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Review on Bioethanol Production using Diverse Substrates and Fungal Strains

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Abstract

The scarcity and unsustainable supply of fossil fuels in reservoirs prompt researchers to explore several alternative and sustainable energy sources from renewable feedstocks. Given the significance of bioethanol being produced in order to meet the energy demand, the available data is scattered, with little effort to condense the findings, which will be imperative to comprehend. This review highlights and summarizes various findings on bioethanol production. Published studies from 2000 to 2024 were reviewed. A total of 3,650 records were collected from various databases and sorted based on the title. Bioethanol has recently seen growing commercialization due to its market stability, low cost, sustainability alternative fuel energy composition, greener output and massive fossil fuel depletion but the major challenges that hindered bioethanol production are due to a lack of optimization which results in a lower yield of bioethanol produced and as a result, it cannot be applied for large scale production. The enzymatic capabilities of fungal strains are essential for Bioethanol production and can be enhanced through modern technologies such as synthetic biology and genome editing. Future research should concentrate on harnessing the capabilities of fungal strains to improve enzymatic hydrolysis and fermentation, particularly emphasizing strain engineering strategies that enhance sugar utilization and resistance to fermentation inhibitors.

Keywords: Fossil-Fuel, Bioethanol, Biomass, Renewable-feedstocks, Fungal strains

INTRODUCTION

The growing consumption of fossil fuels derived from petroleum resources raises questions about environmental impact and energy security (Falano *et al.*, 2014). Researchers are looking for other methods to produce fuels from sustainable bioresources because of a number of issues, such as the global climate change caused by greenhouse gas emissions (Bezerra and Ragauskas, 2016). Socioeconomic progress and development in industrialized and emerging countries rely heavily on fossil fuels for power generation, yet this has various negative consequences (Dogru *et al*. 2020). Aside from these health and environmental concerns, the use of fossil fuels in energy generation systems exacerbates the problem of low power generation, which not only widens the gap between demand and supply but also decreases people's living standards (Gautam *et al.*, 2019). More over 1.1 billion people, or 17% of the world's population, live in poverty and without access to power (Nabipour *et al.*, 2020). Access to clean energy remains a luxury in many parts of the world (Douf *et al.*, 2024). This has led to a surge in research, investment, and innovation in renewable energy technologies like solar, wind, and biofuels (Elia *et al.*, 2021). Recently, there has been an increase in interest in producing bioethanol from biomass materials, which is one of the most cost-effective liquid fuel alternatives to non-renewable fossil fuels (Tekaligne and Dinku, 2019).

Biofuels are believed to have a lower "carbon footprint" than fossil fuels and contribute less to greenhouse gas emissions due to their CO_2-neutral conversion (Osman *et al.*, 2024). However, there are various concerns with biofuels, including the chance that biofuel plants will displace food crops. This has a negative influence on food security, especially in developing nations (Vassilev *et al.*, 2015). Biofuel has gained acceptability as a fuel alternative to fossil fuels as people become more aware of environmental issues. (EPA, 2023). To avoid conflicts between edible resources for human consumption and industrial uses, researchers have investigated the production of bioethanol from lignocellulosic material (Dahnum *et al.* 2015). Additionally, value was added by utilizing lignocellulosic biomass from weeds, agricultural waste, and

micro- and macroalgal biomass (Ambaye *et al.*, 2021).

Bioethanol is a renewable, colorless, less harmful, and rapidly biodegradable fuel derived from biological sources that can be used for heating, electricity, and fuel (Kida *et al.*, 2023). It is now used as an alternative fuel since it is biodegradable, derived from renewable sources, has a high-octane number, and is less harmful than traditional petroleum-based fuels (Akhabue *et al.*, 2018). Biomass energy has the potential to drastically reduce greenhouse gas emissions (Osman *et al.*, 2024). According to Sebayang *et al.* (2016), bioethanol can be manufactured from a variety of raw materials that are divided into three groups based on their chemical composition: sucrose-containing feedstocks, starch materials, and lignocellulosic materials. Although cellulosic materials are more easily available and less expensive, the process of converting them into ethanol is costly due to the several steps required. In these circumstances, employing renewable substrates such as starchy byproducts, lignocellulosic biomass, and agricultural waste necessitates a novel approach (Broda *et al.*, 2024). Many research studies have been conducted on waste items, especially fruit waste. Coconut trash and starchy bio waste (Bello *et al.*, 2014; Hossain *et al.*, 2017; Hashem *et al.*, 2021)

The United States and Brazil are the world's major ethanol producers, accounting for 85% of the total (Alternative Fuels Data Center, 2016). The vast majority of ethanol in the United States is made from corn starch, whereas Brazil generally uses sugarcane (Pattanathu and Rahman, 2017). Thailand and China create bioethanol from cassava (*Manihot esculenta*), which is also an edible feedstock (Deesuth *et al.*, 2015). However, because of their primary importance as food and feed, these traditional crops cannot match the global need for bioethanol production. To reduce human dependence on fossil fuels, efforts are being made to produce bioethanol from non-edible feedstocks such as lignocellulosic and starchy agricultural feedstocks (Aditiya *et al.*, 2016).

Given the importance of bioethanol production around the world in meeting energy demand, data remains scattered, with little effort made to condense the findings, which will be critical to comprehend (Toor *et al.*,2020) in order to identify knowledge gaps and provide a roadmap for future directions. This review summarizes previous research on bioethanol production, including its physicochemical properties, various feedstocks, the role of fungal strains in bioethanol production, common waste biomass, pretreatment methods, and various fermentation conditions for bioethanol production.

2.1 Overview of bioethanol production

Bioethanol is widely produced through a variety of chemical and biological methods (Fan *et al.* 2012). The biological approach entails fermentation of biomass with ethanologenic microbes in anaerobic or semi-anaerobic conditions (Clain *et al.*, 2016; Kumar *et al.*, 2016). Fermentation is an ancient technology that refers to the bioconversion of carbohydrates into acid or alcohol via glycolytic intermediates. The bioprocessing of carbohydrate-containing feedstock is primarily done in two steps (Carvalheiro *et al.*, 2024). The first step is the hydrolysis of polysaccharides into fermentable sugars, which are then converted to bioethanol using appropriate microorganisms (Dave *et al.*, 2019). Furthermore, downstream processing includes bioethanol purification and concentration through the distillation process. A significant limitation of the production process is the lower concentration of bioethanol in the fermentation broth (Lassmann *et al.*, 2014).

Bioethanol outperforms gasoline due to its high compression ratio, shorter burn time, and lean burn engine (Splitter *et al.*, 2016; Carrillo-Nieves *et al.*, 2019; Elshenawy *et al.*, 2023). Octane number measures engine performance, with a higher number indicating better combustion (Ilves *et al.*, 2019), because of its 35% oxygen content, ethanol emits fewer particulates, hydrocarbons, and NOx after combustion (Toor *et al.*, 2020). Furthermore, bioethanol has a higher-octane number and combustion efficiency than gasoline, a small flame luminosity, corrosive nature, lower vapor pressure (making cold starts difficult), water miscibility, and ecosystem toxicity" (MacLean and Lave, 2003). The properties of ethanol are given in Table 1.

2.2 Bioethanol feedstock

A variety of biomass can be used to produce bioethanol, and these feedstocks fall into one of three categories (Table 2). Feedstocks that contain sucrose, such as sugarcane, sugar beet, and sweet sorghum; starchy substances, such as rice, wheat, corn, and barley; and cellulosic biomass, such as wood, forestry residue, straw, and grasses, are examples of the first three.

(Toor *et al.*, 2020). First generation: Biofuels are produced by fermenting sugar-based raw substrates or edible substrates.A refined fuel requires only a few basic processing steps and is typically made from grains, sugars, or seeds of which only a specific (usually edible) portion is used (Azhar *et al.*, 2017; Derman *et al.*, 2018). Second generation: Bioethanol is made from lignocellulosic biomass. The second-generation bioethanol processes use sugars released from cellulose, necessitating the use of enzymes to hydrolyze cellulose (Carrillo-Nieves *et al.*, 2019; Rocha-Meneses *et al.*, 2019). Under the second generation, various agricultural byproducts such as corn stalks or rice husks, wheat straw, rice straw, and non-edible plants such as trees or grasses grown specifically for energy, wood trimmings, sawdust, bamboo, cotton stocks, and other cellulose-containing biomass can be used to produce bioethanol (Derman *et al.*, 2018; Carrillo-Nieves *et al.*, 2019). Third-generation use algae as substrate for the production of bioethanol. It's still in its early stages (Jambo *et al.*, 2019). Algal fuels' appealing characteristics is that, they can be grown with minimal impact on freshwater resources and can produce up to 300 times more oil than conventional crops (Yang *et al.*, 2010). Fourth-generation biofuels use metabolic engineering or systems biology strategies in feedstock modification, such as E. coli gene modifications, which are more efficient than yeasts (Azhar *et al.*, 2017; Jambo *et al.*, 2019). The fourth-generation fuels include solar fuels or those that capture carbon from the process (Rastogi and Shrivastava, 2017).

Table 1: Typical physicochemical properties of ethanol NCBI, (2025)

Property	Value
Molecular Formula	C_2H_6O
Molecular Weight	46.07 g/mol
Appearance	Colorless liquid
Density	0.789 g/cm³ at 20 °C
Melting Point	−114.1 °C
Boiling Point	78.23 °C
Flash Point	12 °C
Vapor Pressure	5.95 kPa at 20 °C
Viscosity	1.2 mPa·s at 20 °C
Surface Tension	22.3 mN/m at 20 °C
Refractive Index	1.3611 at 20 °C
Solubility in Water	Miscible in all proportions
pKa (in water)	15.9
Dipole Moment	1.69 D
Enthalpy of Vaporization	38.56 kJ/mol
Thermal Conductivity	0.171 W/m·K at 25 °C
Specific Heat Capacity	2.44 J/g·K at 25 °C

2.3 Common waste biomass

2.3.1 Agricultural waste

Agricultural waste, among others, has become a major source of pollution in *Niger*ia. The use of agricultural waste as a renewable feedstock for bioethanol production has the potential to generate clean energy (Salisu and Umar, 2023). The agricultural waste contains a lot of carbohydrates that can be converted into bioethanol (Sahman *et al.*, 2020). Agricultural waste is inexpensive, renewable, and abundant. Rice straw is one of the most widely used and abundant lignocellulosic feedstocks worldwide, particularly in Asia and Africa (Singh *et al.*, 2024). Each year, approximately 667.6 million tons of biomass are post-harvested in Asia (Hossain *et al.*, 2017). Along with rice straw, rice husk is being considered as a potential source for bioethanol production via yeast fermentation (Chavan *et al.*, 2024). Bioethanol production from rice husk can reach 3.20 ± 0.36 g/l, with an ethanol yield of 0.27 g/g total sugar (Srivastava & Agrawal, 2014). Coconut waste biomass has been identified as another remarkable source. The maximum bioethanol yield of coconut waste was 90.09% and productivity was 0.21 g/L.h, derived solely from green coconut shell by *Saccharomyces cerevisiae* (yeast) fermentation (Hossain *et al.*, 2017). Commercial bioethanol experiments using coconut waste are being carried out in the Northeast region of Brazil (Goncalves *et al.*,

2015). Bioconversion of sorghum crop residues to ethanol has great potential for improving ethanol yield for sustainable bioethanol production. Sweet sorghum bagasse and juice yielded 157 and 121 L/tons of bioethanol, respectively, based on industrial production output (Nasidi *et al.*, 2016). Sweet potato residue fermented with an amylolytic industrial yeast strain 1974-GA-temA produced 27.27 g/L ethanol (Wang *et al.*, 2024). Steam-exploded corn Stover hydrolysate (SECSH) yielded 0.454 g/g and an ethanol concentration of 22.96 g/L (Wu *et al.*, 2023). Other residues, such as wheat straw, corn straw, and cereal straw, can also be viable candidates for bioethanol production through fermentation (Swain *et al.*, 2019).

Table 2: Bioethanol production from various feedstock.

Generation	Substrate	Ethanol production	Reference
First	*Amorphophallus spp.* (starchy tuber)	8.68 ± 0.91 g/L	Bhuyar *et al.* 2022
First	Sugar beet pulp	12.6 g/l	Berlowska *et al.*, 2017
Second	Banana peels	56.13±1.45 and 59.13±0.49 g/L	Shitophyta *et al.*, 2023
Second	Corn Stover	34.3 g/l	Liu and Chen, 2016
Second	Steam-exploded corn Stover hydrolysate (SECSH)	22.96 g/L	Wu *et al.*, 2023
Second	seed pods of *Bombax ceiba*	72.0 g/L	Ghazanfar *et al.*, 2022
Second	Cellulose-rich corncob	31.96 g/L	Boonchuay *et al.*, 2021
Second	Potato residue	27.27 g/L	Wang *et al.*, 2024
Second	Cassava stems, peels and leaves	263ml/Kg, 200ml/kg dry and 303ml/kg dry biomass	Pooja *et al.*, 2018
Third	*Eucheuma Denticulatum*	11.6 g/g	Alfonsín *et al.*, 2019
Third	*Eichhornia crassipes*		ShakilaBegam *et al.*, 2024
Third	*Kappaphycus alvarezi*	64.30g/L	Hargreaves *et al.*, 2013
Third	*Sargassum crassifolium*	43.92g/L	Widyaningrum *et al.*, 2016

2.3.2 Municipal Plant-based Waste Biomass

In terms of environmental cleanliness and public health safety, the R&D sector is currently focused on recycling and utilizing waste from municipal drainage (Hossain *et al.*, 2017). Korea has already started a bioethanol production project using municipal waste and sludge from a local industrial complex (Park *et al.*, 2010). Meanwhile, Sweden began producing bioethanol through fermentation from starch plants obtained from slurries and streams (Linde *et al.*, 2008). Apart from industrial waste, bioethanol can also be produced from kitchen waste through a fermentation process. The sugars produced after hydrolysis of kitchen waste were mainly attributed to the monosaccharides, glucose (80%) and fructose (20%). The fermentable sugars obtained were subsequently used as a carbon source for bioethanol

production by locally isolated yeasts, *Saccharomyces cerevisiae*, *Candida parasilosis*, and *Lachancea fermentati*. The yeasts successfully consumed the sugar hydrolysate and produced the highest ethanol yield, ranging from 0.45 g/g to 0.5 g/g and productivity between 0.44 gL−1h−1 - 0.47 gL−1h−1 after 24 hours of incubation, which was equivalent to 82.06 - 98.19% of conversion based on theoretical yield (Hafid *et al*., 2016)

2.3 3 Lignocellulosic biomasses

Lignin, hemicellulose, and cellulose are the primary components of biomass cell walls. Lignin is made up of a wide variety of phenolic polymers. Hemicellulose is a polysaccharide that consists of arabinose, acetic acid, and xylose linked together. According to Chundawat (2011), cellulose is a macromolecule composed of ß-linked glucose molecules. All plant cell walls contain these components; the amount of each component varies only slightly, so any plant material can be used as a feedstock for sugar production. Table 3 shows the composition of lignocellulose biomass from various sources

2. 4 Role of fungal strains in bioethanol production

Fungal strains play an important role in bioethanol production. The fungus *Aspergillus niger* can degrade cellulose and convert paper waste into bioethanol, providing the required carbon, nitrogen, vitamins, and amino acids (Darwesh *et al*., 2020; Bellaouchi *et al*., 2021). The secretion of fungal amylase by yeast strains has enabled the conversion of raw substrate into ethEthanolowering production costs (Favaro *et al*., 2015). Furthermore, *phlebioid* fungal species enabled the bioconversion of lignocellulose waste, demonstrating the feasibility of single-step bioethanol production (Mattila *et al*., 2017). Fungi play an important role in both biomass pretreatment and sugar conversion to bioethanol, making them essential for efficient and sustainable bioethanol production. Cellulases and lignocellulolytic enzymes are known to be produced by *Trichoderma, Penicillium,* and *Fusarium*, which break down plant cellulose and hemicellulose into fermentable sugars. Hydrolytic enzymes enable a diverse range of fungi to break down carbon compounds (Lange *et al*., 2017)

Table 3. Composition of Lignocellulose Biomass from various Sources

Source	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Brewer spent grain	23.1	22.9	19.0	Plaza *et al*., 2017
Corn Stover	31.5	18.0	14.1	Vergara *et al*., 2018
Poplar sawdust	46.2	19.3	26.15	Lai *et al*., 2020
Sugarcane bagasse	44	28	21	Ajala *et al*., 2021
Wheat straw	32.8	29.9	13.8	Vergara *et al*., 2018

Previous research (Table 4), has shown that fungal strains play an important role in bioethanol production, from biological pretreatment to the fermentation process. These strains harness the conversion of substrate biomass into ethanol. Oji *et al*. (2024) found that fermenting yeast (g/L) with 6% banana peel yielded 44.68±0.82% bioethanol after 3 days at 5.5 pH and 35°C. Shitophyta *et al*. (2023) reported that Banana peel was fermented with *Saccharomyces cerevisiae* and *Rhizopus oryzae* at room temperature for 120 hours, with yeast concentrations of 2, 3, and 5 g/L. *R. oryzae* produced more ethanol than *S. cerevisiae*. Water Hyacinth (*Eichhornia crassipes*) co-cultured with *A. oryzae*, *A. niger*, and *S. cerevisiae* produced approximately 56% more ethanol than *S. cerevisiae* - single culture and *S. stipitis* - single culture (Shakila Begam *et al*., 2024). Cassava peel prepared with *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Cassava peels produced a high percentage yield of 30% in 45 mL of ethanol (Behingbe et al., 2021). Sweet potato residue fermented with an amylolytic industrial yeast strain named 1974-GA-temA yielded 27.27 g/L ethanol over 8 days (Wang *et al*., 2024). Steam-exploded corn stover hydrolysate (SECSH) produced with *Saccharomyces cerevisiae* had an ethanol concentration of 22.96 g/L and a yield of 0.454 g/g (Wu *et al*., 2023). Ethanol was optimally produced at 12% substrate concentration using rice chaff, at a temperature of 35 °C and pH of 5.0 (Adeyemo *et al*., 2021). Ragi husk as a substrate for *Aspergillus fumigatus* JCM 10253 cellulase production demonstrates potential for value-added industrial products and lignocellulosic bioethanol production (Saroj and Narasimhulu, 2020). A new strain of *Trametes villosa* from the Paranaense rainforest efficiently hydrolyzes barley straw to produce bioethanol, potentially lowering the overall cost of bioethanol production (Coniglio *et al*., 2020). Yeast co-

culturing of *Saccharomyces cerevisiae*, *Pichia barkeri*, and *Candida* in pairs or triples significantly increases bioethanol production from starchy biowastes, reaching 167.80 0.49 g/kg of biowaste during experiments in a 7-L fermenter (Hashem *et al.*, 2021). The maximum ethanol concentration and ethanol productivity values for cellulose-rich corncob (CRC) residence with S. *cerevisiae* were 31.96 g/L and 0.222 g/L/h, respectively (Boonchuay *et al.*, 2021). Lignocellulosic hydrolysate produced by *Aspergillusniger*, *Zymomonas mobilis*, and *Trichoderma longibrachiatum* yields the highest bioethanol yield from lignocellulosic biomass, indicating promising pathways for sustainable biofuel technologies (Bendaoud *et al.*, 2024).

Table 4: Summary of bioethanol production from different fungal strains

Substrate	*Fungal strain*	Brief findings	Reference
Sugarcane molasses	*Saccharomyces cerevisiae isolate MUT15F*, *Saccharomyces cerevisiae isolate MUT18F*, and*Saccharomyces cerevisiae isolate R9MU*	Stress-tolerant yeast strains from traditional Ethiopian alcoholic beverages can effectively produce bioethanol from sugarcane molasses, with potential for industrial use.	Fentahun and Andualem 2024
Sweet potato residue	Amylolytic industrial yeast strain named *1974-GA-temA*	Optimizing fermentation parameters, such as pH, solid-to-liquid ratio, inoculation volume, and enzyme addition, can significantly increase bioethanol production from sweet potato residue	Wang *et al.*, 2024
Lignocellulosic hydrolysate	*Aspergillusniger*, *Zymomonas mobilis* and *Trichoderma longibrachiatum*	*Aspergillusniger* shows the highest bioethanol yield from lignocellulosic biomass, offering promising pathways for sustainable biofuel technologies.	Bendaoud *et al.*, 2024
Corn Stover	S. *cerevisiae*	The engineered S. *cerevisiae* strain YL13-2 effectively produces high-titer bioethanol from steam-exploded corn Stover, overcoming inhibitory compounds and xylose limitations.	Wu *et al.*, 2023
Sugar substrate	*Wickerhamomyces anomalus BT2*, *BT5*, and *BT6*. *Saccharomyces cerevisiae*, *Geobacillus stearothermophilus and Pseudomonas aeruginosa*	*Wickerhamomyces anomalus* strains from traditional Balinese beverages can produce bioethanol from various sugar substrates, with higher ethanol production on glucose substrate than other substrates.	Fathiah *et al.*, 2023
Rice husk	*Aspergillusniger SIF2* and*Aspergillus flavus CMXY22565* *Saccharomyces cerevisiae FJI and Pichia kudriavzevii IPBCC.y.161552*	Bioethanol can be produced from rice husk using a consortium of *Aspergillusniger* SIF2, *Aspergillus flavus* CMXY22565 for hydrolysis and a consortium of *Saccharomyces cerevisiae* FJI and *Pichia kudriavzevii*.	Audu *et al.*, 2023

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Table 4 continued

Substrate	*Fungal strain*	Brief findings	Reference
Saccharum spontaneum biomass	*Aspergillusniger*, *Ganoderma sessile* and*Saccharomycescerevisiae (CDBT2)*	This study demonstrates a cost-effective method for producing bioethanol from *Saccharum spontaneum* biomass by simultaneous saccharification and electro-fermentation using a mixed culture of microbes.	Dhungana *et al.*, 2022
Seed pods of (*Bombax ceiba*)	*Saccharomycescerevisiae*	KOH-steam-treated *Bombax ceiba* seed pods in SSF fermentation with *Saccharomyces cerevisiae* resulted in the highest ethanol production (72.0 g/L) and the highest saccharification (58.6% after 24 h).	Ghazanfar *et al.*, 2022
Agricultural wastes	*T. reesei*, *S. cerevisiae*, and *P. stipites*.	Encapsulating microorganisms in SBP capsules in a continuous bioethanol production process ensures long-term prosperity and activity, with an efficiency of 60-70%.	Rahamim *et al.*, 2022
Sweet potato starch	*Saccharomycescerevisiae*	Fungal amylases from *Endomelanconiopsis endophytica*, *Neopestalotiopsis cubana*, and Fusarium *pseudocircinatum* can potentially improve bioethanol production by *Saccharomyces cerevisiae*, with potential yields of 17.3-88.1 percent.	Romao *et al.*, 2022
Cellulose-rich corncob (CRC) residue	*Saccharomycescerevisiae*	Thermotolerant *Saccharomyces cerevisiae* TC-5 is a promising yeast for bioethanol production from cellulose-rich corncob residue at elevated temperatures, with potential for second-generation substrates.	Boonchuay *et al.*, 2021
Rice chaff	*Aspergillusniger*	*Aspergillusniger* S48 effectively hydrolyzes pre-treated rice chaff to produce bioethanol at 12 percent substrate concentration, 35°C, and pH 5.0, offering a cost-effective and environmentally friendly alternative energy source.	Adeyemo *et al.*, 2021
Cellulose	*Aspergillus sp. DHEF7*	A novel *Aspergillus* sp. DHE7 strain maximizes extracellular-glucosidase production, making it a promising biofuel source and potential food and beverage additive.	El-Ghonemy, 2021

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Table 4 continued

Substrate	*Fungal strain*	Brief findings	Reference
Wheat straw	*Mucor indicus*, *Aspergillus niger* and *Aspergillus fumigates*	Mucor indicus is the most efficient and eco-friendly fungus for producing bioethanol from wheat straw fermentation, with 8.4% production after 15 minutes of UV exposure.	Naqvi *et al.*, 2021
Alkali-pretreated corncob	Acidic *fungal laccases*	Acidic fungal laccases may be a better choice than neutral/alkaline fungal laccases for delignification and detoxification of alkali-pretreated corncob for bioethanol production.	Liu *et al.*, 2021
Starchy biowaste (waste rice)	*Saccharomyces cerevisiae*, *Pichia barkeri*, and *Candida*	Yeast co-culturing in couples or triples significantly enhances bioethanol production from starchy biowastes, reaching 167.80 ± 0.49 g/kg of biowaste during experiments in a 7-L fermenter.	Hashem *et al.*, 2021
Cocoyam, *Xanthosomaroseum*,	*Kluyveromycesmarxian us*and *Pichia stipites*	African wild cocoyam is an excellent feedstock for bioethanol production, with *Kluyveromyces marxianus* and Pichia stipitis strains producing more ethanol when used as coculture at pH 4.5.	Chukwudi *et al.*, 2021
Ragi husk	*Aspergillus fumigatus*	Ragi husk as a substrate for *Aspergillus fumigatus* JCM 10253 cellulase production shows potential for value-added industrial products and lignocellulosic bioethanol production.	Saroj and Narasimhulu, 2020
Distillers' dried grains with solubles (DDGS)	*Aspergillus niger*	Hydrolyzed DDGS can be an economical substrate for *Aspergillusniger* strains to produce cellulase and xylanase, offering a potential solution for bioenergy production.	Iram *et al.*, 2020
Cornstalk	*Trichoderma reesei*	Fungi Trichoderma reesei exposed to gamma rays can optimize glucose content in cornstalks, leading to a 98% increase in bioethanol production by *Saccharomyces cerevisiae*.	Mulyana *et al.*, 2020
cellulosic substrates (barley straw)	*Trametes villosa*	A novel strain of *Trametes villosa* from Paranaense rainforest efficiently hydrolyzes barley straw to produce bioethanol, potentially reducing the total cost of bioethanol production.	Coniglio *et al.*, 2020

Table 5: Review of various pretreatment processes for bioethanol production

Feedstock	Mechanical	Chemical	Biological	Sugar	Reference
Banana peel	The peels were washed with water and air-dried at 45 °C.	1M NaOH, (1-3%) H_2SO_4		42.14± 0.92%	Oji *et al.*, 2024
Sugarcane bagasse	SCB was dried to constant weight and then crushed with a grinder.	2% NaOH, 2% H_2SO_4 and 12% sodium percarbonate/glycerol	Cellulase (10000 U/g)	443.52 mg/g	Ruan *et al.*, 2024
Sorghum	The sorghum was first washed and then dried and sieved		Alpha-amylase (90, 100 and 110 U/g) and amyloglucosidase (36, 51 and 66 U/mL)	175.94 g/L	Sebayanga *et al.*, 2017
Microalgae	The Microalgae sample was sun dried for seven (7) days for milling	1% H_2SO_4, %2 NaOH	0.5% *Aspergillus niger*	0.519 ± 0.239 g/l	Kida *et al.*, 2023
Pineapple waste	The PI wastes were grinded using a grinding mill and blended with a blender		Natural hydrolysis enzymes such as pectinase, cellulase, and hemicellulose, which are naturally present in the fruit	12.67 ± 0.03	Mgeni *et al.*, 2024
Napier Grass	The Grass was chopped into smaller pieces of 1-3 cm in length and oven-dried, ground and sieved	3.0% (w/w) NaOH	*T. reesei* and *S. cerevisiae* co-culture	82%	Mueanschaia *et al.*, 2022
Potatoes	The Potato was cleaned to be free from sand, stones, soil and potato foliage. Thoroughly washed unpeeled potatoes were cooked in a pressure cooker in distilled water containing 0.5% potassium metabisulphite for 30 minutes. Boiled potatoes were mashed, dried		Co-culture		

2.5 Biological conversion of lignocellulosic biomass to bioethanol

2.5.1 Pretreatment process

There are various pretreatment methods (Table 5) increase cellulose reactivity and the potential yield of fermentable sugars (Edeh, 2021). These could be either traditional or advanced pretreatments. Traditional pretreatments are divided into four categories: chemical, physical, physicochemical, and biological, whereas advanced pretreatment methods can be acid-based fractionation or ionic liquid-based fractionation (ILF) (Maurya *et al.*, 2015). Mechanical pretreatment is the process of reducing the size of biomass particles to reduce the crystallinity of the lignocellulose and

Table 6: Various fermentation condition for bioethanol production

Feedstock	Fermentation agent	Nutrient	Condition	Bioethanol	Reference
Potato waste	*Saccharomyces cerevisiae* MTCC170 &*Aspergillusniger* MTCC2196 co-culture	YEPD, CYEA	30°C, 96hr, pH 4.5, 200rpm	1.0234g/mL, 1.0208g/mL	Sagar *et al.*, 2016
Banana peel	Yeast	Dextrose sugar (1g), Urea (1g); Yeast extract (0.2g), MgSO4. 7H2O (1.0g	5.5pH, 3days, 35°C	44.67±0.82	Oji *et al.*, 2024
Sorghum	*Saccharomyces cerevisiae*	1 g of yeast extract, 0.4 g of KH2PO4, and 0.2 g of NH4Cl	181rpm, 35.6°C,	82.11 g/L,	Sebayanga *et al.*, 2016
Algae	*Saccharomyces cerevisiae*	glucose broth media and yeast extract, PDA,	35°C pH of 5.5.	0.142ml/l	Kida *et al.*, 2023
	Trichoderma reesei and *Saccharomyces cerevisiae* co-culture			16.90 g/L.	
Sugarcane molasses	*Saccharomycescerevisiae* designated as R9MU (OR143320.1), R20MU (OR143322.1), MUT15F (OR209276.1), MUT18F (OR209286.1), and R19MU (OR143321.1)	YEPD, molasses	pH 4.5, 30°C, 72 h	13.13 ± 0.08%	Fantahun and Andualem, 2024
Raw corn starch, Broken rice	*S. cerevisiae* L20	4, 1; MgSO4·7H2O, 0.5; NaCl, 0.1; malic acid, 2; tartaric acid, 3. mg/L: biotin, 0.02; D-pantothenic acid, 0.4; myo-inositol, 2; nicotinic acid, 0.4; thiamine, 0.4; pyridoxine, 0.4; p-aminobenzoic acid, 0.2; H3BO3, 0.5; CuSO4·5H2O, 0.04; KI, 0.1; NaMoO4·2H2O, 0.2; ZnSO4·7H2O, 0.4; FeCl3·6H2O, 0.4; CaCl2·2H2O, 100) supplemented with 200 g/L glucose	72hrs, 30 °C	101 g/L	Gronchi *et al.*, 2019

To be continued next page

Table 6 continued

Feedstock	Fermentation agent	Nutrient	Condition	Bioethanol	Reference
cellulose-rich corncob (CRC) residue	*Saccharomyces cerevisiae* TC-5	(0.1 M) supplemented with (NH4)2SO4 4 g/L, yeast extract 1 g/L, NH4H2PO4 1 g/L, and MgSO4·7H2O 0.1 g/L was mixed with 7.5, 10, 12.5, and 15% (w/v) CRC residue.	pH 5.0, 35-40 °C	38.23 g/L	Boonchuay *et al.*, 2021
Rice straw	*T. reesei* NCIM 1052		25.3 g/L		Prasad *et al.*, 2020

increase the accessible surfaces, thereby promoting subsequent hydrolysis. According to Abo *et al.* (2019), lignocellulosic material is typically ground to less than 2 mm fragment size. Biological pretreatment is the use of microorganisms to break down lignocellulosic biomass before further enzymatic hydrolysis by organisms which include white-rot, brown-rot, and soft-rot fungi, as well as bacteria (Hassan *et al.*, 2018). Chemical pretreatment uses a variety of chemical reagents, including acids, bases, and oxidizing agents. The impact on lignocellulosic material varies according to the chemical reagent used (Abo *et al.*, 2019). The primary challenge of these pre-treatment processes is to make cellulose easily accessible while avoiding harsh conditions that could lead to sugar degradation.

2.5.2 Hydrolysis

The hydrolysis process is the first and limited step in converting insoluble biopolymers into soluble organic complexes like oligomers and monomers, depending on the microorganisms used in anaerobic digestion, the hydrolysis step of the process may be rate-limiting (Ma *et al.*, 2013). During the hydrolysis reaction, proteins are degraded into amino acids, carbohydrates are hydrolyzed into monosaccharides, and fatty acids are obtained by hydrolysis of lipids by the enzyme's proteases, cellulases, or amylases, and lipases, respectively (Kumar and Anand, 2019), it is the most important fungus used in biotechnological applications worldwide. It has been discovered that *Aspergillus* strains may produce a range of enzymes (Mostafa *et al.*; 2016; Sattar *et al.*, 2019), including cellulase, and amylase (Ahmad *et al.*, 2024; Saeed *et al.*, 2025).

2.5.3 Fermentation

Fermentation is a biological process in which microorganisms such as yeast, fungi, or bacteria convert the monomeric sugar units obtained during the hydrolysis step into ethanol, and gases (Sharma and Lorrache, 2020). After the biomass has been digested by enzymes, microorganisms such as yeasts and bacteria ferment sugars such as galactose, fructose, glucose, and mannose to produce ethanol (Gonzalez *et al.* 2024). Yeast species can make bioethanol from sugar fermentation, despite *Saccharomyces cerevisiae* being the most common sugar fermenter (Walker and Walker, 2018). While *Scheffersomyces stipitis* uses lignocellulose substrates (Liang *et al.*, 2013) or algal biomass (Obata *et al.*, 2016), According to Parapouli *et al.* (2020), S. *cerevisiae*'s distinct biological characteristics, such as its capacity to ferment and create alcohol and CO2, as well as its tolerance to adverse osmolarity and low pH, make it ideal for biotechnological applications. Biomass with high lignocellulose content is used as feedstock, providing an alternative fermentation method (Mishra *et al.*, 2019). Until recently, combinations of bacteria and yeast (Mishra *et al.*, 2019; Wang *et al.*, 2019), yeast and yeast (Ntaikou *et al.*, 2018; Singh *et al.*, 2014), or fungi and yeast (Paschos *et al.*, 2015; Izmirlioglu *et al.*, 2017) were used in co-cultures for simultaneous saccharification and co-fermentation. Co-cultures have also been investigated as a technique to increase ethanol yield (Mishra *et al.*, 2019; Izmirlioglu *et al.*, 2017). The Table 6 shows some instances of different microorganisms employed in simple sugar fermentation, as well as their corresponding ethanol yields at varying operating conditions.

2.6 Types of fermentation techniques

2.6.1 Simultaneous saccharification and fermentation (SSF)

The simultaneous saccharification and fermentation (SSF) design consists of a single reactor in which both hydrolysis and fermentation take place. Adopting this type of solution overcomes the inhibition problem observed in separate hydrolysis and fermentation (SHF), as glucose and cellobiose are gradually used during their manufacture (Mazzeo and Piemonte *et al.*, 2020). Recently, the simultaneous saccharification and fermentation method has been used, which combines biomass saccharification with simultaneous sugar fermentation in a single reactor (Rastogi and Shrivastava, 2018). Kumagai *et al.* (2014) also reported that the development of an SSF process was ideal for producing ethanol from Hinoki cypress and Eucalyptus after fibrillation via steam pretreatment and subsequent wet-disk milling.

2.6.2 Batch processing or culture

This system involves inoculating a batch of culture medium with microorganisms. After a certain amount of time, the fermentation process is complete, and the product is harvested. At the start of the stationary phase, the culture is disbanded to recover its biomass (cells, organisms) or the compounds that accumulated in the medium (alcohol, amino acids), and a new batch is established (Behl *et al.*, 2023). Due to these inherent disadvantages and lower yields, the commercial market believes in shifting to other fermentation techniques (Puligundla *et al.*, 2018; De Araujo Guilherme *et al.*, 2019; Liu *et al.*, 2019).

2.6.3 Fed-Batch culture

In fed-batch fermentation, the feed rate is limited, so the cell mass density is not increased excessively (Azhar *et al.*, 2017). As a result, the cell mass concentration must be maintained at a specific level to ensure the highest ethanol productivity (Ariyanti *et al.*, 2014; Moshi *et al.*, 2014; Phukoetphim *et al.*, 2018). The fed-batch system adds a fresh aliquot of medium on a continuous or periodic basis, without removing the culture fluid. The fermenter is designed to handle increasing volumes. The system is always in quasi-steady state.

2.6.4 Continuous fermentation

Continuous fermentation produces more ethanol than batch fermentation (Phwan *et al.* 2018). Continuous culture is an open system in which nutrients are added to the bioreactor aseptically and continuously while the culture broth (containing cells and metabolites) is removed at the same time. The volume of the culture broth remains constant due to a constant feed-in and feed-out rate (Kuene, 2019). Continuous operations are generally easier to control and less laborious than batch operations, but there is a serious contamination issue with this operating method (Mahboubi *et al.*, 2017; Carrillo-Nieves *et al.*, 2019).

2.6.5 Solid state fermentation

Solid state fermentation conditions are ideal for growing microbes such as bacteria, yeasts, and filamentous fungi on solid substrates, increasing their potential for use in bioprocesses (Ortiz *et al.*, 2016; Marín *et al.*, 2019; Salom-ao *et al.*, 2019). Solid State Fermentation is the controlled growth of microorganisms in the absence of free water. Solid State Fermentation products include industrial enzymes, fuels, and nutrient-rich animal feeds. The use of modern biotechnical knowledge and process control technologies can result in significant productivity gains from this ancient process. Solid state fermentation reduces the risk of bacterial contamination by eliminating free water; more concentrated enzymes are produced, which can be extracted with a small amount of water (Kapilan, 2015).

2.7 Factors affecting bioethanol production

Temperature, sugar concentration, pH, fermentation time, rate of agitation, and inoculum size are all factors that influence bioethanol production (Zabed *et al.*, 2014). However, one of the most important factors influencing the amount of ethanol produced is the temperature during fermentation. Previous studies (Piarpuzan *et al.*, 2014; Garcia *et al.*, 2015) found that the ideal fermentation temperature ranges from 30 to 38ºC. Thus, the temperature is precisely controlled throughout the fermentation process. The temperature is precisely controlled. Furthermore, high temperatures can denature the tertiary structure of enzymes that regulate microbial activity and the fermentation process, making them inactive (Lopez-Trujillo *et al.*, 2023). There have also been reports of using enzymatic hydrolysis to accelerate sugar release (Piarpuzan

et al., 2014). Several conditions, including steam purging, microwave and ultrasonic wave treatment, have been proposed for acid or alkaline prepretreatmentth hydrochloric acid or aqueous ammonia (Garcia *et al.*, 2014; Gabhane *et al.*, 2015).

2.8 Ethanol Recovery

Bioethanol is produced in a diluted state (Saini *et al.*, 2020), thus water and other contaminants must be removed to obtain a fuel-grade ethanol product (Aditiya *et al.*, 2016). Bioethanol can be recovered at a variety of temperatures: (i) at or near fermentation temperature; (ii) slightly higher than fermentation temperature that does not harm microorganisms or hinder enzyme activity (Saini *et al.*, 2020). Ethanol recovery begins with a standard distillation process, which produces azeotropic ethanol. Furthermore, dehydration and purification stages are used to produce fuel-grade ethanol, which uses a large amount of energy and has high operational costs, limiting the economic feasibility of lignocellulosic ethanol on a commercial scale (Saini *et al.*, 2020). Ethanol separation is the most expensive and energy-intensive phase in the ethanol production process (Zentou *et al.* 2019). The energy required for ethanol recovery and purification varies with the concentration of ethEthanol the feed stream (Saini *et al.*, 2020). The energy required for ethanol separation has been calculated to be between 12-15% and 35% of combustion energy for input streams containing 12 and 4 wt% ethEthanolespectively (Granjo *et al.*, 2020). Membrane technology employs semi-permeable barriers that exploit the principle of selective permeability, which is widely used in the purification of bio-based products (Méireles*et al.*, 2016). Azeotropic distillation helps separate azeotropic mixtures into their pure constituents (Saini *et al.*, 2020). Ethanol purification involves two primary steps: ethanol pre-concentration and ethanol dehydration (Chandra *et al.*, 2018). The mechanism of the separation into these two distinct phases is that ethanol-water mixtures exhibit azeotropic behavior by mass fraction (Habaki *et al.*, 2016).

CONCLUSION AND FUTURE PERSPECTIVE

The world is experiencing significant global warming due to the widespread use of fossil fuels. Bioethanol has recently seen increased commercialization due to its market stability, low cost, sustainability, and greener output, as well as its potential to reduce fossil fuel depletion. However, the major challenges that have hampered bioethanol production are a lack of optimization, which results in a lower yield of bioethanol produced and, as a result, it cannot be used for large-scale production. This review has offered a complete understanding of physicochemical features, diverse feedstocks, the role of fungal strains in bioethanol production, common waste biomass, pretreatment procedures, and various fermentation settings for bioethanol production. The steps necessary for making bioethanol as the cost-effective, reliable, and widely available biofuel for a growing global population. The invention of bioethanol was hailed as a great breakthrough in converting waste biomass to fuel energy, hence lowering the widespread usage of fossil fuels. The production efficiency of bioethanol from diverse substrates, including sugar-based, starchy by-products, cellulosic biomass, and agricultural waste, will necessitate an innovative method. The enzymatic capabilities of fungal strains are critical, and can be further improved by implementing novel technologies such as synthetic biology and genome editing to develop superior microorganisms. Further research should investigate the potential of fungal strains for improved enzymatic hydrolysis and fermentation, with an emphasis on strain engineering to improve sugar usage and inhibitor tolerance.

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