6.1 Microorganisms

Saccharomyces cerevisiae (ATCC 834) and *Pichia Stipitis* (NCIM 3594) for ethanol production were procured from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune (India) in the slant

Table 2 Experimental design with three independent factors (time, temperature and acid conc.) with cellulose and sugar (dependent factors)

Experiments	Acid conc. (%)	Time (min)	Temperature (°C)	Cellulose (%)	Sugar (%)
1	3.1	55	110	19.81 ± 0.47	24.01 ± 3.87
2	0.75	30	120	10.88 ± 1.35	10.36 ± 1.21
3	2.5	80	120	24.55 ± 1.98	19.86 ± 0.0
4	1.63	12.96	110	14.46 ± 0.43	12.75 ± 0.74
5	2.5	30	120	22 ± 3.14	19.88 ± 1.31
6	1.63	55	110	16.02 ± 1.22	15.89 ± 0.34
7	0.75	80	100	9.31 ± 0.58	6.76 ± 0.13
8	1.63	55	110	17.7 ± 1.27	14.99 ± 0.27
9	2.5	30	100	12.84 ± 1.38	13.54 ± 3.82
10	1.63	55	126.82	17.6 ± 1.37	17.02 ± 0.89
11	1.63	55	110	14.9 ± 4.17	16.49 ± 0.52
12	1.63	55	93.18	10.93 ± 2.02	4.47 ± 0.16
13	1.63	55	110	15.56 ± 1.80	14.72 ± 0.28
14	0.75	80	120	14.1 ± 1.51	8.53 ± 0.35
15	1.63	55	110	16.63 ± 0.25	15.33 ± 0.18
16	1.63	97.04	110	15.2 ± 1.25	11.79 ± 0.10
17	2.5	80	100	15.17 ± 0.86	11.22 ± 0.15
18	0.75	30	100	8.45 ± 1.19	4.1 ± 0.11
19	1.63	55	110	16.79 ± 2.48	15.75 ± 0.50
20	0.15	55	110	4.91 ± 0.67	3.07 ± 0.02

form (active culture) at 4 °C. Both of the strains were maintained in MGYP media Petri plates.

2.6.2 Inoculum preparation

S. cerevisiae and *P. stipitis* were grown in the MGYP media (10 g/L glucose, 3 g/L malt extract, 3 g/L yeast extract and 5 g/L peptone) [39]. Seed culture was prepared in the conical flask with 50 mL of media, inoculated with a loopful of cells and incubated in an orbital shaker with 150 rpm at 30 °C. The mid-exponential phase culture was used to inoculate culture media with an optical density of around 0.8, which was measured at 600 nm in a UV–vis spectrophotometer [40]. The fermentative media were inoculated with 5% (v/v) of seed culture for ethanol production.

2.6.3 Fermentation for ethanol production

Fermentation was performed in a 250-mL shake flask with 150 mL working volume containing synthetic media (malt extract 0.3%, yeast extract 0.3%, peptone 0.5% and glucose 1%) and hydrolysate detoxified by $CaCO_3$ in triplet. For fermentation, 5% (v/v) of the seed culture was inoculated in detoxified hydrolysate. All the experiments were performed for 56 h. Sample collection was done periodically for the determination of cell growth, sugar consumption and ethanol production. The ethanol was separated by gas chromatography (Clarus 580, PerkinElmer, USA) equipped with a ZB-wax column (60 m × 0.32 mm internal diameter 0.25 µm; Phenomenex, UK). Oven temperature was set in between 150 and 180 °C. Ethanol data was captured from programmed software (Total Chrome Workstation Ver 6.3) that was pre-installed. The ethanol estimation in the sample was done by comparing it with the standard of ethanol (EMSURE ACS, Sigma-Aldrich, USA). All samples are performed in triplicates, and the concentration was mentioned in the grammes per litre [41].

Ethanol-related kinetic parameters The kinetic parameters related to ethanol yield were analysed based on the aforementioned reports [42–44].

$$\text{Ethanol yield(YE)} = \frac{\text{ethanol concentration}\left(\frac{\text{g}}{\text{L}}\right)\text{in fermentation media(Ef)} \times 1}{\text{sugar consumed } \left(\frac{\text{g}}{\text{L}}\right)}$$

$$\text{Volumetric ethanol productivity}\left(\frac{\frac{\text{g}}{\text{L}}}{\text{h}}\right) = \frac{\text{Ehanol concentation}\left(\frac{\text{g}}{\text{L}}\right)\text{in fermented broth}}{\text{fermentation time (h)}}$$

$$\text{Fermentation efficiency}(\%) = \frac{\text{ethanolyield(YE)} \times 100}{\text{theoretical ethanol yield}}$$

3 Result and discussion

3.1 Composition analysis of raw sample

In the *P. stratiotes*, the carbohydrate concentration is high so that it can be used as a carbon source for ethanol production (Table 1). The amount of cellulose is observed as 25.29% with a low level of lignin (16.73%) in comparison to another study by Sutaryo et al. The lignin composition is 34.85% [45]. In the present study, 18.44% hemicellulose was observed, while Mishima et al. reported 17.3% [46]. These variations in compositions could be due to cultivation conditions, age and location. Approximately 60% of biomass was composed of carbohydrates in this *P. stratiotes*. *P. stipitis* utilizes both pentose and hexose sugar from the hydrolysate by which maximum production of ethanol may be achieved. Considering the convenience and utilization, this plant was taken as a carbon source for bioethanol production.

3.2 Characteristics of pre-treated *Pistia stratiotes*

3.2.1 Acidic pre-treatment

Sulphuric acid pre-treatment has a high impact on the biomass for depolymerization of cellulose and hemicellulose with a maximum amount of sugar found, which is further utilized in the fermentation process for ethanol production. After performing all the experiments, maximum sugar was found at 3.1% acid concentration. After performing the optimization condition, range of sugar content was 3.07 to 24.01%, and the target was to find out 23% sugar content.

Table 3 Analysis of variance for coded coefficients

Source	DF	Adj SS	Adj MS	*F*-value	*P*-value
Model	8	570.888	71.361	34.48	0.000
Linear	3	501.680	167.227	80.80	0.000
Acid conc	1	358.453	358.453	173.20	0.000
Time	1	0.715	0.715	0.35	0.569
Temp	1	142.512	142.512	68.86	0.000
Square	3	61.914	20.638	9.97	0.002
Acid conc.*acid conc	1	8.007	8.007	3.87	0.075
Time*time	1	20.559	20.559	9.93	0.009
Temp.*Temp	1	43.310	43.310	20.93	0.001
2-way interaction	2	7.294	3.647	1.76	0.217
Acid conc.*time	1	1.256	1.256	0.61	0.452
Acid conc.*Temp	1	6.038	6.038	2.92	0.116
Error	11	22.765	2.070		
Lack of fit	6	20.678	3.446	8.26	0.018
Pure error	5	2.087	0.417		
Total	19	593.653			

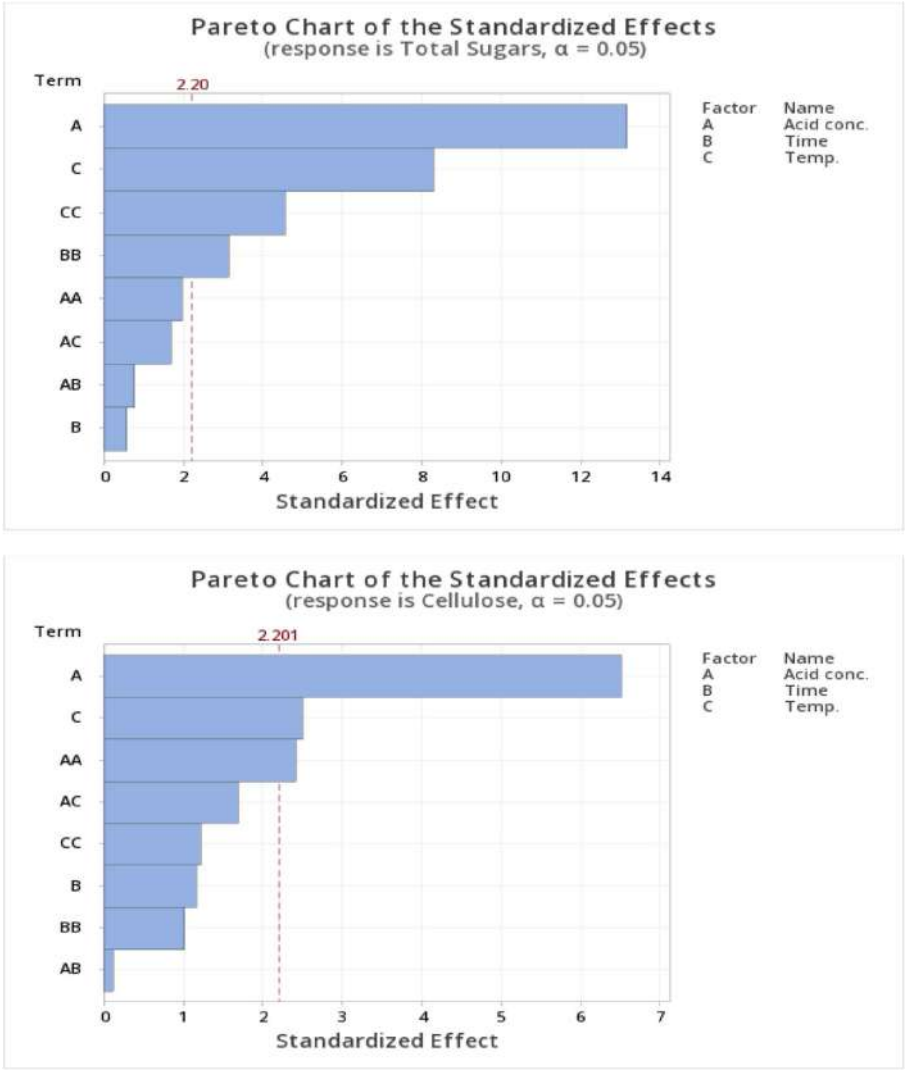


Fig. 1 Pareto chart shows standardized effect of independent factors on sugar and cellulose. Parameters of independent variable combination crossing the reference line shown by dotted red line are statistically significant

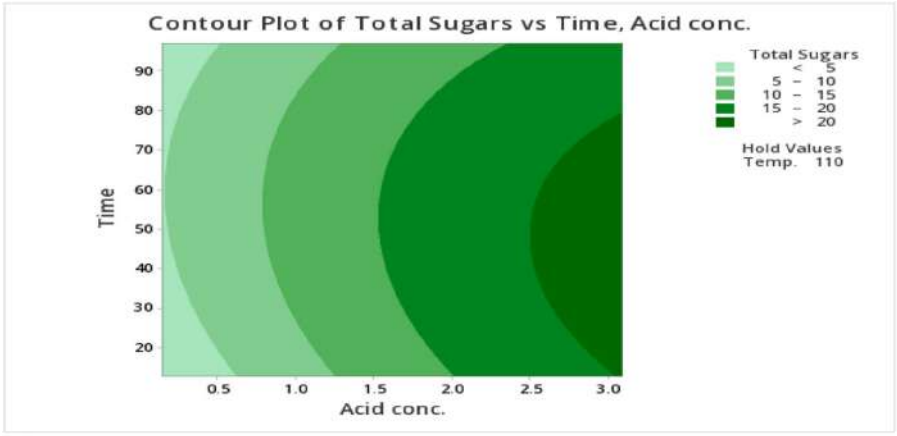
Similarly, range of cellulose content was 4.91 to 24.55%. After the optimization, the best condition was achieved as 2.5% sulphuric acid, 120 °C with a duration of 15 min. by autoclave. The outcome of this experiment was 23.44% sugar with 68% hydrolysate.

All the details of independence and responses from each experiment in experimental design are mentioned in Table 2.

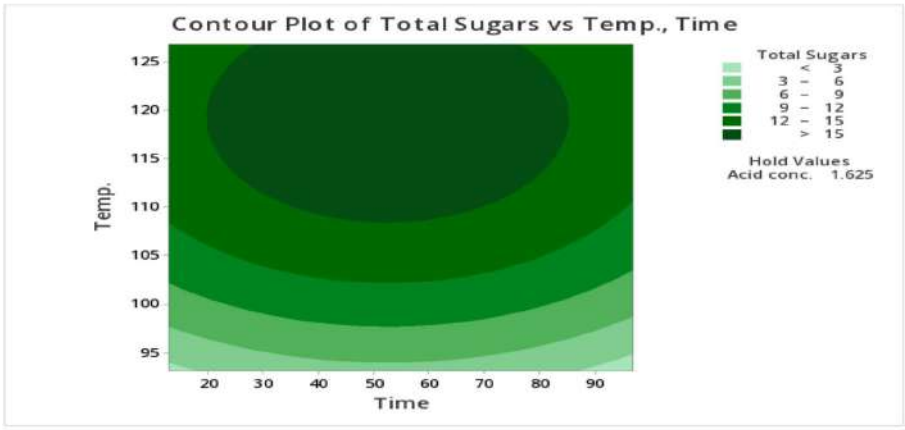
3.2.2 RSM model for pre-treatment

The coefficient of determination and statistical significance were estimated in the current study. The significance and analysis of variance for coded coefficients are represented in Table 3. The model for optimization is significantly based on the *P*-value. Model terms acid concentration, temp., square, time*time and temp.*temp were most significant in the optimization of sugar ($P < 0.05$). This regression model is performed with an alpha value of 1.68.

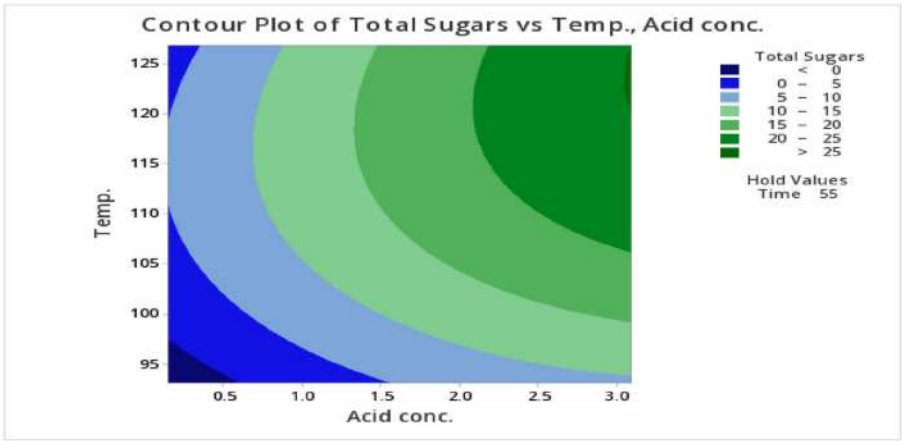
The R^2 (coefficient of determination) values are used to determine the goodness of fit of this statistical optimization model. In this model, the lack-of-fit *F*-value is 8.26 for optimization of the dependent variable (i.e. sugar), which is non-significant. This non-significance of lack of fit suggests that our model is reliable. The regression model in this study gives a regression equation that determines the relationship between the response (i.e. sugar) and the independent variable uncoded values (acid con., temp. and time). The regression equation is shown in Eq. 1.



(a)



(b)



(c)

Fig. 2 Contour plot shows the detailed information of independent and dependent variable correlation. **a** Total sugar vs. time and acid concentration at a constant temperature. **b** Total sugar vs. temp. and time at constant acid conc. **c** Total sugar vs. temp. and acid conc. at constant time

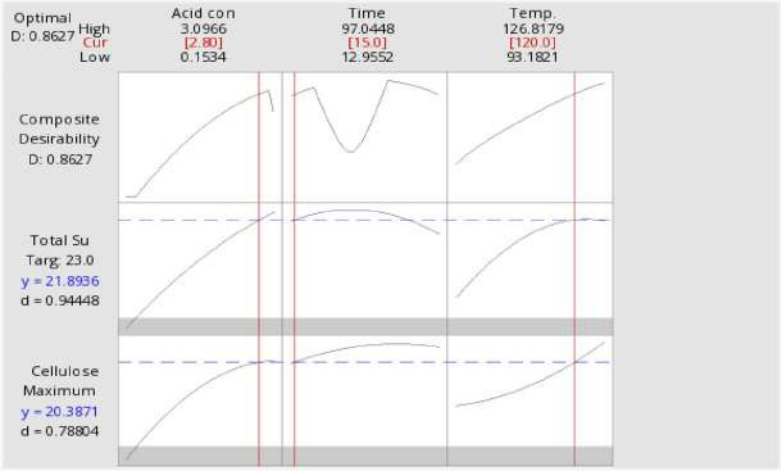


Fig. 3 Optimized condition after performing RSM shows that the composite desirability score is 0.8627 which is good definition that both responses are very close to the entire things in the model. Sugar has high desirability as compared to cellulose shown by the score of 0.94448 for sugar and 0.78804 for cellulose. Outcome of the result find out with 2.8% acid at 120 °C with 15 min

$$\begin{aligned}\text{Total sugars} = &-231.0 - (0.91 \times \text{acid conc.}) + (0.2305 \times \text{time}) \\ &+(3.976 \times \text{ Temp.}) - (0.974 \times \text{acid conc.} \times \text{ acid conc.}) \\ &-(0.001911 \times \text{time} \times \text{time}) - (0.01734 \times \text{Temp.} \times \text{ Temp.}) \\ &-(0.0181 \times \text{acid conc.} \times \text{ time}) + (0.0993 \times \text{acid conc.} \times \text{ Temp.})\end{aligned} \quad (1)$$

Equation no. 1: Regression equation in uncoded units R^2 value is 96.17% (*R*-squared is a statistical measurement of data which shows how close the data is to the regression line) with 93.38% adjusted R^2 value. From R^2 and R^2 (adj.) values, R^2 (pred.) predicted value was confirmed at 80.34% which indicates the regression model predicts the responses in new findings very well.

3.2.3 Pareto chart

The Pareto chart determines the significance of the effect of independent variables on total sugar and cellulose. This

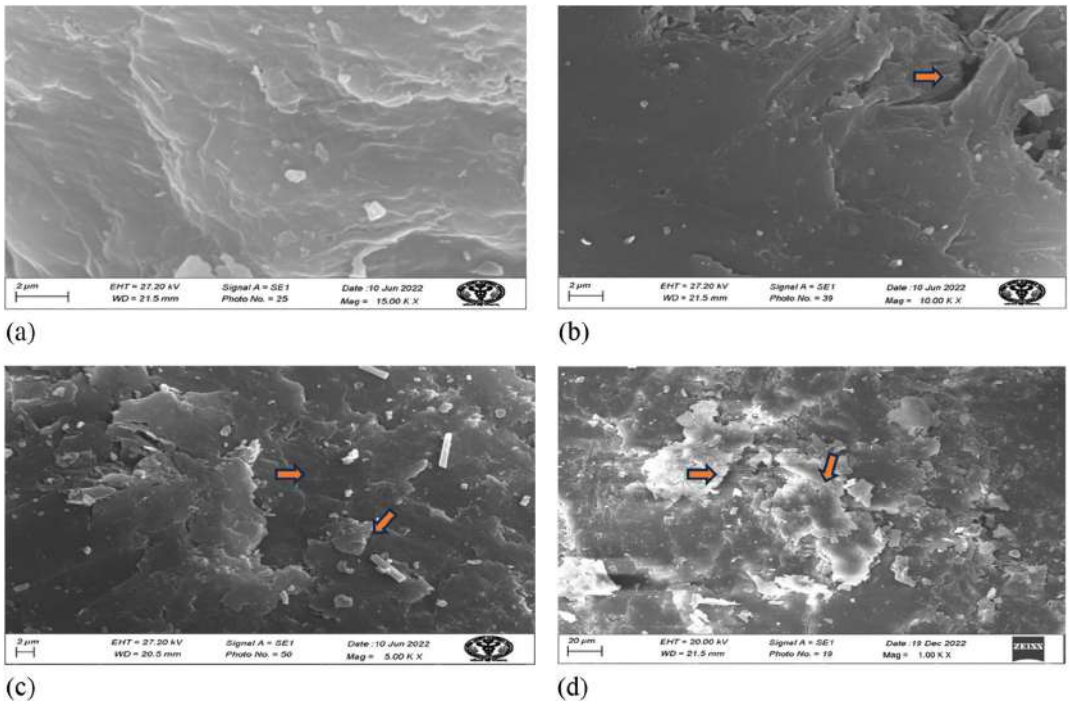


Fig. 4 SEM images of **a** raw biomass, **b** harsh pre-treatment conditions (2.5% acid, 80 min. with 120 °C), **c** mild pre-treatment conditions (0.75% acid, 30 min. with 100 °C) and **d** optimized condition

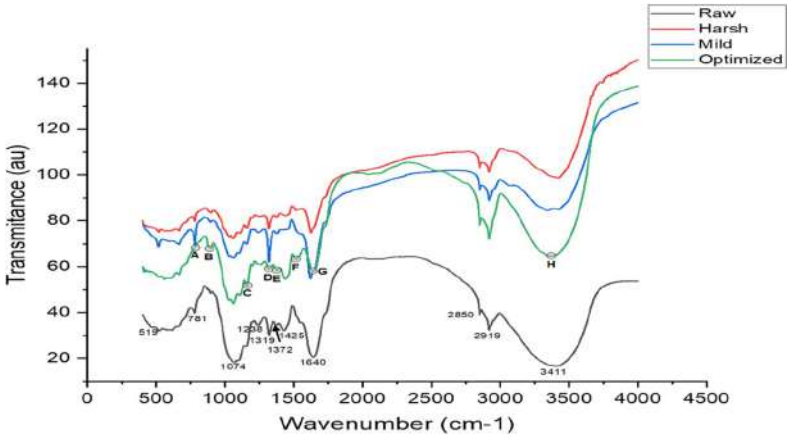


Fig. 5 FTIR spectra of raw, mild (0.75% acid, 30 min, 100 °C), harsh (2.5% acid, 80 min, 120 °C) and optimized pre-treated sample. These spectra show the changes in the functional groups after pre-treatment (A–H) denotes the changes in the peaks which shows the breakdown of the cellulose and removal of lignin

Pareto chart gives all the details of the independent variable in pictorial form. In the Pareto chart of the standardized effect for sugar, the reference line was at 2.20, whereas for cellulose, it was at 2.201. the parameters crossing the reference line were statistically significant. The outcome of the chart determines that the acid conc. (A), temperature (C), temperature*temperature (CC) and time*time (BB) are statistically significant at 0.05 level. On the other hand, significant effect of the independent variable on the cellulose shown in chart no. 2 in Fig. 1 shows acid conc. (A), temperature (C) and acid conc.*acid conc. (AA) are statistically significant. This statistical significance of the independent variable proves that this model is valid for the optimization of pre-treatment.

3.2.4 Contour plot

Contour plot represents the two-dimensional (2D) image of factors and responses and shows the relationship between the independent variable and dependent variables (Fig. 2). While keeping the temperature constant, sugar (> 20%) was obtained at a combination of acid concentration (2.5–2.6%) and time (0–75 min) (Fig. 2a). While keeping the acid conc. constant, sugar conc. (> 15%) was obtained at the temperature (110–125 °C) with time (20–85 min) (Fig. 2b). Similarly, while keeping the time constant, sugar conc. (20–25%) was obtained at acid concentration (2.2–3.0%) and temperature (107–125 °C) (Fig. 2c).

3.2.5 Optimization plot

After performing the optimization of acidic pre-treatment, an optimized condition was found in which independent variables are acid conc. 2.8%, time 15 min with 120 °C. This condition is performed separately to find out the reliability of the condition. After performing the optimized condition, maximum sugar was 23.44% in the hydrolysate which shows the reliability of the model. Minitab software creates a model in which independent variables are added to find the target response. Optimization is calculated by combined desirability ranges from 0–1 [47, 48]. For the optimization experiment, the separate desirability for the entire data is shown in Fig. 3. Sugar has an independent desirability result of 0.94448 as the predicted feedback of 21.8936 which is very close to the target of 23. Cellulose has a moderate desirability outcome that is 0.78804 as the predicted outcome of 20.3871 which is less than the target of 24.55 as mentioned in the parameter table. The composite desirability of 0.8627 is a good score which represents that both the responses were very close in their absolute settings. The optimization of responses did not give an ideal composite desirability score as the sugar and cellulose contents were not in absolute settings but in a justifiable range.

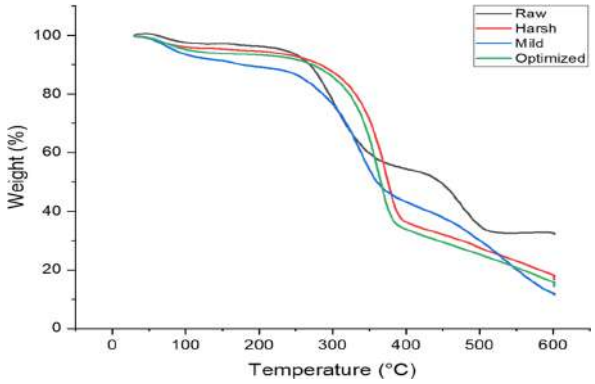


Fig. 6 Thermogravimetric analysis of raw (untreated), mild (0.75% acid, 30 min, 100 °C), harsh (2.5% acid, 80 min, 120 °C) and optimized pre-treated samples confirm the degradation of the compounds in the material or lose the stability of material at a particular temperature optimized condition have high degradation of cellulose and lignin

Table 4 Kinetic parameters for ethanol production by *S. cerevisiae* and *P. stipitis* stains with synthetic media and hydrolysate

Kinetic parameters	*Saccharomyces cerevisiae* in Synthetic media	*Saccharomyces cerevisiae* in hydrolysate	*Pichia stipitis* in synthetic media	*Pichia stipitis* in hydrolysate
Initial total sugar(g/L)	10.04 ± 0.29	10.04 ± 1.36	9.97 ± 0.76	9.99 ± 0.21
Maximum ethanol conc.(g/L)	3.32 ± 0.96	3.26 ± 0.71	3.57 ± 0.31	3.57 ± 2.02
Maximum time (hours)	30	36	32	36
Maximum sugar consumed (%)	97.6 ± 0.29	85.9 ± 1.34	94.9 ± 0.74	87.9 ± 0.21
Ethanol productivity(g/L/h)	0.11 ± 0.04	0.09 ± 0.02	0.10 ± 0.01	0.09 ± 0.06
Growth rate(g cells/L/h)	0.38 ± 0.01	0.30 ± 0.004	0.68 ± 0.03	0.56 ± 0.01
Maximum sugar consumption rate(g/L/h)	0.29 ± 0.01	0.24 ± 0.002	0.24 ± 0.001	0.24 ± 0.00
Ethanol yield coefficient (g/g)	0.39 ± 0.02	0.37 ± 0.06	0.41 ± 0.03	0.39 ± 0.01

The conditions found after this RSM model are mentioned in Fig. 3.

3.3 Characterization of raw and pre-treated biomass

3.3.1 Morphological changes in biomass (SEM analysis)

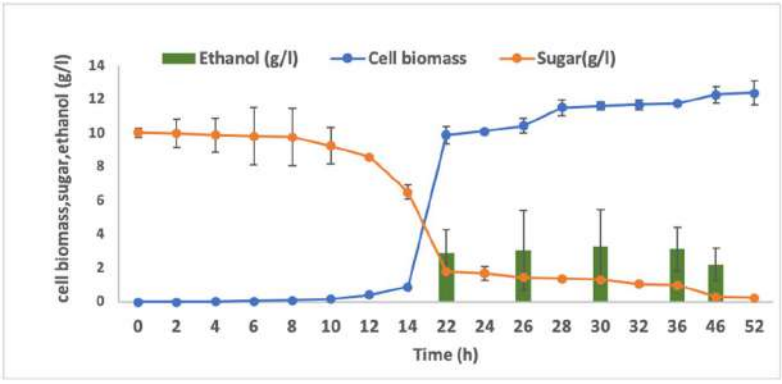
The morphology of *P. stratiotes* changes after acidic pre-treatment. It is more destructive after providing acidic pre-treatment to the sample as compared to the raw material. Maximum disruption was observed in optimized acidic conditions which is denoted by Fig. 4d. However, harsh conditions (2.5% acid conc.) also show a high amount of disruptions denoted by Fig. 4b. Mild condition (0.75% acid conc.) in pre-treatment is shown by Fig. 4c. These disruptions are due to the effect of temperature and acid by which smooth surface of the biomass is converted into the rough surface which shows that the bonds between cellulose and hemicellulose are broken and removal of lignin from the sample increases the surface area of the biomass to increase the accessibility to the enzymes for saccharification. This type of structural change is also seen in the case of elephant grasses when treated with sulphuric acid [49].

3.3.2 FTIR spectrum of raw sample and pre-treated sample

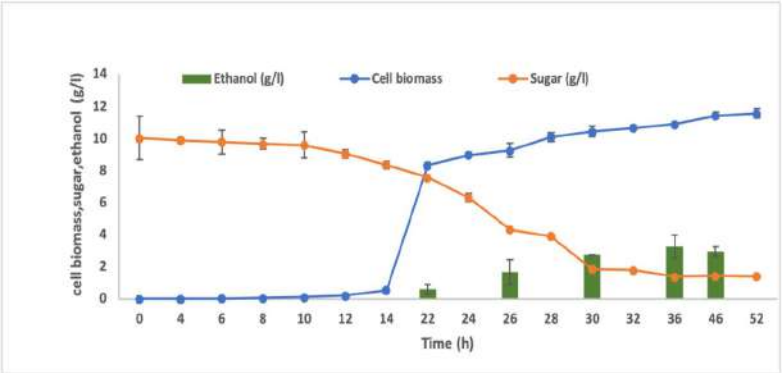
The FTIR spectrum provides detailed information regarding the functional group and its properties. The FTIR spectra of raw, mild (0.75% acid, 30 min, 100 °C), harsh (2.5% acid, 80 min, 120 °C) and optimized pre-treated samples of *P. stratiotes* (Fig. 5) showed a broad peak (H) around 3500–3200 cm^{-1} in the optimized condition, which shows N–H starching of primary amines and free O–H stretching of the OH group present in cellulose [50]. By acidic pre-treatment, these O–H bands were reduced [36]. The peak between 2848 and 2851 cm^{-1} in each pre-treated and raw sample represents the characteristic C–H stretching, which represents the vibration of cellulose [51]. The peak observed at 1640 cm^{-1} (G) shows water absorption, and in the optimized pre-treated condition, the intensity of the peak decreased due to aromatic skeletal vibration giving information regarding lignin removal [52]. In the pre-treated condition, the peak at 1515 cm^{-1} (F) relates to the aromatic skeletal stretching. Acidic pre-treatment reduced the adsorption of bands, but acid-soluble lignin occurred in the solid fraction of the pre-treated sample. The intensity of the peak at 1374 cm^{-1} (E) increased due to breakage in ether groups present in lignin [53]. As compared to the pre-treated sample, the peak at 1316 cm^{-1} (D) in the raw sample is more prominent due to the C–H ring vibration, indicating the presence of cellulose. CH_2 bending, which represents the cellulose with skeletal vibration of the C–C bond, shows the presence of cellulose [54]. The peak between 1200–1000 cm^{-1} (C) displays the presence of cellulose and hemicellulose in the sample with C–O starching and C–H rocking vibration correlating with cellulose structure [55]. The range of the aromatic band is 950–700 cm^{-1} corresponding to the β-glycosidic linkage between sugar units of cellulose and hemicellulose [15]. FTIR indicates the effective removal of other contents apart from cellulose by acidic treatment of *P. stratiotes*. The appearance of a peak at 894 cm^{-1} (B), an absorption peak for cellulose, in the pre-treated sample shows the C–O–C stretching in β-glycosidic linkage in cellulose and hemicellulose [56]. The peak intensity at 781 cm^{-1} (A) is decreased in the pre-treated sample as compared to the raw sample, showing the removal of lignin by the acidic treatment [57].

3.3.3 Thermogravimetric analysis (TGA)

Raw sample, mild (0.75% acid and 30 min at 100 °C), harsh (2.5% acid and 80 min at 120 °C) and optimized pre-treated sample (acid conc. 2.5% and 15 min. at 120 °C) were thermogravimetrically analysed to differentiate their degradation characteristics. *P. stratiotes* biomass contains four types of weight which can be degraded (moisture,



(a)



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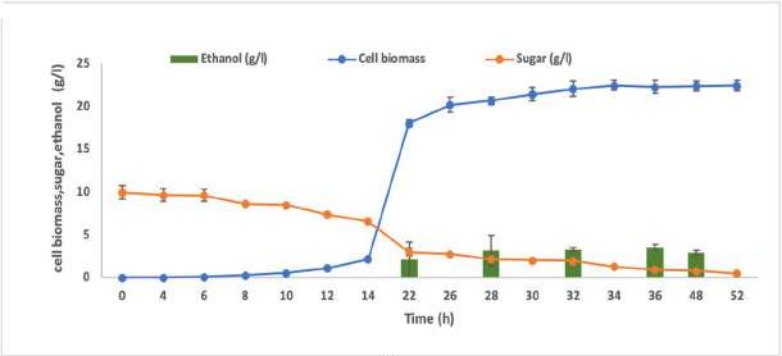

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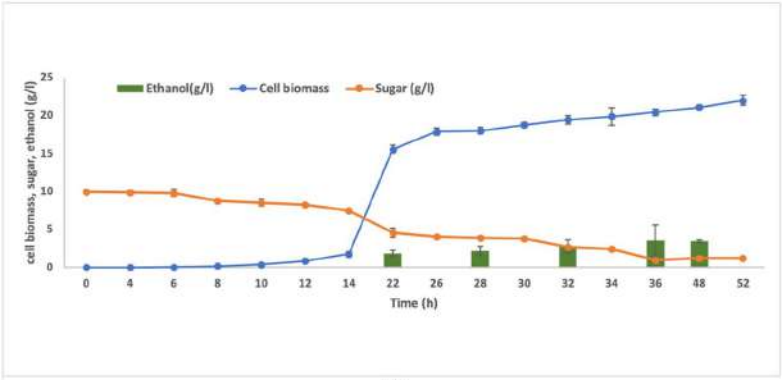

(d)

Fig. 7 The outcome of the fermentation process in four different conditions, using *Saccharomyces cerevisiae* **a** with synthetic sugar and **b** with hydrolysate and by using *Pichia stipitis* **c** with synthetic sugar and **d** with hydrolysate, was done by calculation of cell biomass, sugar consumption and ethanol production

Table 5 Comparison of different lignocellulosic biomass capability of conversion into ethanol

Biomass	Strains	Ethanol yield (g/g)	Reference
Lignocellulosic biomass	Engineered *Saccharomyces cerevisiae*	0.43	[61]
Water hyacinth	*S. cerevisiae*, *P. stipitis* and *C. lusitaniae*	0.32, 0.44, 0.49	[59]
Rubberwood waste	S. cerevisiae	0.14	[62]
Sugarcane bagasse	*S. cerevisiae*	0.41	[63]
Kans grass	*S. cerevisiae*	0.46	[64]
Pistia stratiotes	*S. cerevisiae* and *P. stipitis*	0.37 and 0.39	Present study

cellulose, hemicellulose and lignin) during the TGA shown in Fig. 5 [7]. In all samples, initial weight loss occurred at 100 °C due to evaporation of water. Temperature from 200–300 °C leads the way to depolymerization of hemicellulose and breakage of glycosidic bonds in cellulose. Unexpected loss of weight in mild conditions and untreated raw material at 250 °C is due to the degradation of hemicellulose. This was primarily degraded due to the presence of acetyl group as compared to cellulose and lignin. Lignin degradation occurs simultaneously at the temperature range 200–500 °C and range 250–400 °C is confirmed for cellulose degradation. Maximum weight loss of the pre-treated sample of approximately 53% occurs in the range of 250–400 °C due to degradation of cellulose. It is much higher as compared to the raw sample [34, 58], and on the other hand, optimized and harsh condition degradation goes simultaneously as the same pattern. Degradation in the optimized condition is seen maximum in Fig. 6.

3.4 Fermentation by Saccharomyces cerevisiae and Pichia stipitis

3.4.1 Analysis of ethanol by gas chromatography (GC-FID)

The retention time for the standard of ethanol was 3.61 min with a complete 10-min cycle. *S. cerevisiae* used in fermentation have the ability to utilize hexose for ethanol production, while *P. stipitis* has the capability for the usage of pentose and hexose for ethanol production. The fermentation was done with *S. cerevisiae* NCIM 3594 and *P. stipitis* NCIM 3498 separately to find out the maximum outcomes of the fermentation process. Fermentation done by *S. cerevisiae* and *P. stipitis* utilizes all sugar within 52 h. *P. stratiotes* hydrolysate after pre-treatment contained a maximum of 23.44% sugar, which was further used in the process of fermentation by *S. cerevisiae* and *P. stipitis*. Maximum glucose was consumed within 48 h and converted into ethanol. Starting sugar was taken at 10.04 g/L. The fermentation condition was carried out at 30 °C at 150 rpm. In the fermentation maximum, 86% sugar was consumed from the hydrolysate by the *S. cerevisiae*, while 88% sugar was consumed by *P. stipitis*. During the initial stage of fermentation, intake of sugar by the strains was slower, and cell growth increases after 14 h when sugar consumption increased. Maximum ethanol concentration was found by the *S. cerevisiae* in synthetic media in 30 h, while in hydrolysate, it comes in 36 h (3.25 g/L) with 0.37 g/g ethanol yield. On the other hand, *P. stipitis* strain utilizes both pentose and hexose sugars and the maximum ethanol concentration in synthetic media comes in 32 h, while in hydrolysate, it comes in 36 h (3.57 g/L) with 0.39 g/g ethanol yield (Table 4 and Fig. 7). The ethanol concentration in the hydrolysate was approximately similar the synthetic media because of detoxification of hydrolysate which removes high amount of toxic compound from the hydrolysate by which production of ethanol increased. In the Sunwoo et al. [59] (Table 5) study, *S. cerevisiae* gives less ethanol yield (0.32 g/g) as compared to the present study (0.37 g/g), and *P. stipitis* gives higher production (0.39 g/g) as compared to *S. cerevisiae*. This determines that both the stains give a very good amount of ethanol by this aquatic weed. The kinetic parameters of our study are comparable just like another study on sugarcane molasses [60].

4 Conclusions

P. stratiotes is used as a biomass for ethanol production because of its high carbohydrate content with a maximum amount of cellulose. Acidic pre-treatment enhances sugar content accessible to the microbes in the sample. In previous studies that used *P. stratiotes*, the optimization of acidic pre-treatment was not performed. Thus, we used the response surface methodology (RSM) analysis using Minitab statistical software to optimize acidic pre-treatment conditions to obtain maximum sugar that is affected by three independent variables, viz. acid concentration, time and temperature. The optimized condition (2.5% acid conc. at 15 min and 120 °C) was used to obtain hydrolysate with 23.44% reduced sugar, which was further detoxified with calcium carbonate and subsequently used for ethanol production. *S. cerevisiae* can only use hexose sugar for fermentation, while *P. stipitis* can use both pentose and hexose sugar. *S. cerevisiae* produced

ethanol conc. 3.32 g/L with 0.39 g/g ethanol yield from the synthetic media, and in the hydrolysate, ethanol conc. 3.25 g/L with 0.37 g/g ethanol yield was found. *P. stipitis* produced 3.57 g/L ethanol conc. with 0.41 g/g ethanol yield from the synthetic media, while in hydrolysate, 0.39 g/g ethanol yield was calculated. Bioethanol from lignocellulosic biomass is a sustainable approach as it is a requirement to replace fossil fuels.

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Author contribution All authors read and approved the final manuscript and also confirm the contribution to the research article: SM: investigation, methodology, data analysis and writing—original draft. RK: idea for the article, critically revised and editing the work and supervision. JGS: funding acquisition, resources and supervision.

Data availability Availability of data and materials could be provided if requested.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

References

1. Karunanithy C, Products KM (2011) Optimization of switchgrass and extruder parameters for enzymatic hydrolysis using response surface methodology. Ind Crops Prod 33(1):188–199
2. Sudhakar M, Ravel M, Perumal K (2021) Pretreatment and process optimization of bioethanol production from spent biomass of Ganoderma lucidum using Saccharomyces cerevisiae. Fuel 306:121680
3. Shrivastava A, Sharma RK (2023) Conversion of lignocellulosic biomass: production of bioethanol and bioelectricity using wheat straw hydrolysate in electrochemical bioreactor. Heliyon 9. https://doi.org/10.1016/j.heliyon.2023.e12951
4. Yadav KK, Krishnan S, Gupta N et al (2021) Review on evaluation of renewable bioenergy potential for sustainable development: bright future in energy practice in India. ACS Sustain Chem Eng 9:16007–16030. https://doi.org/10.1021/ACSSUSCHEMENG.1C03114
5. Kothari R, Vashishtha A, Singh H et al (2020) Assessment of Indian bioenergy policy for sustainable environment and its impact for rural India: strategic implementation and challenges. Environ Technol Innov 20:101078
6. Wyman CE, Yang B (2017) Combined severity factor for predicting sugar recovery in acid-catalyzed pretreatment followed by enzymatic hydrolysis. Hydrothermal Process Biorefineries Prod Bioethanol High Added-Value Compd Second Third Gener Biomass 161–180. https://doi.org/10.1007/978-3-319-56457-9_6
7. Kataria R, Woods T, Casey W et al (2018) Surfactant-mediated hydrothermal pretreatment of ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production. Ind Crops Prod 111:625–632
8. Olatunji KO, Ahmed NA, Ogunkunle O (2021) Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: a review. Biotechnol Biofuels 14. https://doi.org/10.1186/S13068-021-02012-X
9. Sinbuathong N (2019) Predicting the increase of methane yield using alkali pretreatment for weeds prior to co-digestion. Energy Sources, Part A Recover Util Environ Eff 41:1124–1131. https://doi.org/10.1080/15567036.2018.1544990
10. Baadhe R, Potumarthi R, Technology NM (2014) Influence of dilute acid and alkali pretreatment on reducing sugar production from corncobs by crude enzymatic method: a comparative study. Bioresour Technol 162:213–217
11. Mikulski D, Kłosowski G (2023) Cellulose hydrolysis and bioethanol production from various types of lignocellulosic biomass after microwave-assisted hydrotropic pretreatment. Renew Energy 206:168–179. https://doi.org/10.1016/j.renene.2023.02.061
12. Phwan CK, Chew KW, Sebayang AH et al (2019) Effects of acids pre-treatment on the microbial fermentation process for bioethanol production from microalgae. Biotechnol Biofuels 12:1–8. https://doi.org/10.1186/S13068-019-1533-5/TABLES/2
13. Dahunsi SO (2019) Liquefaction of pineapple peel: pretreatment and process optimization. Energy 185:1017–1031. https://doi.org/10.1016/J.ENERGY.2019.07.123
14. Hendriks A, Technology GZ (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100(1):10–18
15. Gusain R, Suthar S (2017) Potential of aquatic weeds (Lemna gibba, Lemna minor, Pistia stratiotes and Eichhornia sp.) in biofuel production. Process Saf Environ Prot 109:233–241. https://doi.org/10.1016/J.PSEP.2017.03.030
16. John R, Anisha G, Nampoothiri K, Technology AP (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 102(1):186–193
17. Kaur M, Kumar M, Singh D et al (2019) A sustainable biorefinery approach for efficient conversion of aquatic weeds into bioethanol and biomethane. Energy Convers Manag 187:133–147. https://doi.org/10.1016/j.enconman.2019.03.018
18. Whangchai K, Inta W, Unpaprom Y et al (2021) Comparative analysis of fresh and dry free-floating aquatic plant Pistia stratiotes via chemical pretreatment for second-generation (2G) bioethanol production. Bioresour Technol Reports 14:100651. https://doi.org/10.1016/J.BITEB.2021.100651
19. Myers RH, Montgomery DC, Anderson-Cook CM (2016) Response surface methodology: process and product optimization using designed experiments. Wiley
20. Montgomery DC (2017) Design and analysis of experiments. John wiley & sons
21. Mahalik K, Sahu JN, Patwardhan AV, Meikap B (2010) Statistical modelling and optimization of hydrolysis of urea to generate ammonia for flue gas conditioning. J Hazard Mater 182(1–3):603–610
22. Awoyale AA, Lokhat D (2021) Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production. Sci Rep 11:557. https://doi.org/10.1038/s41598-020-78105-8
23. Sluiter A, Hames B, Hyman D et al (2008) Determination of total solids in biomass and total dissolved solids in liquid process samples. Laboratory analytical procedure (LAP) (NREL)
24. Singh YD, Mahanta P, Bora U (2017) Comprehensive characterization of lignocellulosic biomass through proximate, ultimate and compositional analysis for bioenergy production. Renew Energy 103:490–500. https://doi.org/10.1016/J.RENENE.2016.11.039

25. Bauer S, Ibáñez AB (2014) Rapid determination of cellulose. Biotechnol Bioeng 111:2355–2357. https://doi.org/10.1002/BIT.25276
26. Kumar M, Turner S (2015) Protocol: A medium-throughput method for determination of cellulose content from single stem pieces of Arabidopsis thaliana. Plant Methods 11. https://doi.org/10.1186/S13007-015-0090-6
27. Wolfrum EJ, Lorenz AJ, deLeon N (2009) Correlating detergent fiber analysis and dietary fiber analysis data for corn stover collected by NIRS. Cellulose 16:577–585. https://doi.org/10.1007/S10570-009-9318-9/FIGURES/4
28. Holtzapple MT (2003) Hemicelluloses. In: Second E (ed) Caballero BBT-E of FS and N. Academic Press, Oxford, pp 3060–3071
29. Zhou Y, Zheng J, Gan R-Y et al (2017) Optimization of ultrasound-assisted extraction of antioxidants from the mung bean coat. Molecules. https://doi.org/10.3390/molecules22040638
30. Xia M, Valverde-Barrantes OJ, Suseela V et al (2022) Characterizing natural variability of lignin abundance and composition in fine roots across temperate trees: a comparison of analytical methods. New Phytol. https://doi.org/10.1111/NPH.18515
31. Nomanbhay SM, Hussain R, Palanisamy K et al (2013) Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield. J Sustain Bioenergy Syst 3:7–17. https://doi.org/10.4236/JSBS.2013.31002
32. Zorić AS, Morina F, Toševski I et al (2019) Resource allocation in response to herbivory and gall formation in Linaria vulgaris. Plant Physiol Biochem 135:224–232. https://doi.org/10.1016/J.PLAPHY.2018.11.032
33. Kataria R, Woods T, Casey W et al (2018) Surfactant-mediated hydrothermal pretreatment of ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production. Ind Crops Prod 111:625–632. https://doi.org/10.1016/J.INDCROP.2017.11.029
34. Umesh M, Santhosh AS, Shanmugam S et al (2022) Extraction, characterization, and fabrication of cellulose biopolymer sheets from Pistia stratiotes as a biodegradative coating material: an unique strategy for the conversion of invasive weeds into value-added products. J Polym Environ 30:5057–5068. https://doi.org/10.1007/S10924-022-02511-4/FIGURES/9
35. Gunaraj V, Technology NM (1999) Application of response surface methodology for predicting weld bead quality in submerged arc welding of pipes. J Mater Process Technol 88(1–3):266–275
36. Dahunsi SO, Adesulu-Dahunsi AT, Izebere JO (2019) Cleaner energy through liquefaction of cocoa (Theobroma cacao) pod husk: pretreatment and process optimization. J Clean Prod 226:578–588
37. Ahmed F, Yan Z, Bao J (2019) Dry biodetoxification of acid pretreated wheat straw for cellulosic ethanol fermentation. Bioresour Bioprocess. https://doi.org/10.1186/s40643-019-0260-x
38. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/AC60147A030
39. Bader NB, Germec M, Turhan I (2022) Repeated-batch fermentation of Scheffersomyces stipitis in biofilm reactor for ethanol production from the detoxified and glucose-or xylose-enriched rice husk hydrolysate and its kinetic modeling. Fuel 326:125053
40. Gonçalves FA, Ruiz HA, Silvino dos Santos E et al (2016) Bioethanol production by Saccharomyces cerevisiae, Pichia stipitis and Zymomonas mobilis from delignified coconut fibre mature and lignin extraction according to biorefinery concept. Renew Energy 94:353–365. https://doi.org/10.1016/J.RENENE.2016.03.045
41. Goswami RK, Sharma J, Shrivastav AK, Kumar G, Glencross BD, Tocher DR, Chakrabarti R (2022) Effect of Lemna minor supplemented diets on growth, digestive physiology and expression of fatty acids biosynthesis genes of Cyprinus carpio. Sci Rep 12(1):3711
42. Pereira SC, Maehara L, Machado CMM, Farinas CS (2015) 2G ethanol from the whole sugarcane lignocellulosic biomass. Biotechnol Biofuels 8. https://doi.org/10.1186/S13068-015-0224-0
43. Pooja NS, Sajeev MS, Jeeva ML, Padmaja G (2018) Bioethanol production from microwave-assisted acid or alkali-pretreated agricultural residues of cassava using separate hydrolysis and fermentation (SHF). 3 Biotech 8. https://doi.org/10.1007/S13205-018-1095-4
44. Mithra MG, Jeeva ML, Sajeev MS, Padmaja G (2018) Comparison of ethanol yield from pretreated lignocellulo-starch biomass under fed-batch SHF or SSF modes. Heliyon 4:885. https://doi.org/10.1016/J.HELIYON.2018.E00885
45. Sutaryo S, Sempana AN, Mulya RM et al (2022) Methane production of Pistia stratiotes as a Single substrate and as a co-substrate with dairy cow manure. Ferment 8:736. https://doi.org/10.3390/FERMENTATION8120736
46. Mishima D, Kuniki M, Sei K et al (2008) Ethanol production from candidate energy crops: water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes L.). Bioresour Technol 99:2495–2500. https://doi.org/10.1016/J.BIORTECH.2007.04.056
47. Yosrey E, Elmansi H, Sheribah ZA, El-Sayed Metwally M (2021) Factorial design-assisted spectroscopic determination of oxybutynin hydrochloride. R Soc Open Sci 8. https://doi.org/10.1098/RSOS.211027
48. Auwal SM, Zarei M, Tan CP et al (2018) (2018) Enhanced physicochemical stability and efficacy of angiotensin I-converting enzyme (ACE) - inhibitory biopeptides by chitosan nanoparticles optimized using Box-Behnken design. Sci Rep 81(8):1–11. https://doi.org/10.1038/s41598-018-28659-5
49. Kataria R, Mol A, Schulten E et al (2017) Bench scale steam explosion pretreatment of acid impregnated elephant grass biomass and its impacts on biomass composition, structure and hydrolysis. Ind Crops Prod 106:48–58. https://doi.org/10.1016/j.indcrop.2016.08.050
50. Lu P, Hsieh YL (2010) Preparation and properties of cellulose nanocrystals: rods, spheres, and network. Carbohydr Polym 82(2):329–336
51. Luzi F, Puglia D, Sarasini F et al (2019) Valorization and extraction of cellulose nanocrystals from North African grass: Ampelodesmos mauritanicus (Diss). Carbohydr Polym 209:328–337
52. Mandal A, Chakrabarty D (2011) Isolation of nanocellulose from waste sugarcane bagasse (SCB) and its characterization. Carbohydr Polym 86(3):1291–1299
53. Hsu T, Guo G, Chen W, Hwang W (2010) Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. Bioresour Technol 101(13):4907–4913
54. Jmel M, Anders N, Messaoud G et al (2019) The stranded macroalga Ulva lactuca as a new alternative source of cellulose: extraction, physicochemical and rheological characterization. J Clean Prod 234:1421–1427
55. Alemdar A, Sain M (2008) Isolation and characterization of nanofibers from agricultural residues–wheat straw and soy hulls. Bioresour Technol 99(6):1664–1671
56. Deng Z, Xia A, Liao Q et al (2019) Laccase pretreatment of wheat straw: effects of the physicochemical characteristics and the kinetics of enzymatic hydrolysis. Biotechnol Biofuels 12. https://doi.org/10.1186/S13068-019-1499-3
57. Malik K, Salama E, Kim T, Li X (2020) Enhanced ethanol production by Saccharomyces cerevisiae fermentation post acidic and alkali chemical pretreatments of cotton stalk lignocellulose. Int Biodeterior Biodegrad 147:104869
58. Kale RD, Taye M, Chaudhary B (2019) Extraction and characterization of cellulose single fiber from native Ethiopian Serte (Dracaena steudneri Egler) plant leaf. J Macromol Sci Part A Pure

Appl Chem 56:837–844. https://doi.org/10.1080/10601325.2019.1612252
59. Sunwoo IY, Kwon JE, Nguyen TH et al (2019) Ethanol production from water hyacinth (Eichhornia crassipes) hydrolysate by hyperthermal acid hydrolysis, enzymatic saccharification and yeasts adapted to high concentration of xylose. Bioprocess Biosyst Eng 42:1367–1374. https://doi.org/10.1007/S00449-019-02136-3
60. Sulaiman NMA, El-Mohsen A, Refaat A et al Kinetics of bioethanol production on the molasses-based medium by Saccharomyces cerevisiae. https://doi.org/10.21608/asajs.2022.228846
61. Tran PHN, Jung JH, Ko JK et al (2023) Co-production of ethanol and polyhydroxybutyrate from lignocellulosic biomass using an engineered Saccharomyces cerevisiae. Renew Energy 212:601–611. https://doi.org/10.1016/J.RENENE.2023.05.080
62. Nunui K, Boonsawang P, Chaiprapat S, Charnnok B (2022) Using organosolv pretreatment with acid wastewater for enhanced fermentable sugar and ethanol production from rubberwood waste. Renew Energy 198:723–732. https://doi.org/10.1016/J.RENENE.2022.08.068
63. Irfan M, Nadeem M, Syed Q (2014) Ethanol production from agricultural wastes using Sacchromyces cervisae. Brazilian J Microbiol 45:457. https://doi.org/10.1590/S1517-83822014000200012
64. Kataria R, Ghosh S (2011) Saccharification of Kans grass using enzyme mixture from Trichoderma reesei for bioethanol production. Bioresour Technol 102(21):9970–9975

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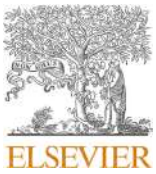

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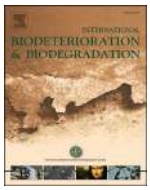

Enhancement in sugar extraction from *Pistia stratiotes* through statistical optimization of alkaline pre-treatment and enzymatic hydrolysis

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ABSTRACT

In lignocellulosic biomass, reducing structural recalcitrance and enhancing hydrolysis efficiency are crucial factors for increasing fermentable sugars and the production of valuable products. This biomass substrate comprises lignin, hemicellulose, and cellulose. In this study, response surface methodology was employed to optimise alkaline pre-treatment followed by enzymatic hydrolysis, aiming to enhance the saccharification of *Pistia stratiotes*. The NaOH concentration during pre-treatment significantly influenced the delignification process, resulting in increased cellulose content. The highest cellulose content was achieved with 2.47% NaOH at 120 °C for 60 min, leading to enhanced cell porosity and facilitating greater enzyme saccharification accessibility. Under these optimized conditions, the sample exhibited a 51.66% cellulose content. The physicochemical characteristics of the cellulose obtained after pre-treatment were analysed using SEM, FTIR, and TGA. After enzymatic hydrolysis of the cellulose with a commercially available cellulase enzyme, 31.06 g/L of reduced sugar was produced after 72 h. This study demonstrates that alkaline pre-treatment of *Pistia stratiotes* significantly increased its cellulose content, leading to a higher sugar yield during enzymatic hydrolysis.

1. Introduction

The primary issue facing the ecological niche that negatively affects aquatic and terrestrial life systems is environmental pollution. Fossil fuel consumption is the primary source of this pollution. The majority of environmental contaminants are hydrophobic so that they dissolve slowly in the water, which restrict microorganisms to utilize them as carbon source.(Gu, 2016).

The need for energy has grown significantly in recent years, placing additional strain on the use of fossil fuels, a resource that is running out quickly. In order to meet the demands of the world's expanding population, significant production and resource extraction will continue to accelerate due to the rising consumption of natural resources, particularly the non-renewable ones, which power most countries' economies today (Gu, 2020). Many scientists are employed worldwide in the field of waste-based renewable energy production By using greener and more innovative methods, renewable energy sources are displacing fossil fuels. Biofuel is a form of renewable energy obtained from sources such as agricultural waste, food waste, algae and municipal waste (Wannapokin et al., 2018). One likely way to achieve the Sustainable Development Goals (SDGs) is through the production of biofuels from lignocellulosic biomass (LCBs) (Nazari et al., 2021). LCBs contain secondary cell wall enriched with sugars that are feasible, sustainable and economically directed to the production of valuable products such as biofuels, polymers and biochemicals (Culaba et al., 2022). Currently United States and Brazil are front runners in bioethanol production using corn as a substrate, thereby creating the deficiency of food crops (Tse et al., 2021). Both on land and in water, a variety of invasive species can be used as a substrate for the long-term, sustainable production of valuable products. These lignocellulosic invasive weed species serve as an alternative to food crops in the same capacity. A lot of work has gone into using terrestrial lignocellulosic substrates to produce reduced sugars that microbes can use to produce biofuels and biogas (Moerman, 1996). The inability of lignocellulosic biomass to grow quickly due to a lack of agricultural resources and land presents a barrier to the industrial production of bioenergy (Chen et al., 2015). Aquatic biomass could be utilised as a substrate for the synthesis of biofuel to address the aforementioned issue. The advantage of aquatic weed over terrestrial lignocellulosic biomass is its lower lignin content and faster growth rate. Because of their components, a variety of aquatic plants have the

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potential to produce bioethanol.

Pistia stratiotes (water lettuce) is a monocotyledonous weed found in fresh water. It can obstruct the water channel due to its dense growth connected with the root nodules. This weed's primary drawback is that it can deteriorate the quality of the water, which is why it's considered an invasive weed. When used as a substrate for the production of bioethanol, this weed's drawback can be turned into a useful resource for financial gain (Jayanth, 2000). Early in the 1990s, *Pistia stratiotes* was discovered to be a valuable carbon source for the creation of products like biogas. In addition to the weed's anaerobic digestion, which yields biogas, continuous fermentation also forms an alternative by producing bioethanol. (Yang et al., 2022). Harvesting the aquatic weeds is the first stage in turning them into biofuels. Compared to alternative strategies, mechanically more efficient methods can be used to remove these weeds (Alam et al., 2021).

Lignocellulosic substrates are highly recalcitrant as a consequence of lignin along with cellulose and hemicellulose in the cell wall of plants. For the saccharifying enzymes to access the substrate, this is a significant barrier. Pre-treatment is necessary to get rid of lignin and hemicellulose and make cellulose more accessible to hydrolysing enzymes in order to improve the saccharification from lignocellulosic biomass (Fillat et al., 2017). Pre-treatment raises the biomass porosity by eliminating lignin and hemicellulose (Asghar et al., 2015). Pre-treatment can be categorized as biological (fungi) like white-rot fungus (Saha et al., 2016), physical (milling, extrusion, microwave heating), chemical (alkali, acidic, ionic, and with organic solvents) and physiological (CO_2 explosion and wet oxidation)(Aguilar-Reynosa et al., 2017). However, none of these meet the requirements in terms of inherent advantages and drawbacks. Thus, to improve the effectiveness of the conversion process, a valuable pre-treatment procedure is evaluative. When fungi are co-cultivated during a biological pre-treatment, their combined growth can sometimes inhibit the growth of the individual fungi, but it also has a synergistic effect on lignin degradation and the expression of lignolytic enzymes (Meehnian et al., 2017).

Alkaline pre-treatment is a more intently studied technique and has many advantages, such as effective lignin solubilization in contrast to other pre-treatments. Alkaline pre-treatment is used for delignification with minimal loss of reduced sugar and unescorted by inhibitory compound formation (Alvira et al., 2010). Saphonication and solvation are the causes of swelling in alkaline pre-treatment. The lignin and hemicellulose eater bonds break down as a result of the saponification reaction. The surface area of cellulose increases as the polymerization and crystallinity decrease as a result of the swelling of the biomass. Alkaline concentration, reaction temperature, and reaction time are some of the parameters that need to be optimized in order to get the most out of the alkaline pre-treatment (Kim and Han, 2012).

Response surface methodology (RSM), a statistical tool, is used for the optimization process, which involves combining the results of each experiment to optimise multiple variables under various experimental conditions (Ali et al., 2015; Manmai et al., 2020). It is a statistical tool that draws on scientific techniques such as analytical and classical methods, producing responses that are influenced by a variety of variables and that ultimately relate to optimization. Numerous lignocellulosic biomass, including rice straw grass, switchgrass, and corncob, are optimized using RSM (Başar et al., 2021; Gundupalli et al., 2022; Selvakumar et al., 2022). The most popular and frequently utilised RSM design is the central composite design (CCD). Enzymatic hydrolysis requires cellulase enzyme for conversion of cellulose into reduced sugar. The source of carbon has the lowest concentration limit at which the balance between newly produced and dead cells keeps the amount of living cells relatively constant (Gao and Gu, 2021).

Therefore, the main goal of this investigation was to optimise the alkaline pre-treatment of *P. stratiotes* starting from raw material to enzymatic hydrolysis. To our knowledge, no pretreatment study on *P. stratiotes* has been reported. The content of cellulose is affected by three distinct factors: temperature, time, and alkali concentration. Central composite design (CCD) and factorial design were used to optimise the pre-treatment process. Fourier transform infrared spectra (FTIR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) were used to further characterise the recovered cellulose fraction following the pre-treatment. The commercially available cellulase enzyme was used to hydrolyse the recovered solid fraction (cellulose). The estimation of reduced sugar was computed after hydrolysis. It is possible to conclude from the study's observations that this is the first study to optimise the alkaline pre-treatment of Pistia stratiotes using RSM, resulting in a higher cellulosic content.

2. Materials and methods

2.1. Collection and preparation of sample

Pistia stratiotes, commonly known as water lettuce, were harvested in August 2022 from a local pond in Delhi, India's Hauz Rani City Forest (28.51590 N, 77.21110 E). The harvested material included all plant parts: roots, stems, and leaves, with an average plant size of 35 cm. Subsequent to collection, the sample underwent a dual cleansing process with tap water to eliminate any impurities. Following this, it was allowed to air-dry for 48 h at room temperature. The air-dried sample was further dehydrated in an oven at 45 °C overnight and subsequently ground using a grinder. The resulting finely ground sample was stored in an airtight polybag for future use.

2.2. Sample composition analysis

2.2.1. Quantification of cellulose

To estimate cellulose content, the Updegraff method was employed. Initially, for washing 70 mg sample was incubated in 1.5 ml of 70 % ethanol at 70 °C for 1 h and then removed out ethanol by an aspirator twice. After that 1 ml of acetone was added for 2 min and then aspirated out and put the sample in a fume hood for 3–4 h, keep the sample in the oven at 37 °C overnight, resulting in what is known as alcohol-insoluble residue (AIR). This sample was stored at room temperature for further use. This AIR was then subjected to a 30-min treatment at 100 °C in a water bath using 3 ml Acetic nitric reagent (80% glacial acetic acid: 10 % nitric acid: 20 % Milli-Q). Following this treatment, the sample underwent rinsing with ethanol and water until cellulose became apparent. After that 4 ml acetone was added to the sample and evaporated in a fume hood. The resulting residue was subsequently placed in an oven overnight at 37 °C. Subsequently, 1 ml of 67 % H_2SO_4 was added to the sample, and the mixture was placed in a shaker for thorough mixing. After the cellulose was effectively mixed with the acidic solution then glucose standards were made from 100 mg/ml stock solution. Anthrone reagent was prepared by mixing 0.3 % anthrone in H_2SO_4. For testing 500 μl sample and blank take out in the Eppendorf and add 1 ml anthrone reagent in each, now boil each for 5 min and take the OD at 620 nm. The anthrone test was employed to estimate the amount of glucose present in the cellulose (Kumar and Turner, 2015).

2.2.2. Hemicellulose and lignin

The fibre analysis method was used to estimate hemicellulose. This methodology starts with the estimation of neutral detergent fibre (NDF), moves on to the calculation of acid detergent fibre (ADF), and subtracts ADF from NDF to find the concentration of hemicellulose (Holtzapple, 2003). National Renewable Energy Laboratory (NREL) protocols are followed for the lignin estimation. The first step in this process was the extraction of the sample with the help of milli Q water using probe sonicator (Branson Sonifier W450 Digital, tip size 6.5 mm) (Zhou et al., 2017). The resulting extracted sample was treated with 72 % H_2SO_4 in the water bath for 1 h. It was then diluted with 4 % milli Q and autoclaved for 1 h at 121 °C and 15 psi. After that, the sample was filtered using a vacuum filtration unit in a crucible that had been preheated. After being dried at 105 °C for 4 h, the residue was weighed and

designated as acid-insoluble residue (AIR). Then the crucible was transferred into muffle furnace at 575 °C for 24 h and weighed once more. The outcome obtained after burning the sample in the muffle furnace gives % of ash in filter (Xia et al., 2022). Acid-soluble lignin was calculated by measuring absorbance at 280 nm (Nomanbhay et al., 2013). All the calculations were performed using the formulas mentioned below Eqs. (A.1, A.2 and A.3).

Eq.(A) was used to calculate the lignin.

$$\%\ AIR = \frac{(Crucible\ plus\ AIR\ weight - Crucible\ weight)}{Sample\ loading\ in\ Crucible} \times 100$$

$$\%\ Ash\ in\ filter = \frac{(Crucible\ plus\ ash\ weight - Crucible\ weight)}{Sample\ weight} \times 100$$

$$\%\ Acid\ insoluble\ lignin = \%\ AIR - \%\ Ash\ in\ filter \quad \text{Eq.(A.1)}$$

$$\%\ Acid\ soluble\ lignin = \frac{UV_{absorbance\ (280nm)} \times Voulme\ of\ filtrate \times dilution}{Absorptivity \times dry\ weight \times pathlength} \quad \text{Eq. (A.2)}$$

$$\%\ Lignin = \%\ Acid\ insoluble\ lignin + \%\ Acid\ soluble\ lignin \quad \text{Eq. (A.3)}$$

2.3. Sample characterization

2.3.1. Fourier-transform infrared (FTIR) spectroscopy

The changes in functional groups after alkaline pre-treatment were determined through the use of FTIR spectroscopy (PerkinElmer 400 FTIR/FTIR). With the control sample, the spectra span the frequency range of 4000 cm^{-1} to 400 cm^{-1}. Pellet preparation involved the use of potassium bromide (KBr), and scanning was done at a resolution of 4 cm^{-1} (Kataria et al., 2018a).

2.3.2. Thermogravimetric analysis (TGA)

The TGA of raw and alkaline pre-treated samples was performed by PerkinElmer TGA 4000. The samples were heated using a nitrogen flow rate of 10 mL/min and a heating range of 10 °C min^{-1} from room temperature to 600 °C (Kataria et al., 2018b; Umesh et al., 2022)

2.3.3. Scanning electron microscopy (SEM)

The changes in surface and fibre morphology of pre-treated sample were examined using Scanning Electron Microscopy (SEM) (Model: EV018 Zeiss, Germany) with 5 kV voltage at 10,000× magnification. SEM analysis was used to identify the structural alterations following cell wall breakdown (Umesh et al., 2022).

2.4. Optimization of pre-treatment using RSM

RSM is a multivariant statistical tool that provides a new approach for determining the ideal pre-treatment state. Design Expert software assisted in the development of a central composite design, which was used to determine the effects of various independent variables on cellulose extraction. Temperature (60, 90, and 120 °C), time (30, 45, and 60 min), and NaOH concentration (0.5, 1.75, and 3 %), were the variables. RSM was responsible for determining the results. The variables in this model were represented by codes, which were represented by the numbers −1,0, and 1. The neighbouring distance from the value of the central point determines how variables are coded. The experiment's independent variables, such as the temperature, time, and NaOH concentration, were transformed into code variables Xi (Eq. (B.1))

$$Xi = 2\left(\frac{OV - V}{\Delta}\right) \quad \text{Eq. (B.1)}$$

Equation 2 comprises the following terms:

Δ (difference between largest and smallest values (range)), OV (original variable), and V (average of the largest and smallest values of variables or mid value). Three independent variables with lower and higher levels are mentioned in Table (A.1).

2.4.1. Statistical analysis and model fitting

The statistical tool known as Analysis of variance (ANOVA) was employed for divination of the statistical factors required in the assessment of the RSM model between the independent variables and response of parameters. Utilising the software Design Expert, regression analysis of trial data was employed to generate the contour plot, pareto chart, and optimized condition. The influence of independent variables on the cellulose concentration is shown by the contour plot and pareto chart. The outcome of the model predicted statistically significant values by F test and p value. If the value was less than 0.05, then the model was stated as statistically significant with lack of fit test that is insignificant in the same model. Independent factors significantly impacting the response were determined by p test value less than 0.05 with confidence level above 95 %. Significance of the model was accessed by R^2 predicted and R^2 adjusted value (Ramaraj and Unpaprom, 2019). To determine the ideal condition divided into linear, quadratic, and interactive components, the effects of independent variables on the dependent variable (response) were estimated using a polynomial equation. Eq. (C.1) displayed the entire cellulose concentration estimation.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + \epsilon \quad \text{Eq. (C.1)}$$

where Y is the predicted response (cellulose concentration); β_o the intercept; β_i the linear constant; β_{ii} the quadratic coefficients; β_{ij} the interaction effect of coefficients; X_i and X_j the coded values used for variable parameters; and ϵ is the random error (Dahunsi et al., 2019).

2.5. Enzymatic hydrolysis

After pre-treatment, 10 ml of citrate buffer (0.05M, pH 4.8) was added to 150 ml test tubes containing 0.5 g of both pre- and untreated samples for enzymatic hydrolysis. The commercially available cellulase enzyme (40000 U/g) was sourced from IndiaMart, and the Filter Paper Unit (FPU) was calculated following NREL protocols. The cellulose loading was adjusted to 25, 50, and 75 FPU/g of dry biomass.

The hydrolysis was carried out in an incubator shaker (New Brunswick Innova 44 series, Germany) with a rotation speed of 150 rpm for 96 h at 50 °C. Samples were withdrawn at 24, 46, 72, and 96 h. The reducing sugar was quantified using the DNSA method as described by Liu et al. (2021). After enzymatic hydrolysis, the sugar yield was determined using Eq. (D.1), and each experiment was conducted in triplicate.

$$Sugar\ yield\ (\%) = \frac{0.9\ X\ reducing\ sugar\ \left(\frac{g}{L}\right) X\ volume\ (L)}{solid\ cellulose\ fibre\ wt.\ (g)}\ X\ 100 \quad \text{Eq. (D.1)}$$

2.6. Reducing sugar extraction

The 3, 5-Dinitrosalicylic acid (DNSA) method was used to estimate the total amount of reducing sugar (Miller, 1959). After preparation of DNSA reagent (10 g 3,5 DNS, 0.5 g sodium sulphate, 182 g sodium potassium tartrate, 10 g sodium hydroxide and 2 g phenol in 1 L), it was kept in an amber bottle for storage. For the standard curve, a stock solution of glucose standard was prepared. Initially, 1 ml DNSA reagent was added to the sample, glucose standards and a blank and put in the water bath and left for 15 min. Then the samples were removed from the water bath and 5 ml milli Q was added for dilution to take the OD of each sample to quantify reduced sugars, the optical density of the samples was measured at 540 nm using a glucose standard.

3. Results

3.1. Composition analysis of the sample

P. stratiotes can be used as a good carbon source for the production of value-added products. In the raw sample, the estimated contents of cellulose, hemicellulose, and lignin were 25.90 %, 18.44 % and 25.25 % respectively. The composition analysis proves that in this weed, a high amount of cellulose content was found compared to hemicellulose and lignin. After the chemical pre-treatment, the composition analysis showed that it might be a useful carbon source for the production of bioethanol. By applying the alkali pre-treatment to remove the lignin content from the raw sample, the cellulose content could be increased.

3.2. Optimization of alkali pre-treatment and validation

After pre-treating *P. stratiotes* with alkali (NaOH), which had an impact on the raw material's depolymerization, cellulose was the final product, with lignin removed. The reduced sugar obtained from the hydrolysis of cellulose can be used to further produce ethanol. The best conditions were achieved by optimising biomass using RSM, which is 2.47 (w/v) NaOH at 120 °C for 60 min after autoclaving. The optimal condition gives the result of 51.66 % cellulose. The result of the alkali pre-treatment under various conditions is cellulose, which is shown in Table (A.2).

The factors that affect the expected changes in the mean response when the factors change from a lower to a higher level, which also establishes the coded coefficients. The alkali concentration shows major positive impact so that this parameter has an effect on increased response (cellulose concentration) proceeding from 0.5 % to 3 %. If the alkali concentration is higher, then the cellulose concentration will also be enhanced. However other factors like time, temperature, alkali conc. *alkali conc. And alkali conc.*time, shows the negative sign which successively impacts on reducing the response.

Table (B) illustrates an ANOVA statistical model for cellulose estimation from CCD. ANOVA and the lack of fit test are used to analyse the model's fitness. When the experimental data fits the model, significant regression and non-significant lack of fit are displayed. This model's statistical advantage was evaluated and attributed to the interactions between the model's factors and p-value. Table (B) shows that the model's p-value for cellulose concentration is 0.000, which is less than 0.050 and suggests that the response to the model is substantial and meaningful. The model is considered highly significant when the p-value is less than 0.001. A p-value greater than 0.1 indicates that the model is not significant. However, because of the large f value (53.79), the model result has a significant impact on the response. This f-value is probably going to demonstrate the significance of the model. There is only a 0.01 % chance in the statistical model that a large f value could arise from (Pashaei et al., 2020).

3.2.1. Model summary

The adjusted R^2 of 96.15 % and the predicted R^2 value of 92.48 % agree rationally, indicating a difference of less than 4 %. This result demonstrates that this model can accurately represent the percentage of cellulose extraction from *P. stratiotes* and can be shown as coded variables, as shown in Eq. (E.1).

3.2.2. Regression equation in Uncoded units

Cellulose = 64.90 + 12.18 Alkali conc. - 0.986 Time - 0.697 Temp.

−2.409 Alkali conc.*Alkali conc.+ 0.00465 Time × Time +0.001267 Temp.*Temp.

−0.1395 Alkali conc.*Time +0.0675 Alkali conc.*Temp. +0.01109 Time × Temp. Eq.(E.1)

3.2.3. Contour plot

The contour plot describes the impact of different variables on the response, and change in cellulose content depending on the temperature and time). The effects of temperature and time on cellulose are interpreted in Fig. (A.1). A notable increase in cellulose is seen as a result of the temperature rising over time. A rise in cellulose content of more than 50% was noted after 55–60 min at 110–120 °C. Conversely, after pre-treatment, a higher amount of cellulose content is obtained by raising the temperature and alkali concentration. The maximum cellulose content, or >50%, is achieved at 120 °C and >2.5 % alkali content, as Fig. (A.2) illustrates.

3.2.4. Pareto chart

The Pareto chart was used to clearly illustrate the significance and magnitude of the impact of independent variables on response. The complete values of the regulated effects, arranged from highest effect to lowest effect, are shown in the Pareto chart. The chart's reference line indicates statistical significance for the effect. The statistical significance is shown by the bar that crosses the reference line. The cellulose Pareto chart is shown in Fig. (A.3). This chart indicates that the parameters are statistically significant because of time × temperature (BC), alkali conc.* time (AB), alkali conc.* temp. (AC), alkali conc. (A), alkali conc.* alkali conc. (AA), temp. (C), and time (B) cross the reference line.

3.2.5. Response (cellulose) optimization

The response observed in the complete process was cellulose. After statistical analysis of the optimization model, the target cellulose content was 50.87 %, and the objective was to maximise the cellulose outcome with a lower cellulose content of 21.17 %. The ideal condition was discovered at 2.4 % alkali concentration with 60 min at 120 °C temperature after multiple responses. Post the optimization experiment the cellulose fit was 51.65 % with 1 % composite desirability.

3.2.6. Response outcome

RSM was used to determine the response (cellulose content) outcome, and it fit 51.66 % of the cellulose content with a standard error of 1.06. With a prediction interval ranging from 47.92 to 55.39 %, the confidence interval fell between the range of 49.92–54.01 % cellulose content.

3.2.7. Multiple response prediction

The optimized condition was discovered through multiple response prediction after carrying out a series of experiments. All variables were then fixed, and after 60 min at 120 °C, the maximum cellulose content was discovered at 2.46 % alkali content.

3.2.8. Optimization plot

Optimization of alkaline pre-treatment was performed, optimized condition considered at 2.47 % NaOH, 120 °C with 60 min. This optimized condition was performed separately to check the reliability. After performing the optimized condition, 51.67 % cellulose was found in the hydrolysate which shows the reliability of the model. A model created by Minitab software in which independent variables are adjoined to estimate the target response. Combined desirability ranges from 0 to 1 are helpful for the calculation of optimization. Cellulose has an independent desirability outcome of 1.0 as a predicted outcome of 51.65 %. The separate desirability of 1.0 is an excellent score which shows that response is immediate to their absolute setting. The optimization of response (cellulose) gives an ideal desirability score as cellulose was an absolute setting that is included in the justifiable range. The optimized condition established after the RSM model is represented in Fig. (A.4).

3.3. Effect of cellulase loading on enzymatic hydrolysis

A comparison of the pre-treated and raw samples' enzymatic hydrolysis shows promising results following pre-treatment. 25 FPU, 50 FPU, and 75 FPU cellulase loadings are used to hydrolyse the raw and pre-treated samples in a shaker at 150 rpm and 50 °C. The sample that was pre-treated and hydrolyzed by 50 FPU of cellulase, displayed a maximum concentration of 25 g/L of sugar reduction (fig. (B.2). With an enzymatic activity of 75 FPU, the pre-treated sample's maximum reduced sugar of 33.62 g/L was formed in 72 h. After 72 h, the pre-treated sample's maximum sugar yield was discovered, with 75 FPU enzymatic activity of 60.53% (Fig. (B.2). In contrast, under the same conditions, the raw sample yields 6.68 g/L of reducing sugar with 50 FPU in 72 h following hydrolysis. With 75 FPU, the maximum reduced sugar of 11.56 g/L was formed in 96 h. After 96 h, the raw sample's maximum sugar yield of 20.80 % was formed, with an enzymatic activity of 75 FPU Fig. (B.1). It demonstrates how successful the pre-treatment is when hydrolysis occurs. Mass balance of the complete study was determined by the total product formation from the substrate (Njoku et al., 2013). The mass balance is shown in Fig. (C).

3.4. Improvisation of the raw and treated sample

3.4.1. Phonological modulation in raw material (SEM analysis)

The sample's initially smooth surface transforms into a rough texture under the influence of elevated temperatures and alkaline conditions, causing the breakdown of cellulose and hemicellulose bonds upon lignin removal. This alteration increases the surface area of the biomass, enhancing its accessibility to enzymes, as demonstrated by Kataria et al. (2017). Post-alkaline treatment, noticeable structural changes in the untreated sample are evident, as depicted in Fig. (D). Comparative analysis with the untreated raw material reveals a more pronounced disruption in the sample following alkaline pre-treatment. In Figure (D.2), the most significant disruption is observed after alkaline pre-treatment. Notably, severe conditions (3 % NaOH concentration) also exhibit substantial disruptions, as illustrated in Fig. (D.4) In contrast, mild pre-treatment conditions (0.5 % NaOH concentration) are shown in Fig. (D.3).

3.4.2. FTIR spectrum of the untreated and treated sample

The functional group and its properties are specifically revealed by the FTIR spectrum. The treated sample's FTIR spectra (Fig. E.1) showed a broad, highly intense peak spanning 3500–3200 cm^{-1} that indicated N–H stretching of primary amines, an increase in free O–H bonds as a result of extractives being removed, and the presence of cellulose (Lu and Hsieh, 2010). When the sample is left untreated, an ester bond-related band at 1740 cm^{-1} indicates that the polymer within is mutually interconnected; however, when the sample is treated with alkali, xylose solubilization takes place and the band vanishes (Sills and Gossett, 2012). The treated sample exhibits more intense peaks at 1430 cm^{-1} and 1320 cm^{-1}, which correspond to C–H_2 and C–H bending, respectively, in relation to cellulose (Sombatpraiwan et al., 2019). The hemicellulose acetyl ester's stretching vibration is reduced in the treated sample, indicating the removal of the xyloglucan acetyl group with the COOH group from the lignin hemicellulose matrix. This is represented by the peak at 1231 cm^{-1} (Trevorah and Othman, 2015) 2015. The peak at 1024 cm^{-1} represents polysaccharides. The treated sample has high intensity at this region because of C–O, C═C, and C–C–O stretching for polysaccharides, which correlate with hemicellulose decomposition (Bano and Irfan, 2019). The aromatic band ranges from 950 to 700 cm^{-1}, which corresponds to the β-glycosidic linkage in cellulose and hemicellulose units (Gusain and Suthar, 2017). Due to C–O–C stretching in the β-glycosidic linkage in cellulose and hemicellulose, which makes cellulose accessible to enzymes, the intensity of the peak at 880 cm^{-1} increases in the treated sample (Deng et al., 2019).

3.4.3. Thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTG)

Fig. (E.2) shows the thermogravimetric analysis of the untreated sample (raw), mild (0.5 % NaOH conc., 30 min and 60 °C), harsh (3 % NaOH Conc., 60 min and 120 °C), and optimized pre-treated condition (2.47 % NaOH conc., 60 min and 120 °C) to determine their degradation characteristics at 0–600 °C. During the TGA analysis shown in Fig. E.2, four distinct types of weight reduction were observed in *Pistia stratiotes*: moisture, cellulose, hemicellulose, and lignin reduction. Water evaporation causes weight loss to begin at 100 °C. At 200–300 °C, hemicellulose depolymerizes and breaks down its cellulosic glycosidic bonds. Water loss causes a mild, raw sample to lose 10–12 % of its weight between 100 and 200 °C. Unexpected weight loss of approximately 50 % was noted in all samples between 250 and 350 °C as a result of hemicellulose, cellulose, and lignin degradation. Lignin breaks down between 200 and 500 °C. The primary thermal degradation region was observed to be between ~325 and 345 °C, indicating the degradation of cellulose and lignin in the sample. (Basak et al., 1993). The range of 200–500 °C was where the optimized condition's 55 % weight loss occurred. This indicates the sample's delignification and is the result of the alkaline pre-treatment. The optimized sample's DTG curves show a peak at 345 °C, respectively, with the hydrolyzed sample showing the strongest signal because of its higher cellulose content. The raw material's maximum thermal stability, which corresponds to its non-cellulosic content, was measured at 325 °C. Fig. (E.3) illustrates the optimized sample's high thermal stability, with the DTG curve indicating a maximum stability temperature of 345 °C. The optimized sample's degradation temperature is higher than that of other lignocellulosic materials that have been reported, such as Napier grass (300 °C) (Reddy et al., 2018), roselle fibres (326 °C)(Kian et al., 2017) and kans grass (340 °C) (Baruah et al., 2020).

4. Discussion

Biofuels can be produced from biomass, which is an environmentally friendly way to mitigate the energy crisis. The sustainable development goal is aided by the bioconversion of lignocellulosic biomass into biogas and biofuel, which lessens the carbon footprints. Lignocellulosic biomasses are composed of lignin, hemicellulose, and crystalline cellulose. Unlike the first generation, which used sugar directly, intrinsic biomass recalcitrance significantly hinders lignocellulose's bioconversion by protecting carbohydrates from degradation. The recalcitrance of wrapped cellulose significantly hinders the recovery of fermentable sugar from it. These types of biomass degrade slowly, so effective pre-treatment and enzyme dosage are necessary (Hu et al., 2023a). Pre-treatment is a crucial stage in the bioprocess technology process as a whole. In modern biorefineries, various pre-treatments such as physical, chemical, and biological are used to overcome the recalcitration of lignocellulosic biomass (Mankar et al., 2021). Physical pretreatment is used to reduce the size of particles and increase the surface area of lignocellulosic biomass by disrupting of structure of cellulose, hemicellulose and lignin for accessibility of enzymes e.g. grinding (Ji et al., 2017) Chemical pretreatment is more effective for increasing the hydrolysis and it is a simple procedure with high efficiency. Different chemicals are used in this process like acids, alkali, organic solvents and ionic liquids, all of these can break down the lignocellulosic biomass. Acidic pretreatment is specific for lignin degradation by using different concentrations of acid, reaction time and temperature. The disadvantage of this pretreatment is its ineffectiveness in the lignin removal and stopping its hindrance effect on cellulose (Rodrigues Gurgel da Silva et al., 2019). Alkali pretreatment is helpful for lignin and hemicellulose removal and increases the accessibility to the enzymes for hydrolysis with a high reduced sugar yield. The expensive neutralization and secondary contamination are its drawbacks (Malik et al., 2020) Ionic liquids are advantageous over acids with non-flammability, thermal stability and chemical adjustability (Abushammala and Mao, 2020).

Biological methods use fungi and bacteria, its advantage over other methods is the low cost of the downstream process, simple procedure, and low energy consumption. The drawback is its low-efficiency limit and time-consuming process (Rezania et al., 2020)(Li et al., 2022). Lignocellulosic biomass includes corn stover, which undergoes enzymatic hydrolysis and pre-treatment before being transformed into a value-added product (Li et al., 2017). One abundant and renewable feedstock for making biofuels is grass. Its growth cycle is short, with low lignin content, and its crystallinity is also less. However, getting a high yield of biofuels from grass is difficult due to the stiff cellulose-hemicellulose-lignin network that resists enzyme attack. Consequently, various pre-treatment methods have been applied to different types of grass in order to boost the yield of biofuels (Jin et al., 2015). Due to their low lignin content, aquatic weeds are classified in the same category as grasses. Numerous aquatic weeds, such as *Eichhornia* sp., *Pistia stratiotes*, *Lemna minor*, and *Lemna gibba*, can serve as good carbon sources for the synthesis of biofuel (Gusain and Suthar, 2017). Each of these weeds has a different composition. According to a previous study, duckweed (*Lemna minor*) contains cellulose 55.2%, hemicellulose 32.6 %, and lignin 12.2% (Yadav et al., 2017). The composition of water hyacinth before pre-treatment showed 1.77% acid-soluble lignin, 6.33% acid-insoluble lignin, 32.84% cellulose, and 24.7% hemicellulose. The results of the thermal pre-treatment were different: by using the autoclave technique, 1.58% acid-soluble lignin, 8.35 % acid-insoluble lignin, 27.8% hemicellulose, 29.26% cellulose were formed (Barua and Kalamdhad, 2017). Organic waste also includes rice straw that is used to optimise the alkali pre-treatment process. The temperature, time, NaOH concentration, and solid loading were the independent variables. The sugar reached its peak at 121 °C, 40 min, 2% w/v NaOH concentration, and 5% loading. With 50.5 g/L of glucose, 13.5 g/L of xylose, and 1.3 g/L of arabinose, the maximum sugar content was 65.3 g/L(Valles et al., 2021). A comparative investigation of diverse chemical pretreatment was performed on sorghum straw. Six different pretreatment were applied to it, namely, 2 % (w/v) NaOH, 2 % H_2SO_4, 2 % (w/v) Na_2CO_3, 2 % (w/v) oxalic acid, 2.15 % (w/v) H_2O_2 and 95% (v/v) glycerol pretreatment. In this study, 2 % NaOH pretreatment was most effective for lignin removal and enzyme-mediated hydrolysis process (Bhati and Sharma, 2023). Another optimization of alkaline pretreatment by central composite design with enzymatic hydrolysis study was performed on the cocoa pod husk. The optimal condition was 5%(w/v) NaOH for 30 min at 120 °C increased the content of cellulose from 27.68 (untreated) to 57 % and then enzymatic hydrolysis was performed resulting in 66.80 g/L reducing sugar yield up to 98.75 % (Hernández-Mendoza et al., 2021) which is high from our study. When alkaline pretreatment was done on the *Brassica juncea* with a low concentration of NaOH (0.5 M) at 160 °C for 30 min with 10 % loading. The delignification occurred in the sample and then enzymatic hydrolysis was done by the mixture of cellulase obtained from *A. niger* MTCC284, *T. harzianum*MTCC8230 and *F. incarnatum*KU377454. Then 62.35 mg/ml reduced sugar was formed after hydrolysis in 48 h at 50 °C with 15 % loading (Pant et al., 2021). Optimization of alkali pretreatment was performed with the help of response surface methodology and maximum cellulose content was formed by 2 % NaOH i.e. 62.7 % and highest 78.3 g/L glucose was obtained at 20 g/L enzyme loading with 50 °C for 72 h (Punia and Singh, 2024). Another study on *C. oleifera* fruit shell and its seed cake used as a substrate for enzymatic hydrolysis after oxalic acid pretreatment. SSF (Solid state fermentation) performance of *A. niger* with 50 °C at 200 rpm for 24 h gives 20.58 g/L reduced sugar which is less than our study (Dessie et al., 2024). *Pistia stratiotes* contains 49.4 % carbohydrates, 17.8 % fibres, and 16.5 % protein with 23.8 % ash content (Pantawong R., Chuanchai A., Thipbunrat P., Unpaprom Y., 2015). The cellulose content of *Pistia stratiotes* was extracted in a previous study under various conditions using bleaching agents. Four distinct conditions were used to optimise the pre-treatment: (1) 2% sodium chlorite + 2% glacial acetic acid; (2) 4% sodium hypochlorite; (3) 4% hydrogen peroxide + 4% sodium hydroxide; and (4) 4% soapnut solution. The results of all the conditions in the form of cellulose yield as a percentage were 38.92, 25.70, 10.7 and none in the fourth condition (Umesh et al., 2022). In another study, 1% alkali (NaOH) and 1% H_2O_2 were used to treat *Pistia stratiotes* and water hyacinth. Following this treatment, the cellulose results in water hyacinth increased from 19.7 to 34.2% and in *Pistia* from 16.5 to 28.4% (Mishima et al., 2008). After the Pre-treatment, besides ethanol, other solid-liquid products are also produced like foliar fertilisers, jet fuels and catalysts from the *Pistia stratiotes* (Yang et al., 2022). The optimization of pre-treatment was done on water hyacinth. Different acids like HCL/H_2SO_4/HCOOH (2% v/v) with varied concentrations were used such as 1%,2%,3% and 4% v/v to obtain a high amount of reduced sugar. Also, 3% v/v NaOH was used for alkali pre-treatment. The observed results gave maximum reduced sugar at 37.89 mg/100 ml filtrate with 4 % H_2SO_4 (dilute acid pre-treatment). With the alkali pre-treatment, 3% NaOH gave 17.185 mg/100 ml sugar concentration (Awasthi et al., 2013). In contrast, the highest amount of reduced sugar detected in our study was 33.62 g/L following 72 h of enzymatic hydrolysis. The pre-treatment of lignocellulosic biomass with enzymatic hydrolysis is crucial. The saccharification of cellulosic biomass or the low degradation of lignocellulosic biomass for the production of valuable products is the bottleneck in this field, necessitating the use of economically used enzymes that are effective in the saccharification process. Enzymes ought to be less expensive to produce at the industrial scale. The cost of using sugars from the lignocellulosic biomass reaches 25–30% of the total cost of producing biofuel (Hu et al., 2023b). Either commercially available or in-house-produced cellulase enzymes can be used for enzymatic hydrolysis. The primary source of fermentable sugars is plant cell walls. Certain bacteria possess the ability to break down cellulose, hemicellulose, and lignin. For example, the bacterium, *Trabulsiella* sp. is capable of efficiently breaking down the lignin model compound, guaiacylglycerol-β-guaiacyl ether (Suman et al., 2016). *Clostridium thermocellum* is an isolated bacteria which is capable for degradation of the cellulose and used for saccharification process. It is isolated by the goat rumen and is a thermophilic bacteria that is active at temperature of 50–70 °C and works on the agro-industrial waste (Hamann et al., 2015). Apart from bacteria, the fungus is also used for the production of enzymes. Different types of fungus used for the production of cellulase enzymes like *Aspergillus* sp. A1C2-06, *Talaromyces verruculosus* A1C2-05 (Fontes et al., 2023), *Aspergillus niger* (Sulyman et al., 2020) and *Trichoderma resei* (Wu et al., 2019). In the previous study, the *A.niger* produced enzymes by using the waste of *C. oleifera* as a substrate and the conditions were 50 °C at 200 rpm for 24 h and produced 20.58 g/L sugar (Dessie et al., 2024). Another study shows that using the same fungus stain and conditions were different than was at 50 °C at 150 rpm for 48 h on the wheat straw after thermal pretreatment produced 32.90 g/L sugar (Infanzón-Rodríguez et al., 2022) Commercially available cellulase enzymes suitable for the saccharification process are also reasonably priced. We employed the inexpensive, readily available commercial cellulase enzyme in our investigation to achieve successful saccharification results.

5. Conclusions

Alkali pretreatment of biomass is a crucial step for lignocellulosic biomass delignification. This lignin removal process results in the exposure of cellulose and the liberation of sugars. Depending on the type of plant biomass, pretreatment process need to be employed. In the present study, alkaline pretreatment was optimized for *Pistia stratiotes* biomass and 51.66 % cellulose was formed. The biomass characterization of pretreated biomass also revealed the delignification. The enzymatic hydrolysis was done after pretreatment by a commercially available enzyme which gives a maximum of 31.06 g/L reduced sugar with 75 FPU in 72 h. Hence, this renewable sugar obtained by hydrolysis may be employed for various microbial metabolites production such as ethanol, bioplastics, organic acids and different metabolites. This makes it a sustainable and cost-effective process.

Statements and declarations

All the authors declare that the work done in the present manuscript is original and done at Delhi Technological University, Delhi, India.

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CRediT authorship contribution statement

Sweeti Mann: Writing – original draft, Methodology, Investigation, Formal analysis. **Jai Gopal Sharma:** Writing – review & editing, Supervision, Resources. **Rashmi Kataria:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the paper. All the authors declare that the work done in the present manuscript is original.

Data availability

Data will be made available on request.

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Appendix

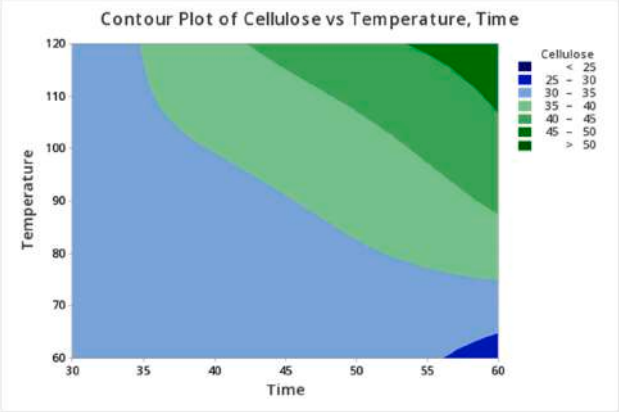

Fig. (A.1). Contour plot shows the effect of temp. And time on the cellulose content. An increase in cellulose content was noticed when temperature increased with time.

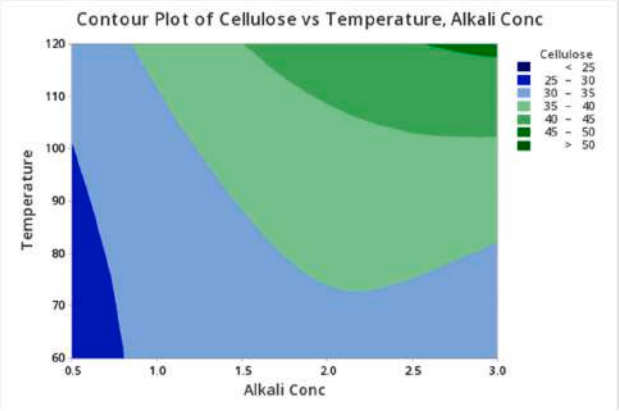

Fig. (A.2). Contour plot demonstrates the effect of alkali conc. And temperature on cellulose content. With an increase in the alkali conc. with temperature, the cellulose content also rises.

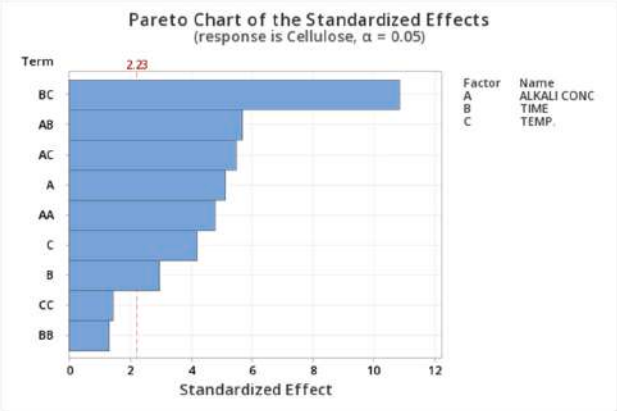

Fig. (A.3). Pareto chart of standard effects of independent variables on response. This chart illustrates that the factors time × temp., alkali conc.*time, alkali conc. *temp, alkali conc., alkali conc.*alkali conc., temp., and time are statistically significant.

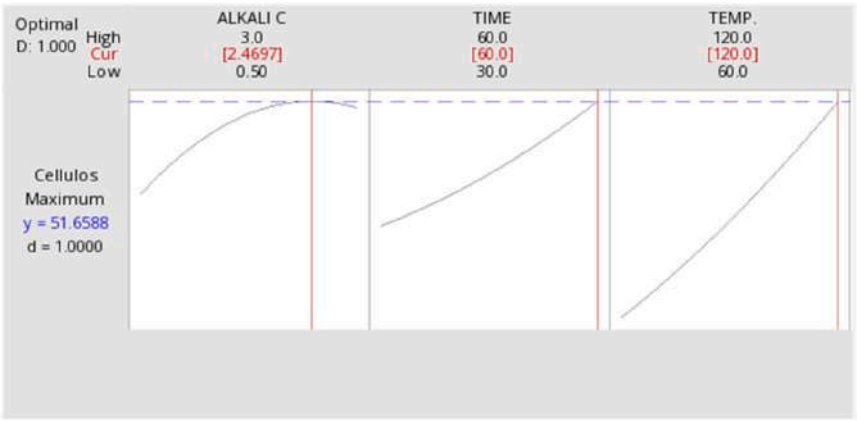

Fig. (A.4). Optimized condition demonstrates when the cellulose substrate is treated with high conc. of alkali (2.47 %) at 120 °C for 60 min then the maximum cellulose content was obtained with optimal density 1 which is statistically significant.

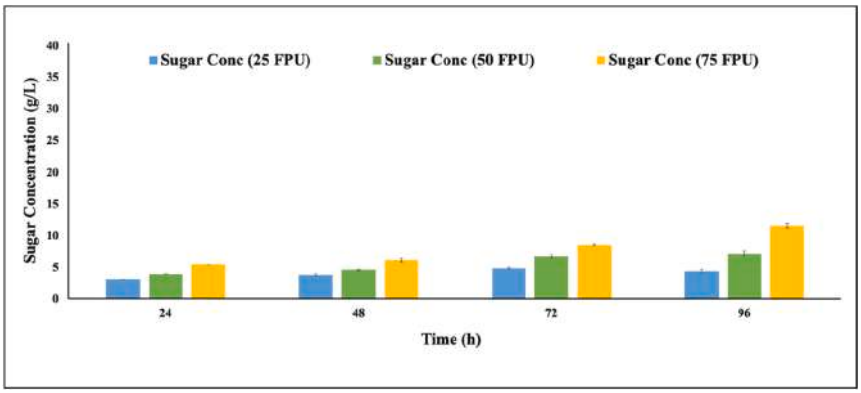

Fig. (B.1). Profile of reducing sugar concentration at the time of enzymatic hydrolysis of the raw sample of *P. stratiotes* at different initial enzyme loading. (50 °C, pH 4.8, rpm 150 with 5 % biomass loading).

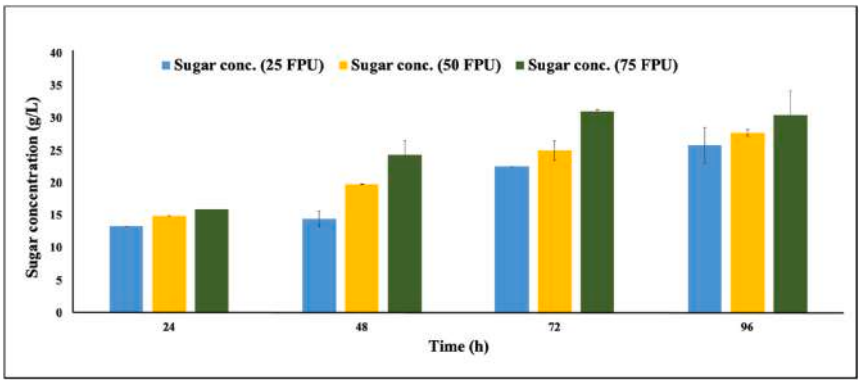

Fig. (B.2). The profile of reducing sugar concentration at the enzymatic hydrolysis optimized pre-treated sample at different enzyme loading conditions (50 °C, pH 4.8, 150 rpm and 5 % pre-treated solid content).

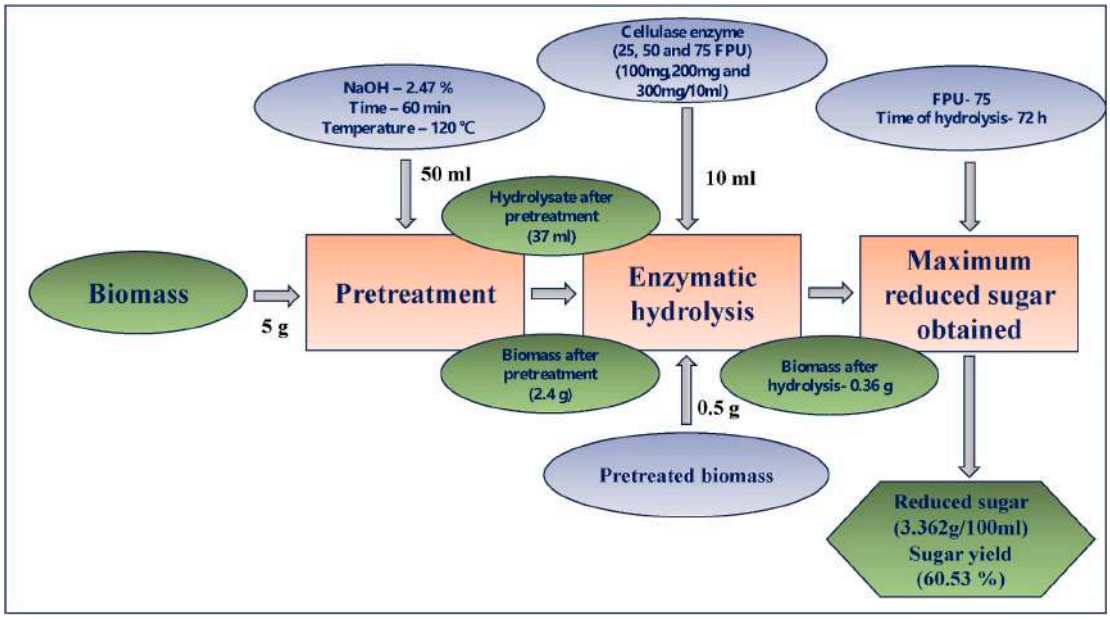

Fig. (C). The mass balance diagram of the complete process from pretreatment to enzymatic hydrolysis.

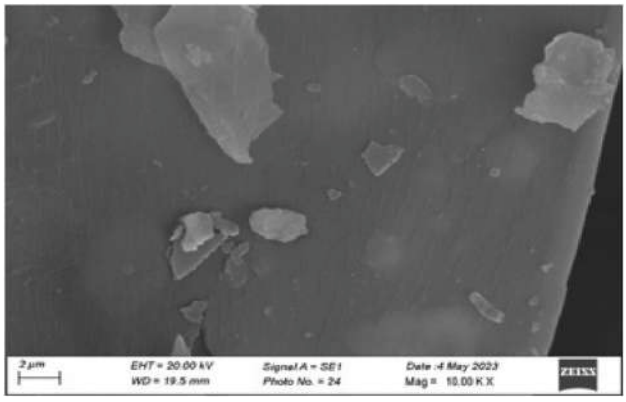

Fig. (D.1). SEM analysis for untreated (Raw).

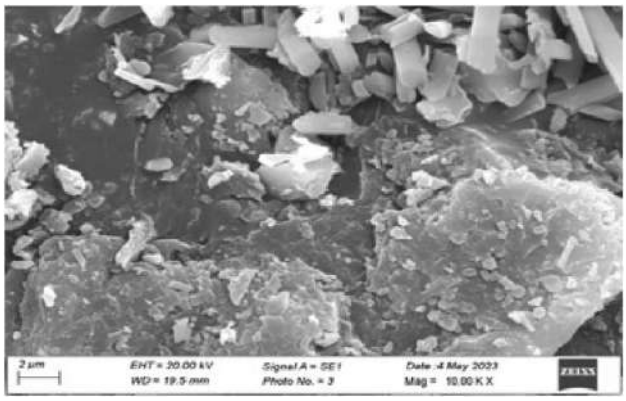

Fig. (D.2). Optimized condition (2.47 % NaOH conc., 60 min and 120 °C).

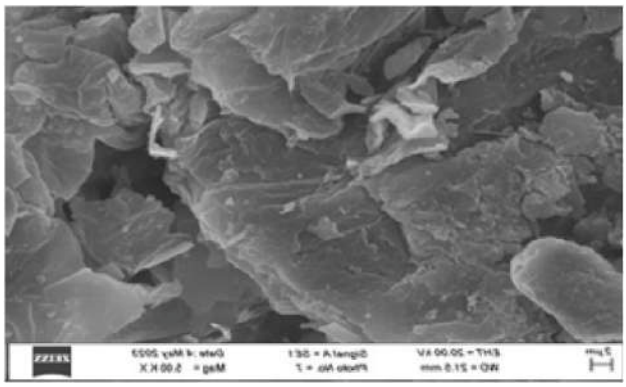

Fig. (D.3). Mild condition (0.5 % NaOH conc., 30 min and 60 °C).

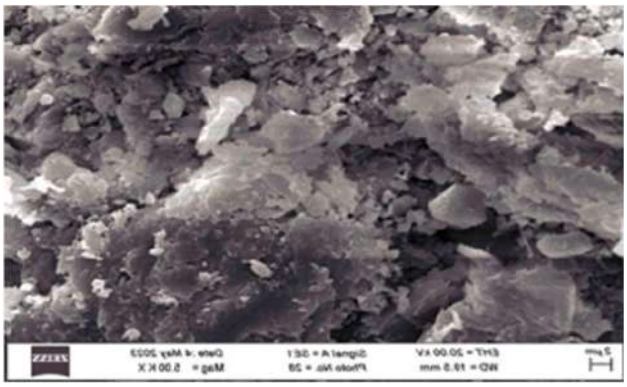

Fig. (D.4). harsh (3 % NaOH Conc., 60 min And 120 °C). Optimized condition shows maximum disruption due to the removal of lignin and hemicellulose.

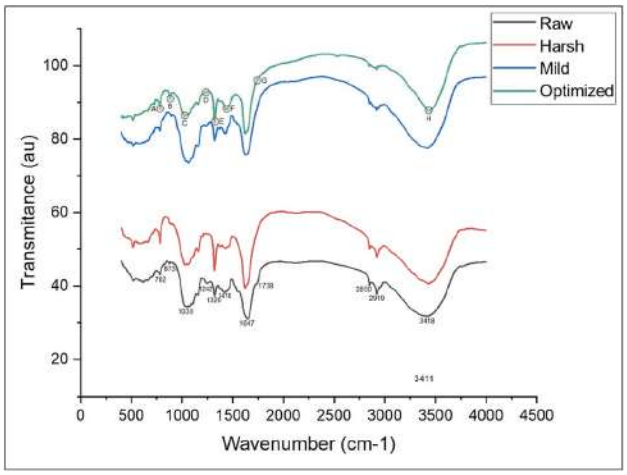

Fig. (E.1). FTIR spectra for untreated (Raw), mild, harsh and optimized conditions. The intensity of the signal in the form of peaks in different wavelengths shows the presence of relevant molecular bonds found in the polymers of samples.

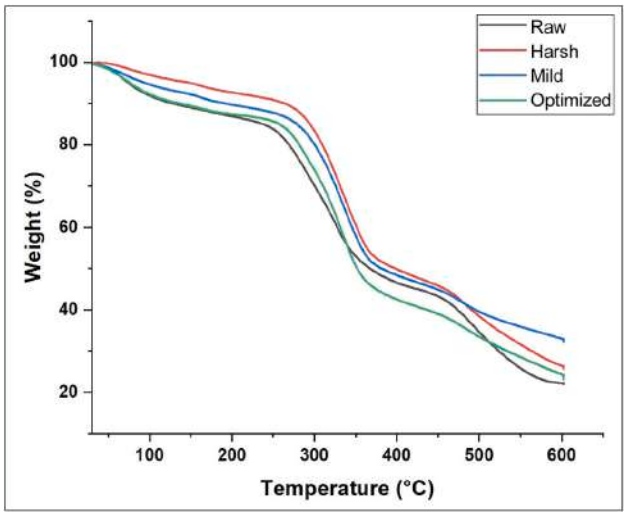

Fig. (E.2). Thermal profile for untreated (Raw), mild, harsh and optimized condition thermogravimetric analysis (TGA) curves represent the thermal degradation of the areas of the major compounds present in the samples.

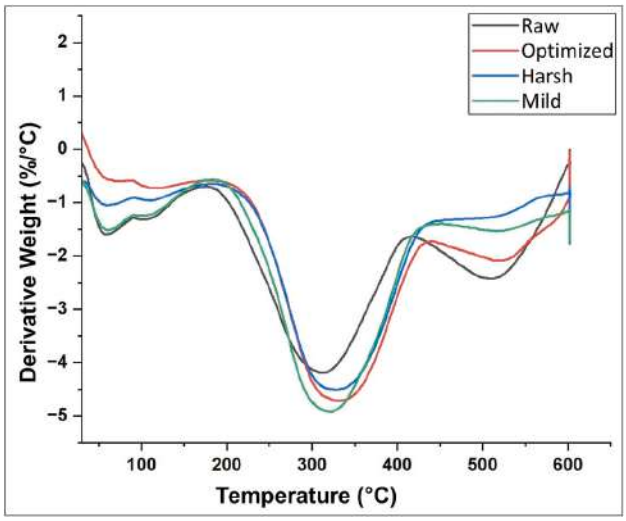

Fig. (E.3). Differential thermogravimetric (DTG) curves represent the rate of thermal degradation of treated and untreated samples.

Table (A.1)
Independent variables with experimental levels in the RSM model

Symbols	Independent variables	Unit	Relation of codes with original independent variables		
			−1	0	1
A	NaOH concentration	°C	0.5	1.75	3
B	Reaction Time	min	30	45	60
C	Reaction Temperature	% w/v	60	90	120

Table (A.2)
Experiment list with different levels of independent variables (alkali conc., time and temperature) with response (cellulose)

Experiment no.	Alkali concentration	Time	Temperature	cellulose (%)
1	1.75	45	90	37.3 ± 2.61
2	1.75	45	120	41.5 ± 3.51
3	1.75	45	90	36.92 ± 2.44
4	3	60	120	50.88 ± 1.40
5	0.5	30	60	27.38 ± 0.93
6	1.75	45	90	35.01 ± 2.95
7	3	30	60	36.31 ± 3.24
8	0.5	60	60	27.64 ± 2.61
9	0.5	30	120	21.17 ± 1.04
10	1.75	45	90	34.39 ± 2.1
11	1.75	45	60	34.2 ± 1.38
12	1.75	45	90	35.27 ± 0.72
13	3	60	60	27 ± 3.83
14	3	45	90	32.57 ± 1.91
15	3	30	120	41.12 ± 2.98
16	1.75	45	90	34.08 ± 2.51
17	1.75	60	90	40.83 ± 3.25
18	1.75	30	90	34.67 ± 2.34
19	0.5	60	120	42.28 ± 3.41
20	0.5	45	90	29.15 ± 2.39

Table (B)
Analysis of Variance (ANOVA) results and statistical parameters of the model quadratic correlation versus alkaline conc., reaction time and reaction temperature

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	818.693	90.966	53.79	0.000
Model	9	79.187	26.396	15.61	0.000
Linear	3	44.593	44.593	26.37	**0.000**
Alkali conc.	1	15.012	15.012	8.88	**0.014**
Time	1	30.042	30.042	17.77	**0.002**
Temp.	1	40.584	13.528	8.00	**0.005**
Square	3	38.954	38.954	23.04	**0.001**
Alkali conc.*Alkali conc.	1	3.007	3.007	1.78	0.212
Time × Time	1	3.576	3.576	2.11	0.177
Temp.*Temp.	1	305.185	101.728	60.16	**0.000**
2-Way Interaction	3	54.702	54.702	32.35	**0.000**
Alkali conc.*Time	1	51.288	51.288	30.33	**0.000**
Alkali conc.*Temp.	1	199.195	199.195	117.80	**0.000**
Time × Temp.	1	16.910	1.691		
Error	10	8.084	1.617	0.92	0.537
Lack-of-Fit	5	8.826	1.765		
Pure Error	5	835.603			
Total	19				

References

Abushammala, H., Mao, J., 2020. A review on the partial and complete dissolution and fractionation of wood and lignocelluloses using imidazolium ionic liquids. Polymers 12, 195. https://doi.org/10.3390/polym12010195.

Aguilar-Reynosa, A., Romaní, A., Ma Rodríguez-Jasso, R., Aguilar, C.N., Garrote, G., Ruiz, H.A., 2017. Microwave heating processing as alternative of pretreatment in second-generation biorefinery: an overview. Energy Convers. Manag. 136, 50–65. https://doi.org/10.1016/j.enconman.2017.01.004.

Alam, S.N., Khalid, Z., Guldhe, A., Singh, B., Korstad, J., 2021. Harvesting and pretreatment techniques of aquatic macrophytes and macroalgae for production of biofuels. Environ. Sustain 4, 299–316. https://doi.org/10.1007/S42398-021-00178-6.

Ali, C.H., Mbadinga, S.M., Liu, J.-F., Yang, S.-Z., Gu, J.-D., Mu, B.-Z., 2015. Significant enhancement of Pseudomonas aeruginosa FW_SH-1 lipase production using response surface methodology and analysis of its hydrolysis capability. J. Taiwan Inst. Chem. Eng. 52, 7–13. https://doi.org/10.1016/j.jtice.2015.02.001.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour. Technol. 101, 4851–4861. https://doi.org/10.1016/J.BIORTECH.2009.11.093.

Asghar, U., Irfan, M., Iram, M., Huma, Z., Nelofer, R., Nadeem, M., Syed, Q., 2015. Effect of alkaline pretreatment on delignification of wheat straw. Nat. Prod. Res. 29, 125–131. https://doi.org/10.1080/14786419.2014.964712.
Awasthi, M., Kaur, J., Rana, S., 2013. Bioethanol production through water hyacinth, Eichhornia crassipes via optimization of the pretreatment conditions. Int. J. Emerg. Technol. Adv. Eng. 3, 42–46.
Bano, A., Irfan, M., 2019. Alkali pretreatment of cotton stalk for bioethanol. Bangladesh J. Sci. Ind. Res. 54, 73–82. https://doi.org/10.3329/BJSIR.V54I1.40733.
Barua, V.B., Kalamdhad, A.S., 2017. Effect of various types of thermal pretreatment techniques on the hydrolysis, compositional analysis and characterization of water hyacinth. Bioresour. Technol. 227, 147–154. https://doi.org/10.1016/J.BIORTECH.2016.12.036.
Baruah, J., Deka, R., Kalita, E., 2020. Greener production of microcrystalline cellulose (MCC) from Saccharum spontaneum (Kans grass): statistical optimization. Int. J. Biol. Macromol. 154, 672–682.
Basak, R.K., Saha, S.G., Sarkar, A.K., Saha, M., Das, N.N., Mukherjee, A.K., 1993. Thermal properties of jute constituents and flame retardant jute fabrics. Textil. Res. J. 63, 658–666. https://doi.org/10.1177/004051759306301107.
Başar, İ.A., Çoban, Ö., Göksungur, M.Y., Eskicioğlu, Ç., Perendeci, N.A., 2021. Enhancement of lignocellulosic biomass anaerobic digestion by optimized mild alkaline hydrogen peroxide pretreatment for biorefinery applications. J. Environ. Manag. 298, 113539 https://doi.org/10.1016/J.JENVMAN.2021.113539.
Bhati, N., Sharma, A.K., 2023. Comparative study of different chemical pretreatments for enhanced enzymatic hydrolysis of sorghum straw. Biomass Convers. Biorefinery 1, 1–9. https://doi.org/10.1007/S13399-023-05185-7/FIGURES/4.
Chen, K.Y., Zheng, Y., Cheng, Y.S., 2015. Integrated alkali pretreatment and preservation of wet lettuce (Pistia stratiotes) by lactic acid bacteria for fermentable sugar production. Biomass Bioenergy 81, 249–255. https://doi.org/10.1016/J.BIOMBIOE.2015.07.007.
Culaba, A.B., Mayol, A.P., San Juan, J.L.G., Vinoya, C.L., Concepcion, R.S., Bandala, A. A., Vicerra, R.R.P., Ubando, A.T., Chen, W.H., Chang, J.S., 2022. Smart sustainable biorefineries for lignocellulosic biomass. Bioresour. Technol. 344, 126215 https://doi.org/10.1016/J.BIORTECH.2021.126215.
Dahunsi, S.O., Adesulu-Dahunsi, A.T., Izebere, J.O., 2019. Cleaner energy through liquefaction of Cocoa (Theobroma cacao) pod husk: pretreatment and process optimization. J. Clean. Prod. 226, 578–588. https://doi.org/10.1016/j.jclepro.2019.04.112.
Deng, Z., Xia, A., Liao, Q., Zhu, X., Huang, Y., Fu, Q., 2019. Laccase pretreatment of wheat straw: effects of the physicochemical characteristics and the kinetics of enzymatic hydrolysis. Biotechnol. Biofuels 12. https://doi.org/10.1186/S13068-019-1499-3.
Dessie, W., Xiao, J., Tang, J., An, B., Luo, X., Wang, M., Liao, Y., Wahab, R., Li, C., Qin, Z., 2024. Maximizing fermentable feedstocks from Camellia oleifera seed oil extraction residues: green pretreatment and enzymatic hydrolysis for effective valorization. Arab. J. Chem. 17, 105815 https://doi.org/10.1016/j.arabjc.2024.105815.
Fillat, Ú., Ibarra, D., Eugenio, M.E., Moreno, A.D., Tomás-Pejó, E., Martín-Sampedro, R., 2017. Laccases as a potential tool for the efficient conversion of lignocellulosic biomass: a review, 2017 Fermentatio 3. https://doi.org/10.3390/FERMENTATION3020017. Page 17 3, 17.
Fontes, D.I., Bezerra, T.S., de Freitas, E.P.B., de Oliveira, M.N., Silva, S. da C., Silva, S.Y. S., Albino, U.B., Santos, D. de A., 2023. Production of cellulases from Amazonian fungi and their application in babassu cellulose hydrolysis. Int. Biodeterior. Biodegrad. 182, 105631 https://doi.org/10.1016/J.IBIOD.2023.105631.
Gao, L., Gu, J.D., 2021. A new unified conceptual framework involving maintenance energy, metabolism and toxicity for research on degradation of organic pollutants. Int. Biodeterior. Biodegrad. 162, 105253 https://doi.org/10.1016/J.IBIOD.2021.105253.
Gu, J.D., 2020. On environmental biotechnology of bioremediation. Appl. Environ. Biotechnol. 5, 3–8. https://doi.org/10.26789/AEB.2020.02.002.
Gu, J.D., 2016. Biodegradation testing: so many tests but very little new innovation. Appl. Environ. Biotechnol. 1, 92–95. https://doi.org/10.26789/AEB.2016.01.007.
Gundupalli, M.P., Tantayotai, P., Panakkal, E.J., Chuetor, S., Kirdponpattara, S., Thomas, A.S.S., Sharma, B.K., Sriariyanun, M., 2022. Hydrothermal pretreatment optimization and deep eutectic solvent pretreatment of lignocellulosic biomass: an integrated approach. Bioresour. Technol. Rep. 17, 100957 https://doi.org/10.1016/J.BITEB.2022.100957.
Gusain, R., Suthar, S., 2017. Potential of aquatic weeds (Lemna gibba, Lemna minor, Pistia stratiotes and Eichhornia sp.) in biofuel production. Process Saf. Environ. Protect. 109, 233–241. https://doi.org/10.1016/J.PSEP.2017.03.030.
Hamann, P.R.V., Serpa, D.L., Barreto da Cunha, A.S., de Camargo, B.R., Osiro, K.O., Valle de Sousa, M., Felix, C.R., Miller, R.N.G., Noronha, E.F., 2015. Evaluation of plant cell wall degrading enzyme production by Clostridium thermocellum B8 in the presence of raw agricultural wastes. Int. Biodeterior. Biodegrad. 105, 97–105. https://doi.org/10.1016/J.IBIOD.2015.08.013.
Hernández-Mendoza, A.G., Saldaña-Trinidad, S., Martínez-Hernández, S., Pérez-Sariñana, B.Y., Láinez, M., 2021. Optimization of alkaline pretreatment and enzymatic hydrolysis of cocoa pod husk (Theobroma cacao L.) for ethanol production. Biomass Bioenergy 154, 106268. https://doi.org/10.1016/J.BIOMBIOE.2021.106268.
Holtzapple, M.T., 2003. In: Caballero, B.B.T.-E., of, F.S. and N. (Eds.), HEMICELLULOSES, Second E. Academic Press, Oxford, pp. 3060–3071. https://doi.org/10.1016/B0-12-227055-X/00589-7.
Hu, Y., Priya, A., Chen, C., Liang, C., Wang, W., Wang, Q., Lin, C.S.K., Qi, W., 2023a. Recent advances in substrate-enzyme interactions facilitating efficient biodegradation of lignocellulosic biomass: a review. Int. Biodeterior. Biodegrad. 180 https://doi.org/10.1016/j.ibiod.2023.105594.
Hu, Y., Priya, A., Chen, C., Liang, C., Wang, W., Wang, Q., Lin, C.S.K., Qi, W., 2023b. Recent advances in substrate-enzyme interactions facilitating efficient biodegradation of lignocellulosic biomass: a review. Int. Biodeterior. Biodegrad. 180, 105594 https://doi.org/10.1016/J.IBIOD.2023.105594.
Infanzón-Rodríguez, M.I., Ragazzo-Sánchez, J.A., del Moral, S., Calderón-Santoyo, M., Aguilar-Uscanga, M.G., 2022. Enzymatic hydrolysis of lignocellulosic biomass using native cellulase produced by Aspergillus Niger ITV02 under liquid state fermentation. Biotechnol. Appl. Biochem. 69, 198–208. https://doi.org/10.1002/BAB.2097.
Jayanth, K.P., 2000. Biological Control of Weeds in India, Biocontrol Potential and its Exploitation in Sustainable Agriculture. CSIRO Publishing. https://doi.org/10.1007/978-1-4615-4209-4_15.
Ji, G., Han, L., Gao, C., Xiao, W., Zhang, Y., Cao, Y., 2017. Quantitative approaches for illustrating correlations among the mechanical fragmentation scales, crystallinity and enzymatic hydrolysis glucose yield of rice straw. Bioresour. Technol. 241, 262–268. https://doi.org/10.1016/j.biortech.2017.05.062.
Jin, S., Zhang, G., Zhang, P., Jin, L., Fan, S., Li, F., 2015. Comparative study of high-pressure homogenization and alkaline-heat pretreatments for enhancing enzymatic hydrolysis and biogas production of grass clipping. Int. Biodeterior. Biodegrad. 104, 477–481. https://doi.org/10.1016/j.ibiod.2015.08.005.
Kataria, R., Mol, A., Schulten, E., Happel, A., Mussatto, S.I., 2017. Bench scale steam explosion pretreatment of acid impregnated elephant grass biomass and its impacts on biomass composition, structure and hydrolysis. Ind. Crops Prod. 106, 48–58. https://doi.org/10.1016/J.INDCROP.2016.08.050.
Kataria, R., Woods, T., Casey, W., Cerrone, F., Davis, R., O'Connor, K., Ruhal, R., Babu, R., 2018a. Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production. Ind. Crops Prod. 111, 625–632. https://doi.org/10.1016/J.INDCROP.2017.11.029.
Kataria, R., Woods, T., Casey, W., Cerrone, F., Davis, R., O'Connor, K., Ruhal, R., Babu, R., 2018b. Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production. Ind. Crops Prod. 111, 625–632. https://doi.org/10.1016/J.INDCROP.2017.11.029.
Kian, L., Jawaid, M., Ariffin, H., Alothman, O., 2017. Isolation and characterization of microcrystalline cellulose from roselle fibers. Int. J. Biol. Macromol. 103, 931–940.
Kim, I., Han, J.I., 2012. Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass Bioenergy 46, 210–217. https://doi.org/10.1016/J.BIOMBIOE.2012.08.024.
Kumar, M., Turner, S., 2015. Protocol: a medium-throughput method for determination of cellulose content from single stem pieces of Arabidopsis thaliana. Plant Methods 11. https://doi.org/10.1186/S13007-015-0090-6.
Li, F., Zhang, P., Zhang, G., Tang, X., Wang, S., Jin, S., 2017. Enhancement of corn stover hydrolysis with rumen fluid pretreatment at different solid contents: effect, structural changes and enzymes participation. Int. Biodeterior. Biodegrad. 119, 405–412. https://doi.org/10.1016/j.ibiod.2016.10.038.
Li, X., Shi, Y., Kong, W., Wei, J., Song, W., Wang, S., 2022. Improving enzymatic hydrolysis of lignocellulosic biomass by bio-coordinated physicochemical pretreatment—a review. Energy Rep. https://doi.org/10.1016/j.egyr.2021.12.015.
Liu, P., Li, A., Wang, Youmei, Cai, Q., Yu, H., Li, Y., Peng, H., Li, Q., Wang, Yanting, Wei, X., Zhang, R., Tu, Y., Xia, T., Peng, L., 2021. Distinct *Miscanthus* lignocellulose improves fungus secreting cellulases and xylanases for consistently enhanced biomass saccharification of diverse bioenergy crops. Renew. Energy 174, 799–809. https://doi.org/10.1016/J.RENENE.2021.04.107.
Lu, P., Hsieh, Y. Lo, 2010. Preparation and properties of cellulose nanocrystals: rods, spheres, and network. Carbohydr. Polym. 82, 329–336. https://doi.org/10.1016/j.carbpol.2010.04.073.
Malik, K., Salama, E.S., Kim, T.H., Li, X., 2020. Enhanced ethanol production by Saccharomyces cerevisiae fermentation post acidic and alkali chemical pretreatments of cotton stalk lignocellulose. Int. Biodeterior. Biodegrad. 147 https://doi.org/10.1016/j.ibiod.2019.104869.
Mankar, A.R., Pandey, A., Modak, A., Pant, K.K., 2021. Pretreatment of lignocellulosic biomass: a review on recent advances. Bioresour. Technol. 334, 125235 https://doi.org/10.1016/J.BIORTECH.2021.125235.
Manmai, N., Unpaprom, Y., Ponnusamy, V.K., Ramaraj, R., 2020. Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation. Fuel 278. https://doi.org/10.1016/j.fuel.2020.118262.
Meehnian, H., Jana, A.K., Jana, M.M., 2017. Pretreatment of cotton stalks by synergistic interaction of Daedalea flavida and Phlebia radiata in co-culture for improvement in delignification and saccharification. Int. Biodeterior. Biodegrad. 117, 68–77. https://doi.org/10.1016/J.IBIOD.2016.11.022.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428. https://doi.org/10.1021/AC60147A030.
Mishima, D., Kuniki, M., Sei, K., Soda, S., Ike, M., Fujita, M., 2008. Ethanol production from candidate energy crops: water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes L.). Bioresour. Technol. 99, 2495–2500. https://doi.org/10.1016/J.BIORTECH.2007.04.056.
Moerman, D.E., 1996. An analysis of the food plants and drug plants of native North America. J. Ethnopharmacol. 52, 1–22. https://doi.org/10.1016/0378-8741(96)01393-1.
Nazari, M.T., Mazutti, J., Basso, L.G., Colla, L.M., Brandli, L., 2021. Biofuels and their connections with the sustainable development goals: a bibliometric and systematic review. Environ. Dev. Sustain. 23, 11139–11156. https://doi.org/10.1007/S10668-020-01110-4.

Njoku, S.I., Ahring, B.K., Uellendahl, H., 2013. Tailoring wet explosion process parameters for the pretreatment of cocksfoot grass for high sugar yields. Appl. Biochem. Biotechnol. 170, 1574–1588. https://doi.org/10.1007/S12010-013-0299-7.

Nomanbhay, S.M., Hussain, R., Palanisamy, K., Nomanbhay, S.M., Hussain, R., Palanisamy, K., 2013. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield. J. Sustain. Bioenergy Syst. 3, 7–17. https://doi.org/10.4236/JSBS.2013.31002.

Pant, S., Ritika, Komesu, A., Penteado, E.D., Diniz, A.A.R., Rahman, M.A., Kuila, A., 2021. NaOH pretreatment and enzymatic hydrolysis of Brassica juncea using mixture of cellulases. Environ. Technol. Innov. 21, 101324 https://doi.org/10.1016/J.ETI.2020.101324.

Pantawong, R., Chuanchai, A., Thipbunrat, P., Unpaprom, Y.R.R., 2015. Experimental investigation of biogas production from water lettuce, Pistia stratiotes L. Emer. Life Sci. Res. 1, 41–46.

Pashaei, H., Ghaemi, A., Nasiri, M., Karami, B., 2020. Experimental modeling and optimization of CO2 absorption into piperazine solutions using RSM-CCD methodology. ACS Omega 5, 8432–8448. https://doi.org/10.1021/acsomega.9b03363.

Punia, P., Singh, L., 2024. Optimization of alkali pre-treatment of sweet sorghum [Sorghum bicolor (L.) Moench] residue to improve enzymatic hydrolysis for fermentable sugars. Waste Manag. Bull. 2, 131–141. https://doi.org/10.1016/j.wmb.2023.12.007.

Ramaraj, R., Unpaprom, Y., 2019. Optimization of pretreatment condition for ethanol production from Cyperus difformis by response surface methodology. 3 Biotech 9. https://doi.org/10.1007/S13205-019-1754-0.

Reddy, K.O., Maheswari, C.U., Dhlamini, M.S., Mothudi, B.M., Kommula, V.P., Zhang, Jinming, Zhang, Jun, Rajulu, A.V., 2018. Extraction and characterization of cellulose single fibers from native african napier grass. Carbohydr. Polym. 188, 85–91. https://doi.org/10.1016/j.carbpol.2018.01.110.

Rezania, S., Oryani, B., Cho, J., Talaiekhozani, A., Sabbagh, F., Hashemi, B., Rupani, P.F., Mohammadi, A.A., 2020. Different pretreatment technologies of lignocellulosic biomass for bioethanol production: an overview. Energy 199. https://doi.org/10.1016/j.energy.2020.117457.

Rodrigues Gurgel da Silva, A., Giuliano, A., Errico, M., Rong, B.G., Barletta, D., 2019. Economic value and environmental impact analysis of lignocellulosic ethanol production: assessment of different pretreatment processes. Clean Technol. Environ. Policy 21, 637–654. https://doi.org/10.1007/s10098-018-01663-z.

Saha, B.C., Qureshi, N., Kennedy, G.J., Cotta, M.A., 2016. Biological pretreatment of corn stover with white-rot fungus for improved enzymatic hydrolysis. Int. Biodeterior. Biodegrad. 109, 29–35. https://doi.org/10.1016/j.ibiod.2015.12.020.

Selvakumar, P., Adane, A.A., Zelalem, T., Hunegnaw, B.M., Karthik, V., Kavitha, S., Jayakumar, M., Karmegam, N., Govarthanan, M., Kim, W., 2022. Optimization of binary acids pretreatment of corncob biomass for enhanced recovery of cellulose to produce bioethanol. Fuel 321. https://doi.org/10.1016/j.fuel.2022.124060.

Sills, D.L., Gossett, J.M., 2012. Using FTIR spectroscopy to model alkaline pretreatment and enzymatic saccharification of six lignocellulosic biomasses. Biotechnol. Bioeng. 109, 894–903. https://doi.org/10.1002/BIT.24376.

Sombatpraiwan, S., Junyusen, T., Treeamnak, T., Junyusen, P., 2019. Optimization of microwave-assisted alkali pretreatment of cassava rhizome for enhanced enzymatic hydrolysis glucose yield. Food Energy Secur. 8 https://doi.org/10.1002/FES3.174.

Sulyman, A.O., Igunnu, A., Malomo, S.O., 2020. Isolation, purification and characterization of cellulase produced by Aspergillus Niger cultured on Arachis hypogaea shells. Heliyon 6, e05668. https://doi.org/10.1016/J.HELIYON.2020.E05668.

Suman, S.K., Dhawaria, M., Tripathi, D., Raturi, V., Adhikari, D.K., Kanaujia, P.K., 2016. Investigation of lignin biodegradation by Trabulsiella sp. isolated from termite gut. Int. Biodeterior. Biodegrad. 112, 12–17. https://doi.org/10.1016/j.ibiod.2016.04.036.

Trevorah, R.M., Othman, M.Z., 2015. Alkali pretreatment and enzymatic hydrolysis of Australian timber mill sawdust for biofuel production. J. Renew. Energy 2015, 1–9. https://doi.org/10.1155/2015/284250.

Tse, T.J., Wiens, D.J., Reaney, M.J.T., 2021. Production of bioethanol—a review of factors affecting ethanol yield, 2021 Fermentatio 7. https://doi.org/10.3390/FERMENTATION7040268, 268 7, 268.

Umesh, M., Santhosh, A.S., Shanmugam, S., Thazeem, B., Alharbi, S.A., Almoallim, H.S., Chi, N.T.L., Pugazhendhi, A., 2022. Extraction, characterization, and fabrication of cellulose biopolymer sheets from Pistia stratiotes as a biodegradative coating material: an unique strategy for the conversion of invasive weeds into value-added products. J. Polym. Environ. 30, 5057–5068. https://doi.org/10.1007/S10924-022-02511-4/FIGURES/9.

Valles, A., Capilla, M., Álvarez-Hornos, F.J., García-Puchol, M., San-Valero, P., Gabaldón, C., 2021. Optimization of alkali pretreatment to enhance rice straw conversion to butanol. Biomass Bioenergy 150, 106131. https://doi.org/10.1016/J.BIOMBIOE.2021.106131.

Wannapokin, A., Ramaraj, R., Whangchai, K., Unpaprom, Y., 2018. Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. 3 Biotech 8. https://doi.org/10.1007/S13205-018-1084-7.

Wu, H., Zhao, X., Adsul, M., Zhong, Y., Qian, Y., Zhong, L., Sun, Y., Sun, N., Zhang, L., Liu, W., Qu, Y., 2019. Enhancement of cellulase production in Trichoderma reesei via disruption of multiple protease genes identified by comparative secretomics. Enhancement of Cellulase Production in Trichoderma reesei via Disruption of Multiple Protease Genes Identified by Comparative Secretomics 10, 2784. https://doi.org/10.3389/fmicb.2019.02784.

Xia, M., Valverde-Barrantes, O.J., Suseela, V., Blackwood, C.B., Tharayil, N., 2022. Characterizing natural variability of lignin abundance and composition in fine roots across temperate trees: a comparison of analytical methods. New Phytol. https://doi.org/10.1111/NPH.18515.

Yadav, D., Barbora, L., Bora, D., Mitra, S., Rangan, L., Mahanta, P., 2017. An assessment of duckweed as a potential lignocellulosic feedstock for biogas production. Int. Biodeterior. Biodegrad. 119, 253–259. https://doi.org/10.1016/J.IBIOD.2016.09.007.

Yang, M., Zhang, Xiaoliang, Wang, K., Zhu, S., Ye, Z., Sheng, K., Zhang, Ximing, 2022. Investigation of cascade valorization of Pistia stratiotes L. by hydrothermal treatment. Fuel 324, 124473. https://doi.org/10.1016/J.FUEL.2022.124473.

Zhou, Y., Zheng, J., Gan, R.-Y., Zhou, T., Xu, D.-P., Li, H.-B., Cravotto, G., Choi, Y.H., 2017. Optimization of ultrasound-assisted extraction of antioxidants from the mung bean coat. Molecules. https://doi.org/10.3390/molecules22040638.

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Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Sweeti Mann** of **Delhi Technological University** has Participated/Presented a Poster Presentation entitled **Optimization of acidic pretreatment of Pistia stratiotes and its application as biofuel** in International Conference on **"Microbial Bioprospecting Towards Sustainable Development Goals"** held on **24th- 25th November 2023** organized by Association of Microbiologist of India-LPU Unit and Society of Chemical and Synthetic Biology at Lovely Professional University, Punjab.

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VALORIZATION 2024

International Conference on Science & Technology Integration for Circular Economy

18-19 January 2024

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This is to certify that **Ms. Sweeti Mann** has presented a paper titled **Water lettuce is an efficient substrate for ethanol production after pretreatment** in VALORIZATION 2024: International Conference on Science & Technology Integration for Circular Economy, organized by the DST-PURSE Team of BITS Pilani Hyderabad Campus from 18th to 19th January 2024.

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CERTIFICATE OF PARTICIPATION

Is Presented to

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For Poster presentation on the title

"**Organic waste as a supplement for the PHA (poly hydroxyalkanoate) production and its application**" with

Co-author/s: Jai Gopal Sharma, Rashmi Kataria

at International Conference on **Recent Advances in Biotechnology (icRAB -2022)**

on 2nd-4th December 2022

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