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REVIEW

Recent advancement in production of bioethanol from waste biomass: a review

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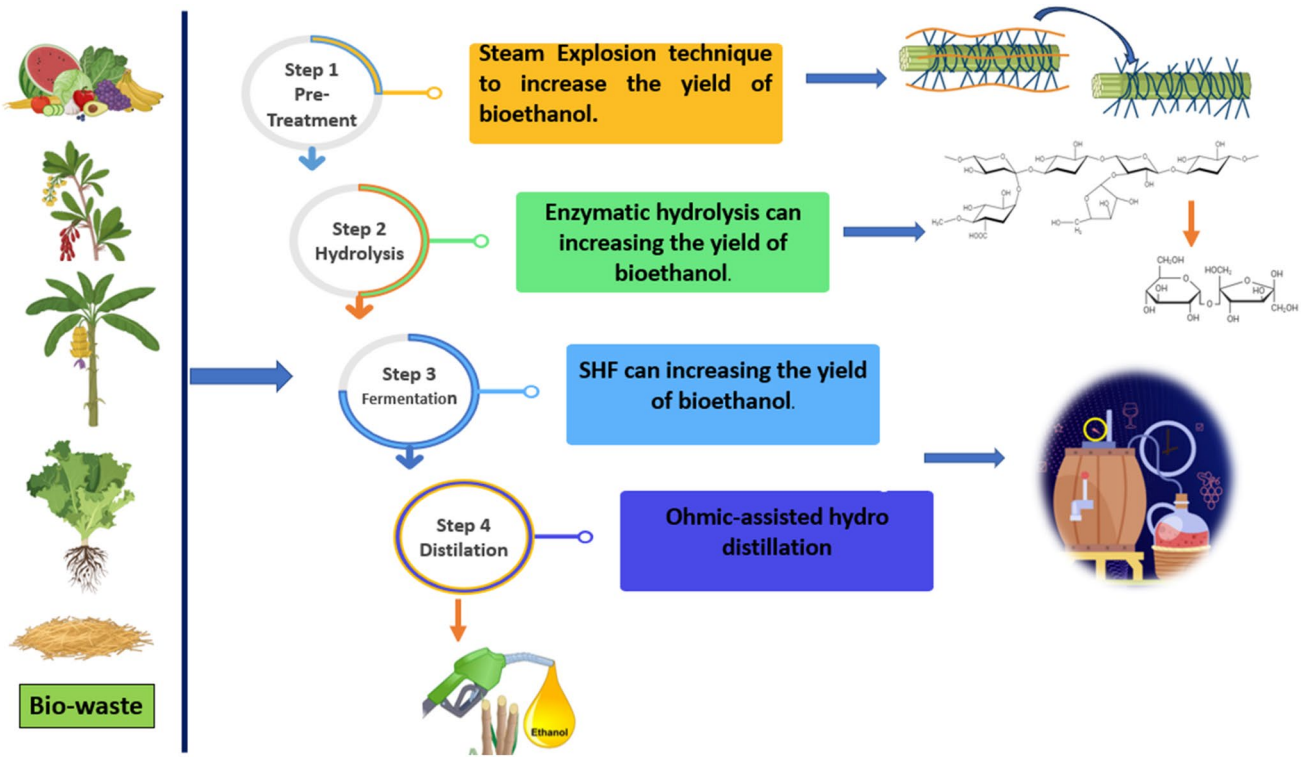
Abstract

It is generally accepted that the world's reliance on fossil fuels had negative implications, such as a decline in crude oil supply, a drop in air quality, an increase in global temperature, unpredictable weather change, etc. Biofuel production is one of the best options for minimizing the quantity of conventional fuel used. This article presents theoretical and practical methods for process improvement, as well as a brief review of the characteristics of bioethanol production and limitations by using various pre-treatment techniques with wastes such as fruit and lignocellulose biomass to ethanol production using various microbe species. Bioethanol has a variety of applications, including petrol blending, solvent use, and distillery sectors. The pH (4–4.3), temperature (32 °C), and kind of microorganisms all have a significant impact on bioethanol production. Many significant phenolic chemicals and bioactive substances have been extracted from waste during bioethanol manufacturing. The approaches discussed in this study, such as pre-treatment, extraction, and distillation, can enhance the yield of bio-ethanol, which can be beneficial in many ways in the future.

Graphical abstract



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Keywords Bioethanol · Fermentation · Fruit waste · Hydrolysis · Lignocellulose biomass · Microorganism

Abbreviations

GHG	Greenhouse gases
SSF	Simultaneous saccharification and fermentation
CBP	Consolidated bioprocessing
SHF	Separate hydrolysis and fermentation
SSCF	Simultaneous saccharification and co-fermentation
GE	Genetic engineering
TS	Total sugar
YPD	Yeast extract, peptone, and dextrose media
LCBs	Lignocellulosic biomass
GC-FID	Gas chromatography-flame ionization detection
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
NIR	Near-infrared spectroscopy
ASTM	American society for testing and materials
EBP	Ethanol blended Program

Introduction

Crude oil is the most significant source of energy. It contributes about 35% of total energy consumption of the world (Dominik and Rainer 2007; Jonathan 2008). After 2010, the demand for crude oil had been increased for multiple applications as per many reports (Ayodele et al. 2020). Petroleum-based goods will remain the dominant source of energy until at least 2030. Despite the expectation for oil reserves, the cost of renewable resources has surely risen, and rural communities have been pushed to boost their economic productivity (Morshabul et al. 2023).

Concerns over conventional fuel depletion (oil, natural gas, and coal), global warming, and the attendant environmental issues have lately spurred academics and politicians to focus on developing alternative energy sources (Izmirlioglu 2016). The dramatic increase in crude oil prices, as well as concerns about its availability, have been cited as major factors driving demand for alternative renewable energy sources that may be cheap and sustainable (Schwab et al. 2016; Robak and Balcerek 2018). India is world's second-largest producer of sugar cane, maize, pulses, cotton, wheat, millets and oil seeds (Saini et al. 2019). Subsequently India has a great potential in field of biofuel production (Harinikumar et al. 2020; Machineni 2020). Consequently, biofuel development could be considered as an alternative to reduce high dependence on non-renewable energy resources (Derman et al. 2018).

Biofuel have been produced from biodegradable wastes, so that nature wise it is nontoxic, sulfur-free and lower GHG (Greenhouse gases) emitter. Biofuel is mainly categorized in to four generations, first-generation biofuels are produced using oil-based plants, sugar, and starch outputs (Ullah et al. 2017). The first generation of biofuels adds to the dispute between fuel and food, although second generation biofuel produced from sustainable lignocellulosic biomass which reduced food safety issues (Li et al. 2017; Jin et al. 2015). Third generation biofuels derived from algae can be generated on a massive scale, absorb CO_2, and are relatively easy to refine, and have received a lot of attention. Engineered cyanobacterial and algal growth is an innovative and rapidly increasing pathway for the fourth generation of biofuels (Adeniyi et al. 2018; Sharma et al. 2020). Biofuels are commonly produced in the form of biodiesel, which is produced from vegetable oils, recycled wax, or animal fats, while bioethanol is produced from plants having sugar and starch through a fermentation process, and biogas, which is produced by anaerobic digestion of biodegradable waste (Xue et al. 2018).

Bioethanol

Any substance that contains sugar molecules such as sugarcane, corn, beetroot, rice straw, sweet sorghum, and algae are able to produce ethanol (Lin and Tanaka 2006). This process is typically divided into three steps: preparing the sugar-containing solution for fermentation; fermenting the sugar-containing solution; and finally recovering the ethanol from the fermented solution (Walker 2010). It should be noted that a number of factors, such as the kind of raw material used and the conversion method, might have an impact on the entire ethanol manufacturing process (Hiben 2013). Bioethanol production, technology, or utilization that have not been well researched or understood yet, these research gaps suggest chances of further research and improvement in this topic (Mukherjee et al. 2016; Melendez et al. 2022). Exploring novel feedstocks and developing efficient conversion technologies could help to enhance the sustainability and economic viability of bioethanol production (Rabbani et al. 2020). The pre-treatment of lignocellulosic biomass and subsequent enzymatic hydrolysis have been observed as critical steps in the conversion of cellulose and hemicellulose into fermentable sugars (Fu et al. 2021). Exploring innovative pre-treatment procedures, discovering superior enzymes, and improving enzymatic efficiency are all topics that need

to be researched further to increase the efficiency and cost-effectiveness of bioethanol production (Ingrao et al. 2021). The fermentation process plays a vital role in converting sugars into ethanol, by improving fermentation efficiency using proper microbial strain we can increase ethanol yields. Bioethanol production generates various co-products and by-products such as lignin, distiller grains, and stillage. Developing novel methods and uses for co-products such as lignin valorization, animal feed formulations, or bio-based chemicals can improve the bioethanol industry's overall economic viability and sustainability (Manikandan et al. 2023). Several techniques have been used in the production of bioethanol (Kaya and Karaosmanoglu 2022). The choice of technique depends on the type of feedstock used and the desired end product. Some techniques can directly effects on the yield of ethanol like saccharification using cellulase and hemicellulase enzymes to convert cellulose and hemicellulose into fermentable sugars. Multiple distillation steps may be used to achieve higher ethanol concentrations. Membrane processes such as prevaporation or vapor permeation can be employed to achieve higher purity levels and reduce energy consumption compared to traditional distillation methods. Simultaneous Saccharification and Fermentation (SSF) in which saccharification and fermentation processes occur simultaneously (Saini and Sharma 2021) offers advantages such as reduced process time, increased ethanol yield, and reduced contamination risks. Consolidated Bioprocessing (CBP) technique aims to combine the saccharification and fermentation processes into a single step, involves certain strains of bacteria or fungi, that possess the ability to break down complex carbohydrates and convert sugars to ethanol (Liu et al. 2021). CBP has the potential to simplify the bioethanol production process and reduce costs. These are just a few examples of the techniques which can be used in bioethanol production. Certain modifications in existing bioethanol production processes can boost bioethanol production output including feedstock selection, pre-treatment methods, fermentation efficiency, and overall process integration. Advancements in technologies such as consolidated bioprocessing (CBP), genetic engineering (GE), and the use of non-food feedstocks hold promise for overcoming these limitations and making bioethanol production more sustainable, efficient, and economically viable. In this review mainly focus on biodegradable waste, such as fruit and lignocellulose biomass, and the suitable microorganisms with different type of pre-treatment techniques in purpose to overcome the drawbacks for new bioethanol advances. This may encourage researchers to work more in this field as bioethanol production from waste provides a comprehensive understanding of the potential, challenges, and opportunities associated with utilizing waste materials as a sustainable feedstock for bioethanol production, ultimately contributing to a more sustainable and circular economy for greener future.

Material and methodology

Bioethanol production pathway in case of fruit waste as a starting material

Fruit waste

Fruit waste, which includes seeds, peels, and other wasted fruit components that are unfit for meals, can be separated from household waste. Fruit waste can be used to make bioethanol instead of being thrown or left to decay. Through a fermentation process, the sugars included in the fruit waste are converted into bioethanol. The gathered fruit waste is a great feedstock for bioethanol synthesis, which contributes to a more sustainable and environmentally friendly approach to waste management and renewable energy generation (Chitranshi and Kapoor 2021).

Alkali pre-treatment

Fruit biomass was used at a loading of 5% (w/v), and different concentrations of sodium hydroxide (NaOH) at 1%, 3%, and 5% were applied. Additionally, a control group with 0% NaOH was used for comparison. The alkali pre-treatment process was carried out at a constant temperature (30 °C for 48 h). The pre-treatment was performed in a steady state within an incubator using 250 mL Erlenmeyer flasks. Following the alkali pre-treatment, 1 N HCl was added to the samples to adjust the pH (4.2–4.5) to the desired level based on the designated hydrolysis conditions. This step aimed to optimize the conditions for subsequent hydrolysis. To analyze the pre-treated samples and determine the content of total sugar (TS) and reducing sugar (RS), the researchers performed analytical tests (Sindhu et al. 2014) (Table 1).

Microorganism and inoculum

Fermentation process with novel Wickerhamomyces strain known as *Wickerhamomyces sp.* UFFS-CE-3.1.2 (GenBank access numbers MF538579 and MF538580). *Saccharomyces cerevisiae,* commonly known as baker's yeast, remains the foremost yeast strain utilized for industrial-scale ethanol production. Yeast growth medium: Yeast extract, peptone, and dextrose media (YPD) were used to prepare the yeast growth medium. The composition of YPD medium is 1% yeast extract, 2% peptone, 2% glucose, and 2% agar. Agar is commonly used for growing microorganisms in a solid form and solidify the media.

Table 1 Main characteristics of pre-treatment methods

Pre-treatment	Advantages	Disadvantages	References
Alkaline	Little interactions with hemicellulose Mild environment conditions Successful delignification	Less effective on species that resist change It is uncoverable to convert alkali to salts Issues about the alkali if it is released in to the environment	Kim et al. (2016a, b) Loow et al. (2016)
Dilute acid	High saccharification yields Flexible method Solubilizing hemicellulose	The generation of inhibitory compounds Corrosiveness of acid High cost of recovering acid	Solarte-Toro et al. (2019)
Steam explosion	Very cheap Simple saccharification and delignification of hemicellulose disturbance of cellulose crystallinity	Partial solubilization of hemicellulose Production of hazardous compounds Incomplete breakdown of lignin-carbohydrate bond Production of chemicals that inhibit the growth of microbes	Singh et al. (2015)
Biological	Low operating conditions Low energy consumption There is no need for recycling operations Little production of inhibitory chemicals	Low downstream yields Long pretreatment time Carbohydrate losses	Zabed et al. (2019)
Ionic liquids	Low operating conditions Almost 100% recovery Green in nature and not harmful Hemicellulose can be solubilized up to 100%	High cost Difficulty in recovery Generation of inhibitors	Kumar and Sharma (2017), Usmani et al. (2020)
Organic solvents	High yield of fermentation Good cellulose recovery Fractionation of hemicellulose is high	Recovery of organic solvents is highly energy-intensive High coast Before washing with water, pre-treated solids should be washed with an organic solvent	Zhao et al. (2009)

Preparing the yeast culture, test tubes containing 10 mL of the YPD medium were inoculated with the *Wickerhamomyces sp.* UFFS-CE-3.1.2 strain. The tubes were placed in an incubator at 30 °C for 72 h on a solid inclined plane. This step allowed the yeast to grow and form colonies on the solid medium. After the incubation period on the solid medium, the yeast was transferred to tubes containing 10 mL of liquid YPD medium without agar. The liquid YPD medium was then incubated for 24 h at 30 °C. This step allowed the yeast to grow and multiply in the liquid medium. After the 24–48 h incubation of the liquid YPD medium, approximately 10% (v/v) of the liquid medium containing the yeast cells was added to the fermentation broth as inoculation of fermentation broth. The fermentation broth is the medium in which the actual ethanol production takes place. The yeast cells act as inoculants, initiating the fermentation process by converting sugars into ethanol. Table 2 describes ethanol production from several types of microorganisms, including ethanol yield, temperature, pH value, carbon–nitrogen sources, and incubation period.

Ethanol fermentation

In the process of producing bioethanol, anaerobic respiration is most commonly observed after hydrolysis. After producing 2% *T. harzianum* hydrolysates, these hydrolysates were used to continue the fermentation process. For this purpose, 2% v/v of *S. cerevisiae* (dry yeast) was injected into the hydrolysates. The fermentation process was initiated by maintaining a temperature 36 °C for 72 h. Samples were collected at 24 h intervals to monitor the production of ethanol. In the study conducted by (Casabar et al. 2019), it was observed that the highest production of bioethanol occurred at 48 h of incubation, resulting in a yield of 5.98 ± 1.01 g/L. At 24 h and 72 h of incubation, the ethanol yields were 5.31 ± 1.76 g/L and 4.5 ± 0.72 g/L, respectively. These findings indicate that the fermentation process using the *T. harzianum hydrolysates* and *S. cerevisiae* as the yeast

Table 2 Yeast species that produce ethanol as the main fermentation product from the fruit waste

Strain species	Temperature (°C)	pH value	Carbon source and concentration (g/l)	Nitrogen sources and concentration (g/l)	Incubation time (h)	The concentration of ethanol produced (g/l)	References
Saccharomyces cerevisiae 27,817	30	5.5	Glucose (50–200)	Peptone and ammonium sulfate	18–94	5.1–91.8	Vallet et al., (1996)
L-041-*S. cerevisiae*	30 or 35	N/A	Sucrose	Urea or ammonium sulfate	N/A	N/A	Pinal et al., (1997)
S. cerevisiae CICC 1308	30	5.0	Glucose or sucrose (50.0)	Peptone (5.0)	48	N/A	Liu and Shen (2008)
K. marxianus DMKU 3–1042	35	4.5	Sugar (50.0–80.0)	Ammonium sulfate (0.5)	72	4.1–63.9	Pattanakittivorakul et al. (2022)
P. kudriavzevii DMKU 3-ET15	40	6.5	Glucose (20.0)	Peptone (20.0)	48	N/A	Yuangsaar et al. (2013)
Z. mobilis ATCC 10988	30	6.0	Glucose (20.0)	Ammonium sulfate (1.0)	24–48	N/A	Tanaka et al. (1999)
UO-1-*S. cerevisiae* (aerobic)	30	5.0	Sucrose (20)	Ammonium sulfate (1)	60–90	N/A	Arora et al. (2015)
V5-*S. cerevisiae*	24	-	Glucose (250)	N/A	36	N/A	Campillo et al. (2012)
ATCC 24860-*S. cerevisiae*	30	4.5	Molasses (1.6–5.0)	Ammonium sulfate (0.72–2.0)	24	5–18.4	Jeppsson et al. (1996)
Bakers' yeast-*S. cerevisiae*	30	4.5	Sugar (150–300)	N/A	192	53	Alvarez et al. (2012)
Fiso-S. cerevisiae	30	5.0	Galactose (20–150)	Peptone, ammonium sulfate and casamino acid (10)	60	4.8–80	Jacobus et al. (2021)
ATCC-32691 *Pachysolen tannophilus*	30	4.5	Glucose (0–25) and xylose (0–25)	Peptone (3.6) and ammonium sulfate (3)	100	7.8	Sanchez et al. (2002)
30,091-*Candida utilis*	30	5.5	Glucose (100)	Peptone (2) and ammonium sulfate (4)	18–94	44.4	Juunchen et al. (2012)
GCB-K5-*S. cerevisiae*	30	6.0	Sucrose (30)	Peptone (5)	72	27	Yang et al. (2022)
Candida shehatae NCL-3501	30	4.5	Sugar (20–100)	Peptone (4.6) and ammonium sulfate (3)	48	0.33	Abbi et al. (1996)
KR18-*S. cerevisiae*	30	6.0	Sucrose (30)	Peptone (5)	72	22.5	Chang et al. (2018)

source was most efficient in producing bioethanol at 48 h of incubation. The ethanol yields slightly decreased at 24 h and 72 h, suggesting that the optimal fermentation time for maximum ethanol production was at 48 h. Figure 1 explains production of bioethanol from lignocellulose biomass.

Chemical composition of lignocellulose biomass

Primary and secondary walls exist in plant biomass, each having distinct functions and compositions. The primary wall is high in pectin and low in cellulose, whereas the secondary wall is mostly lignocellulose, which comprises cellulose, hemicellulose, and lignin. These components are critical in establishing the structure and properties of lignocellulosic substrates (Ornaghi et al. 2020; Cristele 2017). The lignocellulosic biomass is mostly composed of three basic biopolymers: cellulose, hemicellulose, and lignin. Each of these components contributes the overall structure and properties of the biomass, and their arrangement as shown in Fig. 2 (Hernandez-Beltran et al. 2019) (Table 3).

Steps of bioethanol production from LCB (lignocellulose biomass)

In the production of bioethanol from Lignocellulosic Biomass (LCBs), the pre-treatment stage is considered the

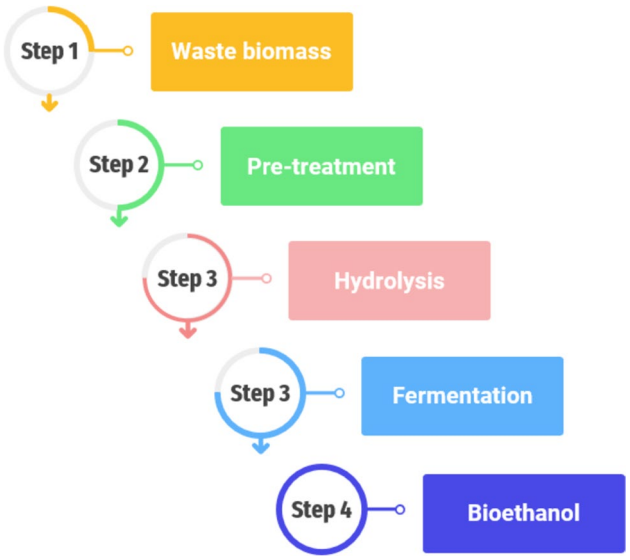

Fig. 1 Production of bioethanol from lignocellulose as a waste feedstock

most expensive and limiting step, followed by hydrolysis and fermentation (Satari et al. 2019). However, appropriate pre-treatment techniques can significantly improve the overall process efficiency by increasing the number of fermentable sugars after enzymatic saccharification (Maurya et al. 2015). Figure 3 illustrated the Structure of lignocellulosic biomass and its biopolymers; cellulose, hemicellulose, and lignin. During the pre-treatment stage, reducing sugars like arabinose, galactose, fructose, and mannose are released from LCBs. Overall, Fig. 4 illustrates the entire procedure for producing bioethanol from lignocellulosic biomass, depicting the various stages involved, including pre-treatment, hydrolysis, and fermentation, to ultimately obtain bioenergy in the form of bioethanol (Fig. 5).

Pre-treatment

Pre-treatment is indeed the initial and critical stage in the production of bioethanol from lignocellulosic biomass. It is considered the most important, challenging, and expensive phase of the entire process (Kumar and Sharma 2017). The basic purpose of any pre-treatment procedure is to delignify the lignocellulosic biomass by changing the structure of cellulose and hemicellulose to make them more accessible to hydrolysis and thereby boosting bioethanol output. The pre-treatment procedure might have three major goals: increasing biomass surface area, dissolving hemicellulose and/or lignin, and lowering biomass particle sizes. To meet the objective of pre-treatment, the structure of LCBs can be changed either chemically or physically. The hydrolysis of cellulose is considerably improved by increasing the accessibility of acids or enzymes to the surface of cellulose (Kumari and Singh 2018). Below Fig. 6 likely provides an overview of different pre-treatment techniques for lignocellulosic biomass and their characteristics, helping researchers to select the most suitable method for the bioethanol production process.

In the production of bioethanol, the fermentation technique faces challenges in incorporating lignin-carbohydrate complexes as they are resistant components of the LCB structure (Bhowmick et al. 2018). To address this, delignification becomes a crucial step in the process, involving the removal of lignin from LCB using various enzymatic, chemical, or natural processes (Miranda et al. 2019). Once the recalcitrant compounds, including lignin, have been removed from the biomass, the LCB can be further degraded chemically or biologically (Molaverdi et al. 2019). Fruit waste, for example, has a high lignin component and accounts for the greatest amount of biomass among non-wood biomass wastes, ranging from 17 to 32% (Arni 2018). Efficient delignification process can be increasing the surface area of

Fig. 2 Composition of primary and secondary cell wall

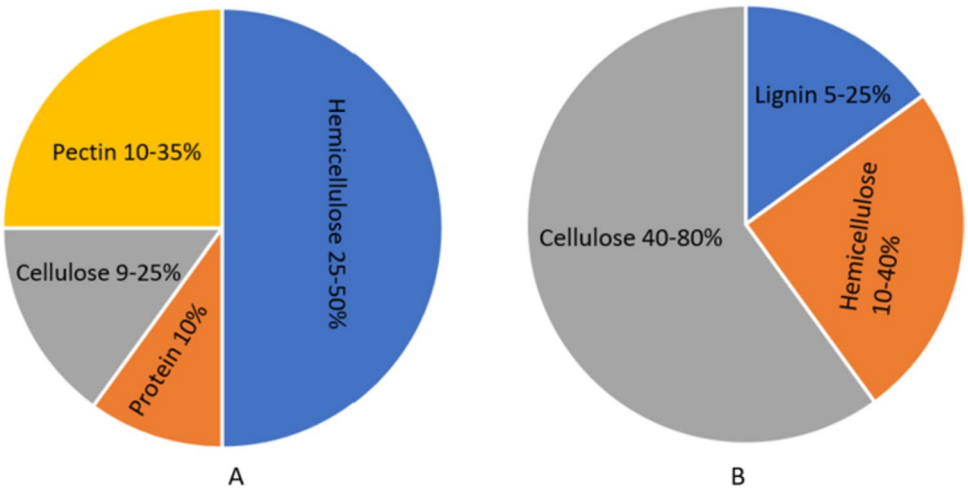

Table 3 Content (%) of cellulose, hemicellulose, and lignin in various lignocellulosic substrates on a dry basis

Lignocellulosic substrates	Composition (% Dry Basis)			
	Cellulose	Hemicellulose	Lignin	References
Eucalyptus	52.07 ± 2.6	24.51 ± 1.1	25.2 ± 1.1	Jinkun et al. (2017)
Corn straw	49.3 ± 1.8	28.8 ± 1.4	7.5 ± 0.4	Song et al. (2014)
Grass	47.12 ± 3.2	36.01 ± 3.17	11.55 ± 0.3	Merino et al. (2017)
Sugarcane bagasse	46.1 ± 0.7	20.1 ± 0.9	20.3 ± 0.6	Mohammad et al. (2018)
Wheat straw	43.4	26.9	22.2	Shah and Ullah (2019)
Poplar	50.8–53.3	26.2–28.7	15.5–16.3	(Isikgor and Becer 2015)
Bamboo stem	43.04	22.13	27.14	Chen et al. (2016)
Corn Stover	42.21	22.28	19.54	Ye et al. (2013)
Corn cob	42.0 ± 0.1	45.9 ± 0.9	2.8 ± 0.2	Noelia et al. (2017)
Meadow grass	41.28 ± 5.3	28.14 ± 3.2	30.14 ± 7.9	Panagiotis et al. (2018)
Cotton Stalk	41.6 ± 0.5	23.6 ± 0.4	23.3 ± 0.7	Yuan et al. (2016)
Giant reed	41.5 ± 2.6	20.5 ± 0.6	18.4 ± 1.4	Jaing et al. (2016)
Birch	40.1 ± 0.6	17.5 ± 0.2	24.2 ± 0.1	Luo et al. (2019)
Oil palm empty fruit bunch	38.5 ± 1.9	26.1 ± 1.1	11.6 ± 1.6	Charnnok et al. (2019)
Maize straw	38.33 ± 0.8	29.76 ± 1.35	3..82 ± 0.5	Khatri et al. (2015)
Pinewood	38.2 ± 0.3	24.1 ± 0.7	34.4 ± 0.3	Salehian et al. (2013)
Rice straw	37.8 ± 0.2	29.6 ± 0.7	14.8 ± 0.4	Mustafa et al. (2016)
Water hyacinth	36.84 ± 0.8	27.7 ± 0.2	27.7 ± 0.2	Barua et al. (2018)
Rye straw	36.5 ± 0.1	NR	21.3 ± 0.1	Smuga et al., (2016)
Corn stalk	36.4 ± 0.1	30.3 ± 0.1	6.9 ± 1.4	Dong et al. (2018)
Miscanthus	36.3 ± 2.1	22.16 ± 1.9	22.55 ± 2.5	Thomas et al. (2019)
Rice hulls	36	12	26	Cabrera et al. (2014)
Willow sawdust	35.6 ± 0.9	21.5 ± 0.9	28.7 ± 0.2	Alexandropoulou et al. (2017)
Oat straw	35	28.2	4.1	Tovar et al. (2012)
Empty fruit bunch	34.77 ± 0.2	22.55 ± 1.2	10.58 ± 2.3	Mardawati et al. (2022)
Hemlocks	47.5 ± 0.8	22.0 ± 0.8	28.5 ± 0.4	Wayman and Parekh (1990)

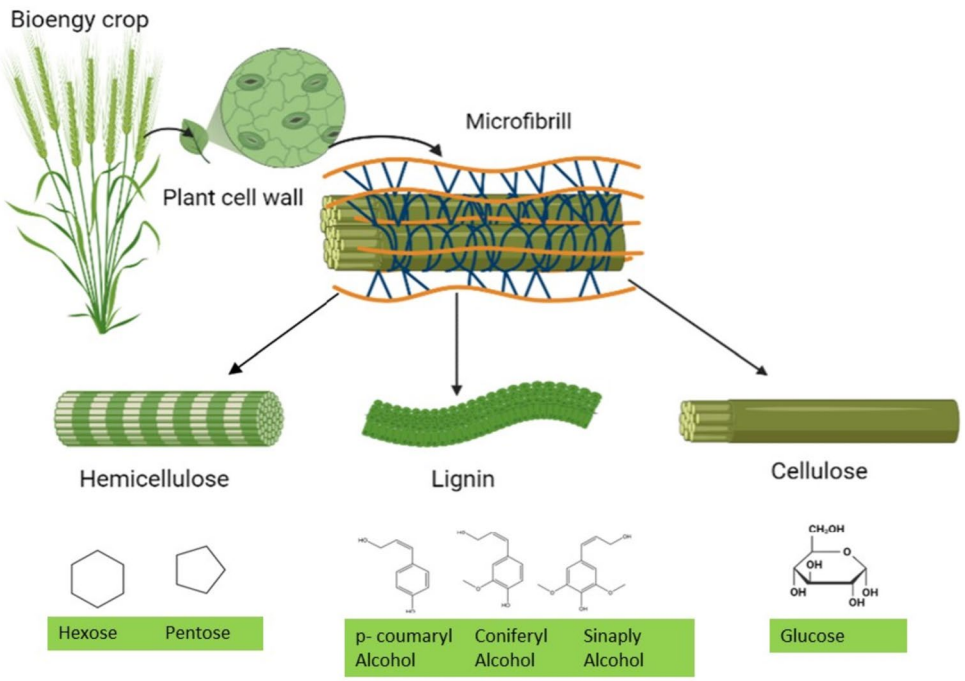

Fig. 3 Structure of lignocellulosic biomass and its biopolymers; cellulose, hemicellulose, and lignin

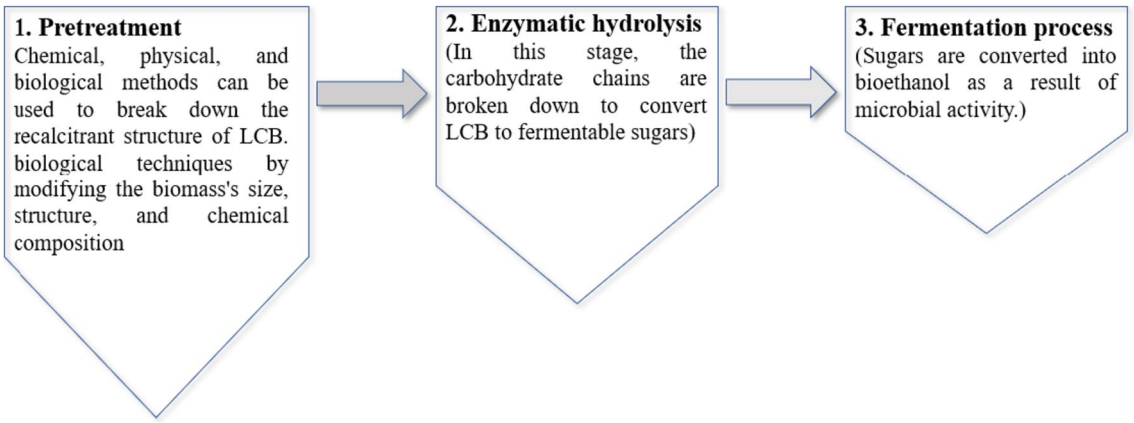

Fig. 4 Conversion of the LCB to bioethanol

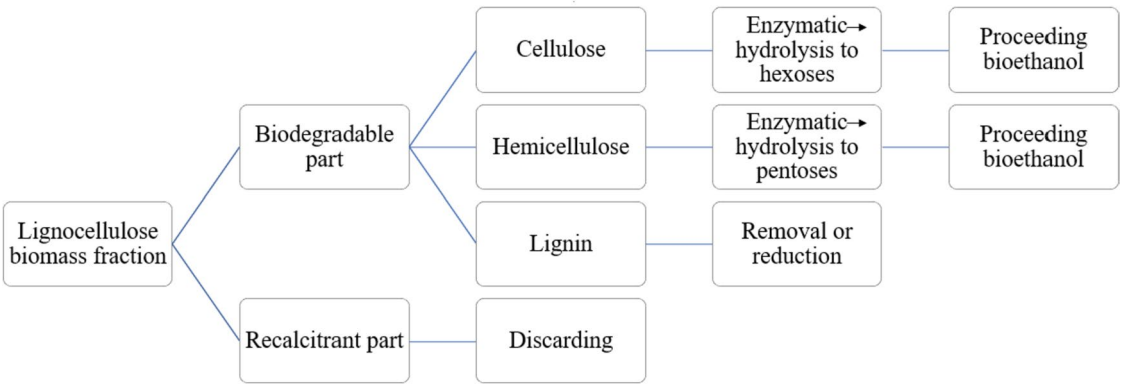

Fig. 5 Fractionating LCB to bioethanol production

the biomass (Singh et al. 2014). Lignin removal is especially essential since it reduces the availability of sugars, and remaining lignin can greatly inhibit the conversion of cellulose to bioethanol. A large proportion of recoverable carbs may be recovered by successfully eliminating lignin (Galbe and Zacchi, Pretreatment: The key to efficient utilization of lignocellulosic materials 2012). Not all pre-treatment processes eliminate lignin. Instead, the structure of lignin may be changed in some situations. Surprisingly, raw biomass can be more digestible than pre-treated biomass, although having a same lignin content (Agbor et al. 2011). Figure 5 describes Fractionating LCB to bioethanol production (Table 4).

Hydrolysis

A critical stage in the manufacturing of bioethanol is hydrolysis, which tries to break down cellulose and/or hemicellulose into readily fermentable sugars. Because of its crystalline form, cellulose provides more hydrolysis issues than hemicellulose. As a result, either the use of an acid, known as chemical hydrolysis or the use of specialized enzymes, known as enzymatic hydrolysis, can help in the breakdown of cellulose (also referred to as biochemical hydrolysis or saccharification) (Mazhad et al. 1995).

Chemical hydrolysis Chemical hydrolysis can be conducted with dilute acid or strong acid. Dilute acid hydrolysis involves the use of sulfuric acid at concentrations of 1–3% for a short duration, typically around 3 min, and temperatures range between 180 to 240 °C (Zhao et al. 2020). However, this approach has drawbacks in that it produces a very low glucose conversion rate of roughly 60% and a considerable amount of pentose sugars are converted into furfuraldehyde, limiting overall efficiency. A two-step hydrolysis technique was devised to overcome these obstacles and improve yields while resolving inhibitor-related concerns. Hemicelluloses are solubilized in the first stage at 140–160 °C, and cellulose is converted in the second step at 160–180 °C. The sugar recovery rate with this method can reach up to 80%. Strong acid hydrolysis, on the other hand, is a two-step process that uses greater acid concentrations of 20–40% and operates at

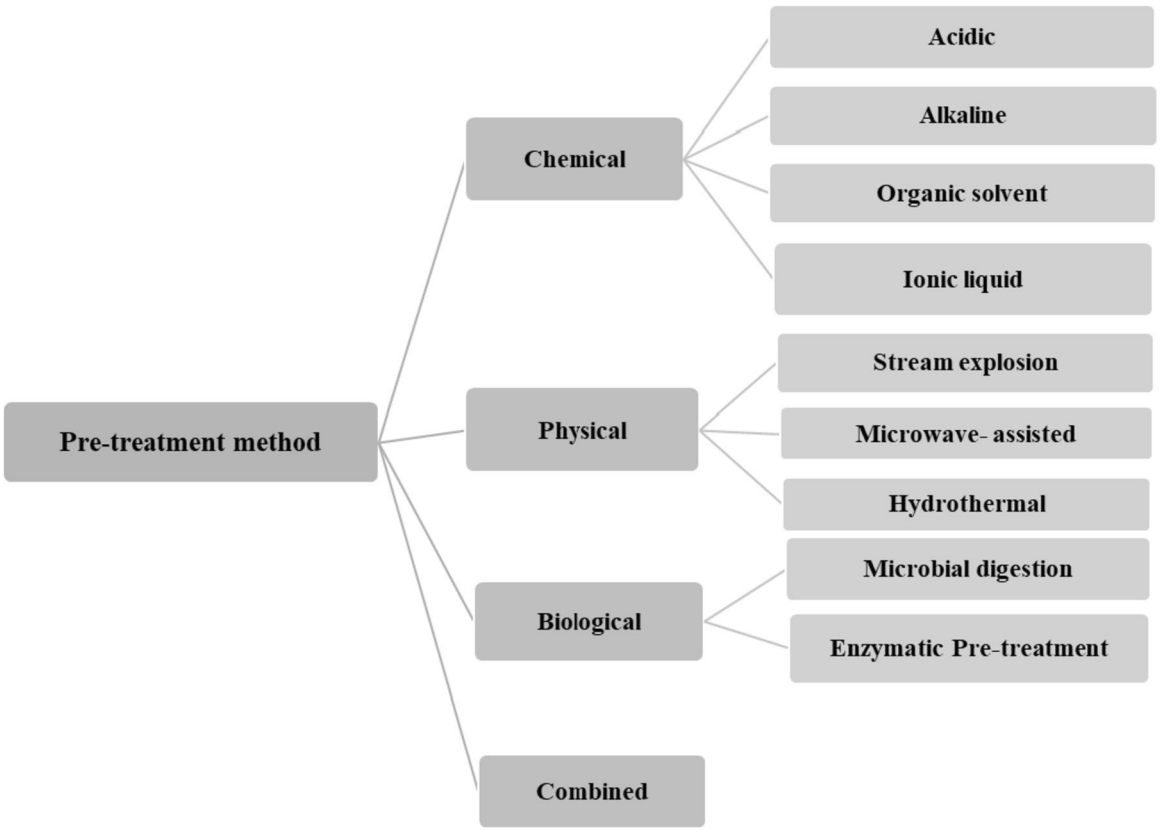

Fig. 6 Pre-treatment methods for bioethanol production

lower temperatures 50–100 °C. This approach has a greater sugar recovery rate of around 90%. However, dealing with acid corrosion and recycling necessitates a larger expenditure. Presently, chemical hydrolysis has become less attractive and less competitive due to the cost of reagents and the production of inhibitors, which may necessitate additional purification steps to obtain the desired bioethanol yield (Hamelinck et al. 2005).

Enzymatic hydrolysis In enzymatic hydrolysis enzymes, which are big proteins with high molecular weights, operate as effective biological catalysts. This method includes the conversion of cellulose and hemicelluloses into simple sugars with the use of particular enzymes released by microorganisms such as bacteria and fungi. Enzymes are extremely selective and adaptable, allowing for full cellulose to glucose conversion without the production of unwanted by-products or inhibitors. This makes enzymatic hydrolysis a very appealing alternative to chemical hydrolysis, which frequently suffers from partial conversion and inhibitor production. Enzymatic hydrolysis acts at lower temperatures and pH levels (about 50 °C and pH 5), avoiding the corrosion difficulties associated with chemical hydrolysis. Despite its potential, enzymatic hydrolysis remains a significant barrier in the lignocellulosic bioethanol sector due to challenges in extracting sugars from hemicellulose breakdown (Galbe and Zacchi 2007). The cost of producing enzymes and the large quantities required for transforming lignocellulosic materials create hindrances to the development of second-generation bioethanol. To address these issues, research and development efforts are being directed on lowering enzyme manufacturing costs, increasing enzyme activity, and generating novel cellulolytic enzyme combinations (Abo et al. 2011). The objective is to match or come close to the yield and cost levels of enzymatic hydrolysis in amylaceous (starch-based) substrates used in first-generation ethanol production. As pointed out, the economic feasibility of bioethanol from lignocellulosic materials is strongly reliant on advancements in the enzymatic hydrolysis step (Abo et al. 2019).

Different processes of bioethanol conservation

Bioethanol synthesis from lignocellulosic biomass requires a biochemical route involving multiple hydrolysis and fermentation techniques. These procedures might be carried out simultaneously in a single reactor or separately. These methods include Simultaneous Saccharification and Fermentation (SSF), Separate Hydrolysis and Fermentation (SHF),

Table 4 Shows the different kind of pre-treatment techniques for ethanol production

Pre-treatment type	Raw material	Sub-type of pre-treatment	Pre-treatment condition (temperature and time)	Reducing sugar	Delignification Rate	fermentation condition	Ethanol yield	References
Chemical Pre-treatment	Sugar bagasse	Na_2CO_3 (5%)	140 °C, 1 h	Glucose:97.6%	40–59%	37°;42 h	7.27 g/L	Nosratpour et al. (2018)
	Rice straw	NaOH	50–90 °C	Glucose: 81%	64.51%	37 °C; 72 h	0.032 g/L	Ahmed et al. (2017)
	Wheat straw	$NaOH/H_2O_2$	50 °C, 3–15 h	61.9 g/L	60%	37 °C; 96 h	31.1 g/L	Yuan et al. (2018a, b, c)
	Rice straw	Na_2CO_3	93 °C, 3–10 h	77.4 g/L	54.5–62.7%	37 °C; 120 h	83.1 g/L	Molaverdi et al. (2019)
	Wheat straw	H_2SO_4 (2%)	180 °C; 10 min	43 g/L	N/A	30 °C; 72 h	0.44 g/L	Prasad et al. (2018)
	Rice straw	H_2SO_4	200 °C; 1 min	NA	N/A	30 °C; 48 h	2.3 g/L	Lin et al. (2016)
	Rapeseed straw	H_2SO_4	180 °C; 10 min	66–80%	N/A	35 °C; 72 h	35.44 g/L	Tsegaye et al. (2019)
Physical Pre-treatment	Wheat straw	Microwave-associated NaOH	160 °C; 15 min	718 mg/g	69.78%	30 °C; 108 h	6.82 g/L	Zhang et al. (2018)
	Rice straw	Microwave-assisted alkali	60 °C; 25 min	N/A	N/A	30 °C; 24 h	1.383 g/L	Mikulski et al. (2019)
	Maize	Microwave-assisted H_2SO_4	50 °C; 20 min	104 mg/g	N/A	50 °C; 24 h	0.511 g/L	Kandasamy et al. (2017)
	Cotton stalks	Microwave-assisted acid	210 °C; 10 min	N/A	81%	210 °C; 10 min	15.9 g/L	Katsimpouras et al. (2017)
	Sugarcane bagasse	Ultrasound	120 °C; 30 min	N/A	N/A	49 °C; 12 h	6.38 g/L	Monschein et al. (2016)
	Corn Stover	steam explosion	200 °C; 10 min	84.7%	N/A	50 °C, 72 h	78.3%	Kim et al. (2016a, b)
	Wheat straw	steam explosion	165 °C; 10 min	Glucose: 38.7 Xylose: 24.1	36.0%	50 °C, 48 h	N/A	Imman et al. (2015)
	Corn stover	Hydrothermal	180 °C; 4 min	Glucose: 89 Xylose: 134	52.6%	62 °C; 72 h	N/A	Huang et al. (2016)
	Rice straw	Hydrothermal	40–180 °C; 5–20 min	Glucose: 71.8	N/A	50 °C; 72 h	N/A	Yang et al. (2019)
	wheat straw	Hydrothermal	180 °C; 40 min	Cellulose: 84.15	23.52%	50 °C; 48 h	84.15%	Wu et al. (2016)
	Bamboo	Hydrothermal	170 °C; 2 h	Cellulose: 70	N/A	50 °C; 72 h	N/A	Fonseca et al. (2018)
Biological Pre-treatment	Rice straw	fungi/ enzyme (T.reesei Aq-5b and T. viride NSW-XM)	28 °C; 2–4 days	22.74 g/L	N/A	30 °C; 48 h	2.17 g/L	Torreiro et al. (2016)
	Rice straw	Yeast hydrolysis (Saccharomyces cerevisiae)	25 °C; 30 min	N/A	N/A	30 °C; 24 h	0.24 g/L	Arora et al. (2016)
	Wheat straw	Fungi Enzyme (White-rot fungus Irpex lacteus)	121 °C; 20 min	11.5%	45.8%	50 °C; 94 h	12.5 g/L	Sreemahadevan et al. (2018)
	Paddy straw	Fungus enzyme (Trametes hirsuta)	30 °C	52.91 g/L	71.34%	50 °C; 24 h	0.86 g/L	Martines-Patino et al. (2018)

Table 4 (continued)

Pre-treatment type	Raw material	Sub-type of pre-treatment	Pre-treatment condition (temperature and time)	Reducing sugar	Delignification Rate	fermentation condition	Ethanol yield	References
Combined pre-treatment	Rice straw	Microwave-alkali-acid	28 °C; 14 days	8.11 g/L	50.65%	30 °C; 24 h	0.38 g/g	Akhtar et al. (2017)
	Sugarcane bagasse	HC-assisted alkaline hydrogen peroxide	60 °C; hydrogen peroxide	Xylose:38 g/L Glucose:80 g/L	63.3%	70 °C; 20 min	0.49 g/g	Hilares et al. (2018)
	Corn Stover	Ethanol–water + diluted sulfuric acid	130–170 °C; 60–90 min	50–60%	30–66%	50 °C; 72 h	N/A	Vergara et al. (2018)
	Wheat straw	Alkaline + alkaline peroxide	50 °C; 7 h	N/A	0.5–3.4%	30 °C; 24 h	31.1 g/L	Yuan et al. (2018a, b, c)
	Olive tree	Fungal and dilute acid	120 °C; 15 min	Glucose: 9.9%	10.3%	50 °C; 72 h	N/A	Arora et al. (2016)
	Bamboo	Alkaline pre-extraction + alkaline hydrogen peroxide	100 °C; 30–80 min	Glucose: 17.6 g/L Xylose 8.5 g/L	62.9%	30 °C; 24 h	4.6% w/v	Yuan et al. (2018a, b, c)

Consolidated Bioprocessing (CBP), Dilute Acid Hydrolysis, Enzymatic Hydrolysis, Fermentation, Distillation, Dehydration, and Rectification. The choice of process is determined by factors like the nature of the feedstock, scale of production, available technology, and economic feasibility. Each method presents its own advantages and challenges in the production of bioethanol from lignocellulosic sources. Table 5 summarizes the various ways of converting lignocellulosic biomass to ethanol.

Simultaneous saccharification and fermentation (SSF) The most commonly used process for bioethanol production is the SSF method (Zabed et al. 2016). It involves conducting enzymatic hydrolysis and ethanol fermentation together in the same reactor. This approach offers several advantages, including lower investment costs compared to using separate reactors (Balat 2011). Because the sugars are fermented immediately after synthesis, the possibility of enzyme inhibition and contamination by hydrolysis end products (glucose and cellobiose) reduces (Krishna et al. 2001). However, one significant disadvantage of the SSF technique is that the enzymatic yield is not ideal due to the lower temperature employed (less than 37 °C) to maintain the yeasts, which are temperature sensitive (Triwahyuni et al. 2015). Some researchers have employed thermophilic yeasts to progressively increase yield to solve this issue. SSF and SHF are the two primary configurations of lignocellulosic biomass bioethanol manufacturing technologies. Overall, the SSF process has been considered to be superior to the SHF method in terms of ethanol output and concentration several supports the same (Kim et al. 2010; Niklitschek et al. 2010; Rana et al. 2014). However, by optimizing the hydrolysis

Table 5 Different processes for lignocellulosic biomass to ethanol conversion

Process name	Description	Ethanol conc. g/L	Refeences
Simultaneous saccharification and fermentation (SSF)	Hydrolysis and fermentation occur simultaneously in the same reactor	13.6–12.6	Kang et al. (2015), Li et al. (2009)
Separate hydrolysis and fermentation (SHF)	Hydrolysis and fermentation take place in separate reactors	39.4–69.2	Tavva et al. (2016), Ask et al. (2012)
Consolidated bioprocessing (CBP)	A single microorganism performs both hydrolysis and fermentation in a single step	N/A	Pejo et al. (2008), Khramtsov et al. (2011)
Simultaneous saccharification and co-fermentation (SSCF)	Combination of enzymatic hydrolysis (saccharification) and fermentation (co-fermentation) in a single step	21.3–32.9	Olofsson et al. (2010)

and fermentation temperatures, some researchers have produced higher yields with the SHF technique. While the SHF process can offer greater yields under certain conditions, the SSF method has shorter processing durations, resulting in higher ethanol production. The decision between SSF and SHF is influenced by process factors, substrate properties, and target ethanol output and productivity (Cantarella et al. 2004).

Separate hydrolysis and fermentation (SHF) In this method, the hydrolysis and fermentation reactions occur in separate reactors, allowing for optimal conditions for each stage (Ask et al. 2012). The hydrolysis of cellulase, operates at 45–50 °C, while the fermentation takes place at 30–37 °C. Despite the advantages, there are several drawbacks to this approach (Paulova et al. 2015). Cellulase and β-glucosidase inhibition can occur due to the accumulation of cellulose and glucose in the hydrolysate. Additionally, using two reactors can increase the investment required, although a second reactor may not always be necessary if operated in batch mode (Khramtsov et al. 2011). Nevertheless, the SHF process provides the advantage of being able to recycle yeast fermentation, which is not always feasible with other methods (Roca and Olsson 2003).

Direct conversion (Consolidated bioprocessing) (CBP) Direct conversion is a one-step process that integrates enzyme synthesis, saccharification, and fermentation in a single operation by a specialized microorganism or microbial consortia. Notably, this approach does not need substrate pre-treatment, while some researchers have referred to this technique are needed enzyme addition separately when pre-treatment is not required (Zerva et al. 2014). CBP is intriguing because it eliminates the need for several reactors, simplifies overall operation, and increases the competitiveness of lignocellulosic bioethanol production. However, its effectiveness is dependent on the development of an appropriate and effective microbial organism or consortium, which can be difficult. Although CBP is an efficient one-step method for lignocellulosic bioethanol synthesis, the creation of a suitable microbial system is critical to its effective deployment (Paulova et al. 2015).

Simultaneous saccharification and co-fermentation (SSCF) Many bioethanol production systems either do not use xylose or need two stages of fermentation. The Simultaneous Saccharification and Co-Fermentation (SSCF) technique is used to overcome this and further minimize investment costs. The SSCF method enables the simultaneous hydrolysis and fermentation of two sugars, glucose, and xylose, in the same reactor. However, bacteria are capable of efficiently absorbing both glucose and xylose with high yields are necessary for successful SSCF. While the yeast *S. cerevisiae* is well-known for its excellent glucose fermentation, it has a low potential for xylose fermentation. Various attempts to enhance xylose fermentation have resulted in the discovery of microorganisms and systems capable of fermenting both glucose and xylose. Researchers use genetic engineering to add specific metabolic pathways for pentose utilization into yeast strains, which is critical in this attempt. One such study by Ohgren et al. involved using a recombinant TMB3400 strain of *S. cerevisiae*, which showed a significant increase in ethanol yield from 54 to 64% in a batch reactor without prior detoxification. The study also highlighted that the glucose concentration affects xylose consumption and suggested that the fed-batch mode is advantageous to prevent glucose accumulation. The SSCF process enables simultaneous fermentation of glucose and xylose, but its success relies on the development of microorganisms with improved xylose fermentation capabilities. Genetic engineering plays a crucial role in enhancing these fermentation processes, leading to more efficient bioethanol production from lignocellulosic biomass (Ohgren et al. 2006).

Distillation process

Distillation is a technique used to separate chemicals based on differences in their rates or ease of evaporation to produce bioethanol (Karimi et al. 2021). Before starting the distillation process, the sample is pasteurized by heating it at 80 °C for 10 min to destroy any germs or contaminants. Once pasteurized, the distillation process can activate. During distillation a high-quality bioethanol product will be obtained (Li and Li 2020).

Determination of bioethanol production

Testing the quality of bioethanol involves a range of measurements and assessments to ensure it meets the required standards for various applications. Some of the key quality tests for bioethanol include Density and Content Measurement: The density of bioethanol, which is the mass per unit volume, is measured to determine its concentration and purity. The content of ethanol in the bioethanol sample is also analyzed to ensure its purity. Specific Gravity (SG) and API Gravity: Specific gravity is the ratio of the density of a substance to the density of a reference substance (usually water). API gravity is a specific gravity scale used in the petroleum industry to measure the relative density of liquids. These measurements provide insights into the density and composition of bioethanol. Calorific Value: The calorific value, also known as the heat value or energy content, is determined to assess the energy density of bioethanol. It quantifies the amount of heat energy released during the combustion of bioethanol and is crucial for evaluating its

fuel efficiency. Yield Measurement: The yield is calculated as the percentage of bioethanol obtained from the raw material or feedstock used in the production process. It reflects the efficiency of the conversion process and the overall quality of the bioethanol. pH Testing: The pH of bioethanol is checked to ensure it falls within the acceptable range. The pH value affects the stability and reactivity of bioethanol in various applications. Physical Characteristics: The physical properties of bioethanol, such as color, odor, appearance, and clarity, are examined to detect any signs of contamination or impurities.

These tests and assessments play a crucial role in determining the quality of bioethanol and ensuring it meets the required specifications for its intended use, whether it be as a fuel additive, industrial solvent, or other applications. Regular and accurate quality testing is essential to maintain the consistency and safety of bioethanol production.

Determination of ethanol using gas chromatography-flame ionization detection (GC-FID)

In the analysis of ethanol concentration in the supernatant obtained after centrifugation, Gas Chromatography with Flame Ionization Detector (GC-FID) was employed. The GC-FID apparatus used in this study is equipped with a DB-wax column with specific dimensions: 30 m in length, 0.25 mm in internal diameter, and a film thickness of 0.25 µm. The FID detector is utilized for detecting and quantifying the ethanol present in the sample (Sanchez, et al. 2020). The temperature conditions for the column oven during the GC-FID analysis like (a) 40 °C for 4 min, (b) 100 °C with a temperature ramp of 5 °C per minute, and (c) 200 °C at a rate of 10 °C per minute. These temperature conditions are crucial for the separation and detection of ethanol in the sample. The different temperature settings allow for the efficient resolution of ethanol peaks and accurate quantification. To determine the concentration of ethanol in the supernatant, a calibration curve was established using several ethanol standards. These standards were prepared by diluting 99.999% ethanol in various ratios to create a range of known concentrations (Setyo et al. 2020). By comparing the peak areas of the ethanol standards with those of the sample, the ethanol concentration in the supernatant can be identified and quantified accurately. This GC-FID analysis, along with the calibration curve, provides a reliable and precise method for determining the ethanol content in the supernatant, allowing researchers to assess the efficiency of the bioethanol production process (Shah et al. 2019). Fourier transform infrared spectroscopy (FTIR) is a commonly employed technique for identifying functional groups in intricate organic mixtures and for comparing similarities between different substances. The wavenumbers at 3298, 3318, 3275, and 3292 cm^{-1} in the FTIR spectrum result from vibrations of the -OH groups, which originate from alcohol and pectic acid constituents found in the biomass (Orozco et al. 2014).

Statistical analysis – significance test

The accurate determination of ethanol content is crucial for assessing the efficiency of the bioethanol production process and ensuring the quality of the final product. In the study, the statistical significance of the experimental outcomes was assessed using Origin Pro software. A paired t-test was performed, and a Tukey test with a significance threshold of P less than 0.05 was used to determine the differences in significance between the Control (C), Dewaxed (D), Pretreated (P), and D + P samples (Trejo et al. 2022). These tests allow researchers to compare the results of different treatments and combinations to identify any significant variations in the data. For the determination of ethanol content in the samples, various techniques can be employed, each has different advantages and limitations. Some of the commonly used methods include Densimetric methods (Lachenmeier et al. 2010), Potassium dichromate (Breisha 2010), Biosensor potentiometry (Rotariu et al. 2003), Gas chromatography (GC) (Wang et al. 2003), Capillary electrophoresis. Additionally, other techniques such as flow injection analysis (Rangel and Toth 2000), high-performance liquid chromatography (HPLC) (Shih and Smith 2009), Raman spectrometry (Oliver et al. 2014), near-infrared spectroscopy (NIR), and electrophoresis (Nosratpour et al. 2018) can also be utilized for ethanol determination. The selection of a specific method depends on factors like the required sensitivity, accuracy, and the nature of the sample matrix. Researchers may choose the most appropriate technique based on their experimental requirements and available resources.

The testing of these properties will be performed using specific ASTM (American Society for Testing and Materials) standard methods, which ensure standardized and reliable measurements (Hadeel et al. 2011). The ASTM standards used for testing the chemical properties are; Ethanol content—ASTM D5501, Methanol content—ASTM D1744, Water content—ASTM D1688, Copper (Cu) concentration—ASTM D512, Chlorine (Cl) concentration—ASTM D2622, Gum content—ASTM D381 (Olugbenga 2023). For the physical properties testing, ASTM standards will be applied like Heating value—ASTM D1613, Density—ASTM D240, Viscosity—ASTM D1298-99, Flashpoint—ASTM D445 and D93 (Tenkolu et al. 2022).

By following the ASTM standards, the analysis of these chemical and physical properties will be conducted with accuracy and consistency. These tests are essential to ensure the quality, safety, and compliance of bioethanol with established standards and regulations (Saleh and Al-Azzawi 2023).

Technology trends and barriers to ethanol production

Many process phases of bioethanol production are currently being improved, including pre-treatment, pentose fermentation, simultaneous saccharification and fermentation (SSF), downstream processing, and by-product reduction (Su et al. 2020). The pre-treatment stage, in particular, is an expensive phase, accounting for several studies 11 to 27% of ethanol production expenses, depending on the pre-treatment method used. It needs to offer high sugar yields for later fermentation, have a low inhibitor concentration to prevent inhibiting fermentation, and successfully separate lignin and hemicellulose for further processing during the pre-treatment stage. Reducing costs in lignocellulosic ethanol production also involves optimizing extraction, washing, and neutralization stages, minimizing energy, reagents, catalysts, and water usage. Steam explosion stands out as one of the most efficient and cost-effective methods, particularly for agricultural residues and lignocellulose biomass. The presence of hemicellulose during hydrolysis results in the creation of pentoses, which are less fermentable than glucose. Because pentose derivatives can account for a large amount of biomass weight, efficient pentose fermentation is critical to lowering ethanol production costs. SSF processes outperform SHF processes in terms of investment costs and cellulase inhibition induced by cellobiose build-up (Broda et al. 2022). However, temperature has a crucial role in saccharification and fermentation. Reducing water usage is also essential to minimize environmental impact, and research is exploring the possibility of fermentation in more concentrated media. The efficient separation of biomass constituents after pre-treatment and the valorization of lignin and hemicellulose are promising research directions (Zabed et al. 2016). The by-product of the fermentation process can be used as animal feed, in biogas plants, various valuable biochemicals, and bioplastics; which adds economic value to the industry. However, the overall cost is affected by challenges related to the use of organic solvents, which can be extracted for the next batch to make the final product cost-effective.

Advantages and future perspective of bioethanol

Bioethanol has major advantages and applications in future as it overcomes the problems occurred by fossil fuels (Himmel et al. 1997). At present, the majority of commercial bioethanol comes from sugarcane and corn. However, these sources alone cannot satisfy the growing demand for bioethanol or can't replace significant amounts of conventional fuels consumed worldwide. In this study, main focus is to obtain bioethanol from waste or other sources, especially from lignocellulosic biomass. When compared to gasoline, bioethanol has the potential to reduce greenhouse gas emissions significantly and carbon cycle neutral. Bioethanol production can be a boon for rural economies and diversify the agricultural sector. The combustion of bioethanol produces fewer pollutants such as sulfur oxides, particulate matter, and volatile organic compounds compared to conventional gasoline, leading to improved air quality and reduced health risks (Das et al. 2023). Current biofuel technology research and development may result in more efficient production methods, better energy outputs, and enhanced performance of bioethanol blends in current engines. Currently, most bioethanol is produced from starch or sugar-rich crops, but research is ongoing to develop technologies for cellulosic ethanol production. Advancements in biotechnology and fermentation processes could lead to higher conversion efficiencies. More countries are searching for efficient bioethanol production as awareness of climate change and the demand for sustainable energy sources grows, creating a global market for this renewable fuel. The government has set a target of 10% blending (10% bioethanol 90% petrol E10) of bioethanol with petrol by 2022 under the Ethanol Blended Program (EBP). Furthermore, they intend to boost this blending ratio to 20% (20% bioethanol 80% gasolineE20) by 2030 (Dhande et al. 2021). Government policies, incentives, and requirements targeted at boosting renewable energy sources can play an important role in promoting the widespread use of bioethanol and other biofuels.

Conclusion

The depletion of fossil fuels, which has increased greenhouse gas emissions, has motivated the search for alternative energy sources. Bioethanol stands out as a potential and sustainable renewable energy alternative among these. The depletion of fossil fuels, which has increased greenhouse gas emissions, has motivated the search for alternative energy sources. Bioethanol stands out as a potential and sustainable renewable energy alternative among these. The prospects of bioethanol are closely intertwined with advancements in genetic engineering of microorganisms, non-food crops, and waste biomass used in the fermentation process. The main goal of bioethanol is to develop microbes capable of efficiently breaking down lignocellulosic waste biomass or non-food crops into sugars for bioethanol production. Fruit and vegetable residues contain abundant amounts of simple and

complex carbohydrates that can serve as raw materials for bioethanol production using microbial cultures. However, there is a rising emphasis on utilizing lignocellulosic biomass to produce bioethanol. To make this process economically viable, significant emphasis has been paid to the development of cellulolytic enzymes, the cost of which accounts for more than half of the total biomass saccharification expenditures. These enzymes can be improved by molecular engineering or genetic engineering of the microorganisms, resulting in lower bioethanol production costs. SHF is preferred optimal conditions independently in the field of bioethanol production and this technique has an advantage compared to SSF. Co-culturing two or more microbes can be employed to achieve a high yield of bioethanol. The study discussions demonstrated that the highest yield of bioethanol can be improved under specific conditions: pH 4, temperature of 32 °C, and using 3 g/L of yeast. Utilizing fruit and vegetable waste biomass have the benefit of being economically feasible and widespread availability, making it an ideal and ecologically beneficial solution for bioethanol production. The study inspires researchers to work further in the field of green chemistry to produce bioethanol at a lower cost.

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Data availability Enquiries about data availability should be directed to the authors.

Declarations

Conflict of interest The authors declared that there are no known competing financial interests or personal relationships that could influence the work reported in this article.

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