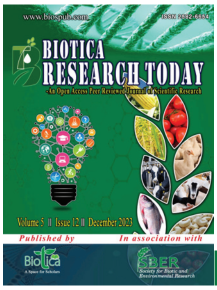

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Sustainable Biofuel Production: Second Generation Ethanol from Agricultural Waste

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Abstract

As the global demand for sustainable energy continues to rise, there is a growing focus on researching and developing second-generation ethanol obtained from agricultural waste. Increasing concerns on climate change and energy security prompted the significant attention towards utilizing non-food biomass for bioethanol production. Second-generation (2G) ethanol, derived from non-food sources like agricultural waste, forest residues and other non-edible plant parts, is considered more sustainable compared to first-generation ethanol, which comes from food crops like sugarcane and corn. Agricultural waste, comprising crop residues, straw, husks and other organic materials remaining after harvest, stands out as a promising raw material for second-generation (2G) ethanol production. Sustainable production of second-generation (2G) ethanol from agricultural waste presents an environmentally friendly and economically feasible alternative to conventional fossil fuels.

Keywords: Enzymatic hydrolysis, Fermentation, Pretreatment, Second-generation ethanol

Introduction

Biofuels, particularly ethanol, have garnered significant attention as an alternative energy source aimed at reducing reliance on fossil fuels, thereby contributing to environmental and climate protection. Production of second-generation biofuels, specifically cellulosic ethanol derived from lignocellulosic biomass, holds potential to spearhead bio-industrial revolution. Lignocellulosic biomass presents sustainable solutions for energy security, environmental well-being, opportunities for economic development and potential socioeconomic benefits for rural economies. Constituting 75% of carbohydrates and 25% lignin, lignocellulosic biomass forms the basis for second-generation ethanol production. The typical process involves biomass pre-treatment, enzymatic hydrolysis, fermentation and distillation. Biomass pre-treatment aims to break down the intricate lignocellulosic structure into simpler sugars, facilitating efficient enzymatic hydrolysis. Various pre-treatment methods, including steam explosion (SE), wet oxidation, alkali pretreatment, acid pretreatment and biological pretreatment are employed for effective conversion of lignocellulosic biomass. Cellulose and hemicellulose are then converted into fermentable sugars utilizing enzymes, which microorganisms subsequently ferment to produce ethanol. Finally, ethanol is separated and purified through distillation. Despite its promising potential, production of second-generation ethanol faces challenges such as high production costs, low conversion efficiency and the need to develop robust enzymatic cocktails for efficient biomass hydrolysis.

Production of Ethanol from Second-Generation Agricultural Waste

Lignocellulosic biomass, composed of cellulose, hemicellulose, lignin and little amounts of ash and extractives, serves as a valuable energy feedstock. Cellulose, is a homopolysaccharide comprising anhydroglucopyranose linked by ß-1,4-glycosidic linkages. The arrangement of cellulose chains involves orderly hydrogen bonds and Van der Waal's forces, results in a parallel alignment and crystalline structure (Zheng *et al*., 2007). Hemicellulose, is a polymer primarily containing pentoses with hexose and dispersed throughout and forming a short-chain polymer that intertwines with cellulose and lignin, resembling as a glue. Hemicellulose acts as a protective coating over the cellulose

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fibril and is thermo-chemically sensitive. Lignin, an aromatic polymer with unique characteristics, naturally protects plant cell walls averse to microorganisms along with resistant to digestion by animal enzymes. The content of lignin differs among different plants. To enhance the conversion of cellulose into fermentable sugars, lignin removal prior to hydrolysis is essential, given that lignin covalently bounds to hemicellulose by ferulic acid ester linkages. Conversion of lignocellulosic biomass (LCB) into ethanol and chemicals from biobased feedstocks involves three processes such as pretreatment, saccharification and fermentation.

Pretreatment Process

The pretreatment process, regarded as the pivotal step in converting lignocellulosic biomass into bioethanol, is indispensable for modifying the structure of cellulosic biomass. This alteration aims to make cellulose more available to enzymes and enable the conversion of carbohydrate polymers into fermentable sugars. Biomass pretreatment should address the following criteria: (i) maximize pentose yield in a non-degraded form; (ii) generate reactive (cellulosic) fibers; (iii) obtain hydrolysates without significant inhibition of fermentation; (iv) minimize feedstock size reduction; (v) design reactors with high solid loading, constructed from cost-effective materials; and (vi) achieve zero waste production.

Various Pretreatment Technologies

Pretreatment of agricultural residues can be carried out using concentrated acids, with sulfuric acid being a particularly effective choice. Alternative acids, including hydrochloric and nitric acids, can also be employed. The use of weak acids in acid hydrolysis results in release of hydronium ions from the feedstock. The drawback of acid hydrolysis is generation of various inhibitors of carboxylic acids and furans.

Steam explosion, applied to various lignocellulosic materials for ethanol production. In this process, comminuted biomass is introduced and high-pressure saturated steam added, which initiates hydrolysis reaction. Subsequently, pressure is rapidly reduced, leading to explosive decompression of feedstock. This step enhances enzyme accessibility to the biomass structure. However, limitations of steam explosion include the generation of volatile compounds due to partial destruction of xylan fraction and disruption of lignin-carbohydrate matrix.

Alkali treatment emerges as a promising pretreatment technique, bringing about alterations in structural properties such as accessible surface area and substrate crystallinity. This modification enhances the enzymatic hydrolysis. Wet oxidation, accomplished by subjecting biomass to high temperatures with water and oxygen for short durations, which results in the extensive solubilization of hemicelluloses, mostly recovered as oligosaccharides. Concurrently, lignin undergoes fragmentation and oxidation, yielding aliphatic carboxylic acids and phenolic compounds. Integration of wet oxidation with alkaline compounds minimizes the formation of inhibitors.

Saccharification

Following pretreatment, the subsequent step is enzymatic saccharification, achievable through either chemical or enzymatic methods. Cellulases (EC 3.2.1.4) stand out as the most utilized enzymes for hydrolyzing cellulose into glucose. The primary objective of saccharification is to achieve the maximum yield of glucose from the intricate polysaccharide cellulose. The cellulose content of the feedstock plays a crucial role in saccharification, with its presence of both crystalline and amorphous forms. Ratio of these forms is significant, as amorphous cellulose is more accessible to hydrolysis than crystalline cellulose. The effectiveness of enzymatic saccharification is influenced by factors such as type of substrate pretreatment and the catalytic activity of the enzymes.

Enzymes Engaged in the Breakdown of Cellulose and Hemicellulose

Endoglucanase functions by breaking down the inner portion of the β-1,4 glucose linkage within cellulose. Exoglucanase, on the other hand, hydrolyzes the outer part of the β-1,4 glucose linkage in cellulose, resulting in the production of cellubiose units. β-glucosidase is responsible for breaking down cellubiose units into glucose, while β-xylosidase performs the hydrolysis of xylobiose units to produce xylose. Additionally, endo-xylanase is involved in breaking down the inner part of the β-1,4 xylose linkage of xylan, while exo-xylanase hydrolyzes the outer part of the β-1,4 xylose linkage of xylan, yielding xylobiose units.

Fermentative Process

The third step in ethanol production from saccharified biomasses is fermentation. During this stage, microorganisms namely yeast (*Saccharomyces cerevisiae*) and bacteria (*Zymomonas mobilis*) are employed to convert hexoses and pentoses into ethanol. Typically, fermentation processes are conducted at mesophilic temperatures ranging from 25 to 37 °C. The two distinct approaches for this process are: separate hydrolysis and fermentation and simultaneous saccharification and fermentation. Figure 1 delineates the production process of second-generation ethanol from agricultural waste.

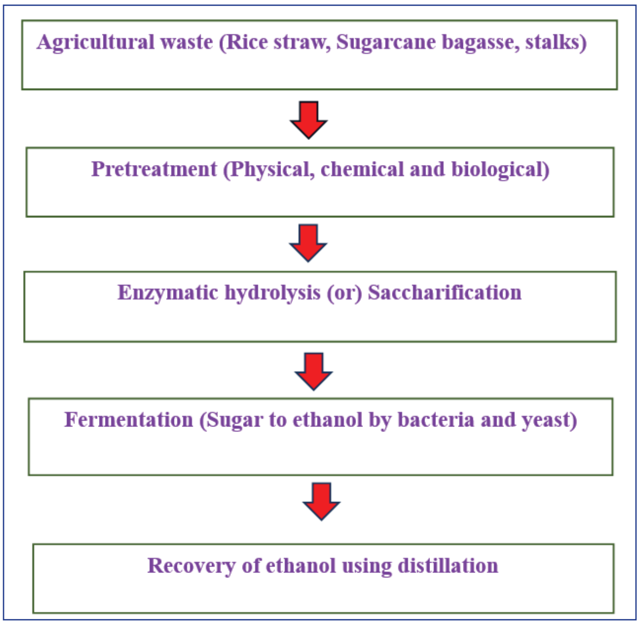

Figure 1: Flow diagram depicting the process of second-generation (2G) ethanol production from agricultural waste

FTIR Analysis of Pretreated Biomass

Absorption band observed at 3440 cm^{-1} associated with stretching of -OH groups found in cellulose, hemicelluloses and lignin. Meanwhile, the absorption band at 1718 cm^{-1} was linked to C=O stretching of unconjugated ketones, primarily occurring in the side chain of lignin structural units. Notably, the intensity of the band at 1239 cm^{-1} decreased in the pretreated samples, indicating the removal of xylan and lignin during pretreatment process.

Scanning Electron Microscopy (SEM) Analysis of Pretreated Biomass

Scanning Electron Microscopy analysis highlighted alterations in the surface of biomass sample post-pretreatment. The structure of lignocellulose underwent disruption, resulting in a notably irregular and rough surface of the residues. The alkali pretreatment, which involved removal of hemicelluloses and lignin, pointed to formation of linear cavities.

Ethanol Overview

Ethanol, also known as grain alcohol, is a transparent, colorless liquid that naturally results from plant fermentation and is soluble in water. It finds application in cosmetics, beauty products, serving as a preservative in lotions, hand sanitizers, paints, lacquers, varnish and food coloring. The FDA has classified ethanol as a "Generally Recognized as Safe" substance for use in food products. To render it unsuitable for human consumption, denaturants such as bitter flavorings are added, while not altering the other properties of substance.

Second Generation Ethanol (2G)

A Second Generation (2G) ethanol plant has the capability to transform agricultural residues such as rice straw, wheat straw, energy crops and more into ethanol. Considering the annual surplus of approximately 160 million metric tonnes of agricultural residues in India, second generation ethanol plants present a substantial opportunity in the country. A plant with a daily capacity of 100 kiloliters can effectively utilize 200,000 tonnes of agricultural residue annually, producing around 30 million litres of ethanol $year^{-1}$.

The government has initiated the Pradhan Mantri Ji-VAN Yogana to offer viability gap funding, providing the initial impetus to establish Second Generation (2G) ethanol capacities in the country. The implementation of the Ethanol Blended Petrol (EBP) program covers the entire nation, excluding the Union territories of Andaman Nicobar and Lakshadweep islands. Currently, petrol in India includes a 10% ethanol blend, a proportion the government aims to double nationwide by 2025. The increased use of ethanol is expected to result in 50% reduction in carbon monoxide emissions for two-wheelers and around 30% for four-wheelers.

Environmental Impact

Vehicular emissions, comprising pollutants such as Carbon Monoxide, Hydrocarbons and Oxides of Nitrogen, pose a significant threat to human health and environment. These pollutants, released from the combustion of fossil fuels in internal combustion engines, contribute to air pollution, smog formation and climate change. Carbon Monoxide interferes with the oxygen-carrying capacity of hemoglobin, affecting human respiratory systems, while Hydrocarbons and Oxides of Nitrogen contribute the formation of ground-level ozone and respiratory issues. The adoption of ethanol-blended gasoline has emerged as a promising strategy to mitigate these environmental impacts. Ethanol, derived from renewable sources such as corn or sugarcane, burns more cleanly than traditional gasoline, reducing CO, HC and NO_x emissions. Additionally, ethanol possesses higher oxygen content, leading to complete combustion and decrease in harmful pollutants. It is essential to recognize the positive strides made in addressing the environmental consequences of vehicular emissions and continue to explore sustainable alternatives to further enhance air quality and protect our planet (Sarwal *et al.*, 2021).

India's First Cellulosic Second-Generation Ethanol Unit

India has inaugurated its initial second-generation (2G) ethanol facility, known as the Centre for Energy Biosciences, established in Kashipur, Uttarakhand. This pilot demonstration plant, set up in collaboration with the Department of Biotechnology and the Institute of Chemical Technology, Mumbai is located on the premises of India Glycols Limited.

Conclusion

The development of second-generation ethanol from agricultural waste signifies a promising pathway for sustainable biofuel production. By utilizing non-edible plant materials like agricultural residues and waste, this approach addresses concerns related to competition for food crops and land-use changes. Additionally, converting agricultural waste into biofuel contributes to the reduction of greenhouse gas emissions and promotes a more circular and resource-efficient agricultural sector. Given the urgent imperative to diminish reliance on fossil fuels and address climate change impacts, the exploration of second-generation ethanol from agricultural waste emerges as a vital strategy for fostering a more environmentally friendly and secure energy future.

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