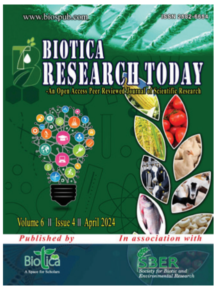

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Bioethanol Production from Residual Biomass of Plants: Prospective and Challenges

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Abstract

The transportation sector faces urgent challenges due to climate change and declining fossil fuel reserves, necessitating viable alternatives to petroleum. This article focuses on second-generation bioethanol production, which utilizes lignocellulosic biomass, offering a significant advancement over previous biofuel generations. Biomass containing Lignocellulose *i.e.,* cellulose, hemicellulose and lignin, undergoes pretreatment, enzymatic hydrolysis, fermentation, distillation and dehydration for conversion. Pretreatment enhances carbohydrate accessibility and reduces inhibitors, while enzymatic hydrolysis releases fermentable sugars like glucose and xylose. However, hydrolysates may require detoxification before fermentation, because of inhibitors. Moreover, effective saccharification involves exogenous hemicellulases and cellulolytic enzymes. Genetically engineered microorganisms are essential for fermenting xylose, as conventional yeast cannot. Moreover, genetic engineering facilitates the acquisition of pentose-fermenting microorganisms by optimizing xylose utilization from the hydrolysate. Utilizing residual biomass for bioethanol production offers substantial potential as a renewable energy solution, capable of combating climate change, bolstering energy security and promoting rural development.

Keywords: Bioethanol, Biomass, Enzymatic hydrolysis, Fermentation

Introduction

The world faces urgent challenges such as climate change and nutritional and energy security. With population growth and industrial expansion driving up energy demands, the finite nature of conventional fossil fuels like petroleum exacerbates concerns, given their significant greenhouse gas emissions. Consequently, there is a pressing need for sustainable and environmentally friendly energy alternatives to meet future requirements. Biofuels, encompassing cellulosic bioethanol, butanol and biodiesel, have emerged as pivotal areas of interest for researchers, industries and governments alike. Particularly, bioethanol presents itself as a promising substitute for gasoline in transportation (Antar *et al.*, 2021). The integration of ethanol into gasoline has notably diminished CO_2-equivalent emissions, effectively equivalent to removing millions of cars from the roads annually. Beyond environmental benefits, bioethanol diminishes dependence on imported petroleum, thereby bolstering energy security. Furthermore, its production fosters job opportunities in rural areas and catalyzes economic expansion (Antar *et al.,* 2021; Mishra *et al.,* 2023).

This article provides a thorough exploration of the transformation process involved in converting lignocellulosic biomass into second-generation bioethanol. It delves into each phase of the process, starting from biomass pretreatment to enzymatic hydrolysis for sugar production, fermentation, distillation and dehydration. It explores biocatalysts used in alcoholic fermentation and the development of tailored fermentative microorganisms for efficient bioethanol production. Genetic engineering plays a crucial role in optimizing bioethanol yield, enhancing pentose and hexose fermentation and conferring tolerance to inhibitory compounds and high ethanol concentrations. Moreover, addressing the prospective and challenges of bioethanol production requires interdisciplinary collaboration among scientists, engineers, policymakers and

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stakeholders to develop innovative solutions that maximize the benefits while minimizing the risks associated with this promising technology.

Bioethanol Generations

First Generation Bioethanol

Bioethanol of the first generation, primarily sourced from starch and sugar-rich food crops, serves as a liquid fuel for road vehicles. Its production necessitates hydrolysis of both starchy and lignocellulosic materials due to the inability of *Saccharomyces cerevisiae* to break down complex carbohydrates. Common feedstocks include corn, wheat, sugarcane and sugar beet, raising socio-economic and environmental concerns. First-generation biofuels exhibit lower greenhouse gas emissions compared to fossil fuels (Mishra *et al.,* 2023).

Second Generation Bioethanol

Second-generation biofuels, like bioethanol, primarily utilize non-food sources such as lignocellulosic biomass or industrial byproducts, mitigating concerns regarding competition with food resources. Lignocellulose, present in various plant materials, provides a renewable and abundant carbon source that is locally available and sustainable. However, converting lignocellulose into reducing sugars presents greater challenges compared to starch. Researchers explore a diverse range of plant biomass, including dedicated energy crops like Miscanthus and switchgrass, agricultural residues like cereal straw and bagasse, forest-based woody wastes, industrial byproducts like brewer's spent grains and municipal solid wastes such as food waste and paper sludge, as potential sources for biofuel production (Mishra *et al.,* 2023).

Third Generation Bioethanol

The third generation of biofuels centers on microalgae and single-celled organisms like cyanobacteria, such as *Cyanidium caldarium*. This process reduces waste streams from industries and contributes to lowering greenhouse gas levels by sequestering CO_2 and converting it into biofuels. Certain microalgae strains like *Chlorella*, *Nanochloropsis* and *Botryococcus* produce biofuels, with *Botryococcus braunii* generating hydrocarbons convertible into gasoline-like fuels. Microalgae also yield high-value carbohydrates, pigments and lipids for various applications. While algae-derived oils offer an alternative for biodiesel, optimization of extraction technologies is necessary. Algae biomass can be converted into eco-friendly energy carriers like bioethanol and biogas, though bioethanol production yields from algae are currently low and biohydrogen production needs improvement (Mishra *et al.*, 2023).

Major Steps Involved in Conversion of Biomass into Ethanol

Bioethanol production generally encompasses several key stages. Initially, raw materials like starch-rich crops or lignocellulosic biomass are gathered and readied for processing. Starch-based feedstocks undergo enzymatic hydrolysis to break down starch into fermentable sugars, whereas lignocellulosic biomass requires pretreatment to break down complex carbohydrates into simpler sugars. These sugars are subsequently fermented using yeast or other microorganisms, transforming them into ethanol and carbon dioxide. Following fermentation, ethanol is separated from the fermentation broth via distillation, followed by dehydration to eliminate any residual water, yielding pure ethanol (Mishra *et al.*, 2023). The major steps in bioethanol production are indexed in figure 1 and these steps are subsequently elaborated.

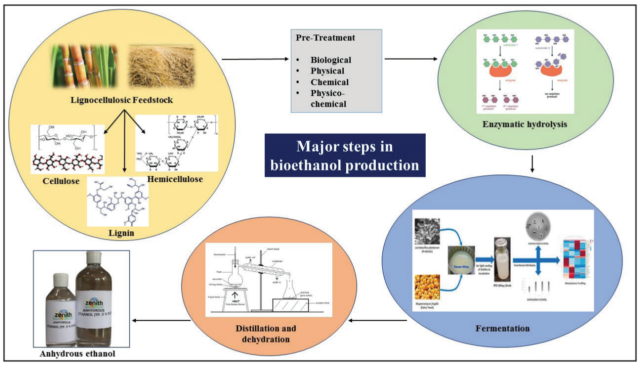

Figure 1: Major steps in bioethanol production

Lignocellulosic Feedstock

Lignocellulosic biomass offers promise for bioethanol production due to its resilience and variable composition influenced by factors like species and growth conditions. Ethanol yield relies on high cellulose, high hemicellulose and low lignin content in the biomass. Despite its complexity, effective pretreatment is necessary for efficient conversion, with holocellulose being a significant component of the biomass (Robak and Balcerek, 2018).

Cellulose

Lignin surrounds cellulose, which is composed of d-glucose units linked by β-1,4-glycosidic bonds, creating a β-glucan structure. The crystalline nature of cellulose forming insoluble microfibrils in the secondary cell wall, necessitates enzymatic hydrolysis at high temperatures and pressures to convert it from a rigid to an amorphous structure, exceeding the conditions required for starchy materials. The process involves a liquefaction step at 95-105 °C and a saccharification step at 60-65 °C, with pH adjustments (Robak and Balcerek, 2018).

Hemicellulose

Hemicellulose consists of various sugar units, including branched-chain sugars and uronic acids like d-glucuronic and d-galacturonic acids. C_5 sugars such as xylose and arabinose are liberated from plant cell wall polysaccharides like xyloglucan, xylan, arabinan and arabinogalactan (Robak and Balcerek, 2018). Xylan is the primary hemicellulose constituent, with hardwoods typically containing 35% hemicellulose and softwoods around 28% (Robak and Balcerek, 2018).

Lignin

Lignin's resistance to biodegradation presents challenges in plant cell walls, impeding enzymatic activity. It acts as a binding agent in plants, composed of phenolic and non-phenolic compounds. Despite its durability, lignin can be directly utilized for energy generation through combustion in biorefineries. Efforts are ongoing to explore new applications for lignin, given its significant production volume in commercial lignocellulosic ethanol production. Efficient extraction techniques during pretreatment are crucial for enhancing the mechanical properties of lignin-based carbon fibers, with potential improvements achievable through genetic engineering (Robak and Balcerek, 2018).

Pre-Treatments

Thermochemical and biochemical pathways are employed to convert lignocellulosic biomass into second-generation ethanol and biofuels, where thermochemical processes involve biomass conversion into intermediate gases or liquids using heat or non-biological catalysts, which are then refined into fuels like methanol, ethanol, hydrogen, or synthetic diesel (Mishra *et al.*, 2023). Gasification and pyrolysis are common thermochemical techniques, producing syngas or liquid intermediates like pyrolysis oil, respectively (Robak and Balcerek, 2018). Biochemical conversion, meanwhile, focuses on producing bioethanol and involves pretreatment of biomass, enzymatic hydrolysis to break down polysaccharides into fermentable sugars, fermentation by microorganisms and distillation with dehydration to obtain bioethanol (Robak and Balcerek, 2018). Pretreatment involves treatment with biological, physical, or chemical catalysts, while enzymes aid in hydrolysis and fermentative microorganisms like yeast or bacteria facilitate fermentation. Both routes offer distinct advantages, with thermochemical methods providing versatility and biochemical processes offering high selectivity and efficiency in bioethanol production (Robak and Balcerek, 2018). Lignocellulosic biomass derived from vegetable waste holds significant promise for bioethanol production (Mishra *et al.*, 2023).

Although pretreatment aims to enhance the accessibility of cellulose and hemicellulose to hydrolytic enzymes, the use of complex pretreatment technologies can escalate ethanol production costs. Goals of pretreatment include increasing sugar yields, avoiding sugar loss, reducing inhibitor formation, recovering lignin for co-products and minimizing heating and power costs, achieved through biological, chemical and physical processes often used in combination (Robak and Balcerek, 2018). Biological treatment with fungi, especially white rot fungi, enhances enzymatic hydrolysis efficiency without generating toxic byproducts, presenting an environmentally friendly approach (Mishra *et al.*, 2023). Various chemical treatments, including bases, acids, ionic liquids and oxidizers, alongside physical methods like milling or grinding, are employed to reduce cell wall crystallinity and particle size; while physicochemical pretreatment methods, such as steam explosion, liquid hot water and ammonia fiber explosion enhance cellulose digestibility and hemicellulose hydrolysis efficiency, albeit with steam explosion potentially, leading to partial sugar and lignin degradation and the production of soluble inhibitors (Mishra *et al.*, 2023).

Enzymatic Hydrolysis

Enzymatic hydrolysis plays a vital role in releasing monosaccharides from polysaccharides such as cellulose and hemicellulose in the plant cell wall, generating fermentable sugars essential for ethanol fermentation. The sugar yield from hydrolysates depends on factors such as the type of raw material, particularly lignocellulosic waste and the pretreatment methods employed, with glucose and xylose being the main products utilized by microorganisms as carbon sources during fermentation (Mishra *et al.*, 2023). Hydrolytic enzymes, often sourced from filamentous fungi such as *Aspergillus* and *Trichoderma* species, degrade cellulose and hemicellulose, with key enzymes including endoglucanases, exo-cellobiohydrolases and β-glucosidases involved in cellulose breakdown; despite challenges posed by the high production costs of cellulases, which are beneficial for degrading cellulose-hemicellulose matrices. Enhancing enzymatic hydrolysis efficiency involves utilizing hemicellulases and accessory enzymes like endoxylanases and esterases, which reduce enzyme requirements and costs. Optimization of the hydrolysis process is achieved through interactions between cellulases and hemicellulases (Wang *et al.*, 2021).

Fermentation, Distillation and Dehydration

Fermentation involves the conversion of hexose and pentose sugars into ethanol through the action of fermenting microorganisms such as yeasts, although their effectiveness may be influenced by factors such as lignocellulosic hydrolysates and fermentation conditions. Stress conditions induced by ethanol accumulation, pH reduction, anaerobic growth and nutrient depletion in lignocellulosic hydrolysates can affect the yields of microorganisms fermenting xylose. Additionally, osmotic stress and alcohol accumulation can inhibit yeast growth and viability, potentially leading to osmotic shock, while severe conditions like oxidative and ethanol stress can impact bioethanol fermentation efficiency.

Microorganisms, especially yeasts, are crucial in industrial bioethanol production, requiring traits such as thermotolerance, high fermentative activity, ethanol yields and resistance to stressors and inhibitors like furfural and 5-hydroxymethyl furfural (Wang *et al.*, 2021). The search for ideal ethanol-producing candidates emphasizes traits like high ethanol yield, tolerance to ethanol concentrations exceeding 40 g L^{-1}, minimal nutrient requirements and resilience to stress and acidic pH environments. Research efforts center on isolating and assessing yeast strains capable of efficiently fermenting glucose and xylose, evaluating their performance in the presence of inhibitors and various pretreatment methods.

Specific yeast strains, such as *Saccharomyces cerevisiae* and *Candida tropicalis*, are recommended for lignocellulosic hydrolysate fermentation following alkali and acid pretreatment, respectively. Despite its widespread use, *S. cerevisiae* faces limitations in pentose sugar metabolism, whereas *Zymomonas mobilis* exhibits high ethanol productivity from starch but lacks the ability to ferment pentoses efficiently (Wang *et al.*, 2021). Genetic modification strategies aim to enhance microbial resistance to fermentation conditions and inhibitors, as well as improve tolerance to ethanol and high sugar concentrations.

However, efficient pentose fermentation remains a challenge due to the absence of reaction intermediates and efficient pentose transporters (Wang *et al.*, 2021). Finally, distillation and dehydration are essential steps in bioethanol production (Wang *et al.*, 2021).

Current Status of Ethanol Production from Lignocellulosic Biomass

Steam explosion is preferred for its energy-saving benefits over mechanical comminution and pretreatment methods are selected with environmental impact considerations. Controlling enzyme dosage and activity is crucial for efficient hydrolysis, with ongoing research exploring methods like protein engineering or discovering new cellulolytic organisms to reduce costs. Surfactants play a role in improving enzymatic hydrolysis efficiency by preventing enzyme inactivation by lignin. Genetic engineering is utilized to develop xylose-fermenting microorganisms, enabling full utilization of raw materials. Simultaneous saccharification and fermentation (SSF) or simultaneous saccharification and co-fermentation (SSCF) methods are implemented to reduce enzyme loading and processing time, thereby enhancing ethanol production efficiency (Zoghlami and Paës, 2019).

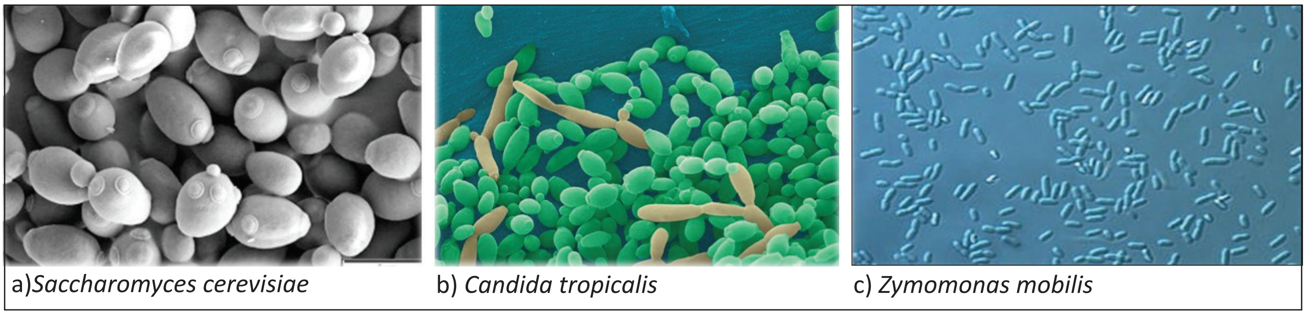
a)*Saccharomyces cerevisiae* b) *Candida tropicalis* c) *Zymomonas mobilis*

Figure 2: Fermentative microorganisms

Conclusion

Transitioning the transportation sector from its reliance on petroleum and gasoline to more sustainable energy sources like second-generation bioethanol presents a significant engineering challenge. Enhancements in the pretreatment, enzymatic hydrolysis and fermentation phases are crucial to improve the cost-effectiveness of lignocellulosic bioethanol production and enable its transition from laboratory to industrial scales. A primary objective is to boost fermentation efficiency to ensure that all sugars from pretreatment and hydrolysis, including pentoses and hexoses, are fully converted into ethanol. Challenges including biomass variability, inhibitor generation, end-product inhibition and ethanol accumulation pose obstacles to second-generation biofuel production, but ongoing advancements indicate that these hurdles may soon be surmounted, leading to optimization of the biochemical pathway for liquid bioethanol production.

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