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Renewable Fuel Production

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ABSTRACT

The growing global energy demand and the environmental concerns associated with fossils fuels have sparked a shift towards renewable and sustainable energy sources, such as bioethanol. Bioethanol, a renewable liquid biofuel is a potential solution to address the challenges of energy security and climate changes. Conventionally first-generation biofuels can be produced through fermentation of starch-rich biomass i.e sugars to ethanol. The article provides a comprehensive overview of the bioethanol production process, encompassing pretreatment, enzymatic hydrolysis, fermentation by various microorganisms, and downstream processing. This focuses on the bioethanol production using microorganisms from starch-rich feedstock such as corn, wheat, potato, cassava and agricultural residues. Overall, the review emphasizes the potential of microbial bioethanol production towards a more sustainable energy landscape.

Key words: *Biofuel; Bioethanol; Starch; Microbial fermentation.*

Introduction

Fossil fuels, such as coal, oil, and natural gas, are finite, dominant energy resources and their continued exploitation contributes to environmental pollution, including greenhouse gas emissions and climate change (Adekunle *et al.,* 2020). Bioethanol, a renewable biofuel gained significant research interest as a promising alternative to fossil fuels, owing to its eco-friendly nature and potential to mitigate greenhouse gas emissions (Zabed *et al.,* 2017; Naik *et al.,* 2010). Bioethanol primarily produced by the microbial fermentation of sugars derived from various starch-rich substrates, making it a first-generation biofuel (Jiang, 2022).

India, being an agricultural-based economy and as one of the world's largest producers of starchrich crops, has actively promoting the production and utilization of bioethanol as a renewable and sustainable transportation fuel (Surendra *et al.,* 2018). The Indian government has implemented various policies and initiatives to promote the development and adoption of biofuel, including the National Policy on Biofuel (2018) and the Ethanol Blending Petrol Program (2003), mandates the production and blending of bioethanol with conventional transportation fuels, aiming to reduce dependence on imported fossil fuels (Zabed *et al.,* 2020; Sarris *et al.,* 2016). Sugarcane molasses and grains like corn, barley and sorghum, are the primary starch-rich materials used for bioethanol production in the country (Adekunle *et al.,* 2020; Shukla *et al.,* 2015).

Starch, a polymeric carbohydrate composed of amylose and amylopeptin, is abundantly found in various plant sources (Nair *et al.,*2018). Starch-rich feedstock such as corn, wheat, cassava, potato peels, beets, various agricultural residue, and industrial by-products offer a sustainable and economically viable source for bioethanol production, undergo multiple steps to break down the complex starch molecules into simpler sugars (Padmanabhan *et al.,* 2019; Nigam *et al.,* 2011).

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Each feedstock has its own unique characteristics which influence the bioethanol production process (Lee *et al.,* 2020) and contributing to addresses the challenges of waste management, energy security, resource utilization, and the development of a circular bioeconomy (Surendra *et al.,* 2018; Balat and Balat, 2009).

Generations of Biofuels: Biofuels can be categorized into different generations based on the feedstock and conversion technology used. The first-generation biofuels are produced from edible sugar-rich and starch-rich biomass, using fermentation by microorganisms (Mohapatra *et al.,* 2019). The second-generation biofuels utilize lignocellulosic biomass and employ cellulolytic bacteria and fungi for the conversion processes (Demirbas, 2009). The third generation biofuels are derived from microalgae and the fourth generation biofuels are synthesized using the genetically modified microorganisms, through advanced fermentation techniques and metabolic engineering (Kumar *et al.* 2018).

Bioethanol Production using microorganisms: Numerous microorganisms have been explored for their ability to efficiently convert starch-rich feedstock into bioethanol. Some of the commonly studied strains include:

Process of bioethanol production from starch- rich feedstock

Production of bioethanol from starch-rich feedstock is a multi-step process facilitated by microorganisms, includes starch hydrolysis, microbial fermentation and bioethanol purification.

Starch Hydrolysis: Starch-rich feedstocks, such as grains, tubers, corn stover, wheat straw, rice husks or agricultural residues, often require physical pretreatment methods such as milling, grinding, and extrusion (Nair *et al.* 2018; Adekunle *et al*., 2020). Pretreatment step aims to disrupt the structural integrity of the starch-rich feedstock, facilitating subsequent enzymatic hydrolysis (Zabed *et al.,*2020; Alvira *et al.,* 2010).

Table 1. Microorganisms used for the bioethanol production from starch-rich materials.

Microorganism	Substrate used	Ethanol Yield/Production (g ethanol/g starch)	References
Saccharomyces cerevisiae	Brewery spent grain, potato protein liquor	0.46 g	Nair *et al.* (2018)
Saccharomyces cerevisiae	Cassava bagasse	0.61g	Zhang *et al.,* (2021)
Saccharomyces cerevisiae (metabolically engineered)	Raw starch	0.48 g	Padmanabhan *et al.* (2019, 2022)
Pichia stipitis	Coffee industry waste	0.41 g	Mussatto *et al.* (2020)
Kluyveromyces marxianus	Cashew apple bagasse	0.44 g	Mussatto *et al.* (2021)
Saccharomyces cerevisiae	Corn stover	0.47g	Rana *et al.* (2021)
Saccharomycopsis fibuligera	tuber crops	0.45 g	Murugan *et al.* (2022)
Aureobasidium pullulans	corn, potato, wheat, rice	0.35-0.48 g	Singh *et al.* (2020)
Lipomyces starkeyi	Tuber crops	0.45 g	Murugan *et al.* (2022)
Zymomonas mobilis	Food waste	0.42 g	Kwan *et al.* (2018)
Escherichia coli (metabolically engineered)	Starch	0.44 g	Padmanabhan *et al.* (2019)
Candida shehatae	Rice straw	0.39 g	Zabed *et al.* (2020)
Bacillus subtilis	Corn Starch,	0.25 g	Gu (2009)
Scheffersomyces stipitis	Wheat straw	0.41 g	Zabed *et al.* (2020)
Pachysolen tannophilus	Cassava starch	0.44 g	Murugan *et al.* (2022)
Pichia kudriavzevii	Sweet potato	0.46 g	Adekunle *et al.* (2020)
Kluyveromyces marxianus	Potato waste	0.42 g	Adekunle *et al.* (2020)
Schizosaccharomyces pombe hydrolysate	`Wheat bran	0.40 g	Adekunle *et al.* (2020)

After pretreatment, the hydrolysis step converts the starch into fermentable sugars, primarily glucose. The enzymatic hydrolysis of starch typically involves two main steps, liquefaction and saccharification (Rana *et al.,* 2021; Kang *et al.,* 2014). During liquefaction, amylase breakdown starch granules into shorter oligosaccharides, dextrins (Padmanabhan *et al.,* 2022). During saccharification, the liquefied starch, dextrin is further hydrolyzed into fermentable sugars i.e glucose through the action of glucoamylases or other amylolytic enzymes. The resulting glucoserich solution is then suitable for microbial fermentation.

Microbial Fermentation: The fermentation process involves inoculum preparation, fermentation setup, fermentation monitoring and optimization. The selection of the appropriate microbial strain for the fermentation process is of utmost importance, as it influences ethanol yield and productivity (Adekunle *et al*., 2020; Murugan *et al*., 2022). The resulting glucose-rich hydrolysate is inoculated with potent ethanol producesing microorganism to convert sugar into bioethanol through various metabolic pathways (Nair *et al.,* 2018; Mussatto *et al.,* 2020). The fermentation process is carried out under anaerobic conditions at controlled and pH to facilitate optimal microbial growth and ethanol production. The fermentation conditions, including temperature, agitation, oxygen levels, pH, inoculum size, nutrient content, incubation period and rotating speed are carefully controlled to optimize microbial growth and ethanol production. Various fermentation strategies, including batch, fed-batch, and continuous processes, can be employed for the ethanol production depending on the specific process requirements (Padmanabhan *et al.,* 2022; Kwan *et al.,* 2018).

One of the key advancements in the field of bioethanol production is the concept of simultaneous saccharification and fermentation (SSF) where the enzymatic hydrolysis and microbial fermentation steps are combined into a single reactor (Murugan *et al.* 2022). The hydrolytic enzymes (amylases) convert starch into fermentable sugars, which are simultaneously utilized by the fermenting microorganisms, like *Saccharomyces cerevisiae* or amylolytic yeasts, to produce bioethanol (Rana *et al.* 2021; Padmanabhan *et al.,* 2022). This offer several advantages over conventional separate hydrolysis and fermentation processes, including reduced processing time, improved substrate utilization, and the potential for higher ethanol yields, by minimizing the risk of product inhibition(Adekunle *et al.,* 2020; Nair *et al.* 2018).

Consolidated Bioprocessing (CBP) is another advanced approach that combines enzymatic hydrolysis, saccharification, and fermentation into a single reactor using a single microorganism or a consortium capable of producing bioethanol (Zabed *et al.* 2020; Adekunle *et al.,* 2020). CBP offers several advantages including reduced operational costs due to the elimination of externally added enzymes, improved process economics through the integration of multiple steps, and minimized product inhibition (Murugan *et al.* 2022; Rana *et al.* 2021; Kwan *et al.* 2018). Metabolic engineering strategies, such as the expression of amylolytic enzymes and the optimization of metabolic pathways, have been employed to enhance the CBP capabilities of microorganisms (Padmanabhan *et al.* 2019; Mussatto *et al.* 2020)

In addition to this several approaches like enzyme immobilization, genetic engineering of microorganisms, integrated biorefinery concepts have been explored to improve the efficiency and sustainability of bioethanol production from starch-rich feedstocks (Adekunle *et al.* 2020; Padmanabhan *et al.,* 2022; Zabed *et al.,* 2020).

Ethanol Purification: After the fermentation process, the resulting fermentation broth is subjected to downstream processing to obtain purified bioethanol. Purification methods used are as follows:

Distillation: Distillation, a primary method for the separation and purification of bioethanol, which exploits the difference in the volatility of ethanol and water (Kwan *et al.* (2018). This The fermentation broth is heated, and the ethanol is vaporized and subsequently condensed to obtain a more concentrated ethanol solution (Rao *et al.,* 2020; Lin and Tanaka, 2006).

Membrane Separation: Membrane separation processes, such as pervaporation and reverse osmosis involves the selective permeation of ethanol through a semi-permeable membrane for the recovery and purification of bioethanol from the fermentation broth (Mussatto *et al.,* 2021).

Adsorption: Adsorption technique utilizes adsorbents like zeolites or activated carbon to selectively remove water and other impurities from the ethanol-water mixture (Adekunle *et al*., 2020).

Molecular Sieves: The ethanol-water vapor mixture is passed through the molecular sieve bed, particularly zeolites where water is selectively adsorbed, leaving behind purified ethanol vapor

(Adekunle *et al.,* 2020; Saxena *et al.,* 2016).

Future Aspects: The future of bioethanol production from starch-rich materials holds promising opportunities. Ongoing research and development efforts aim to improve process efficiency, reduce costs, and expand the utilization of diverse starch-rich feedstocks (Rana *et al.,* 2021; Mussatto *et al.,* 2021). Advancements in feedstock pre-treatment, enzyme engineering, strain improvement, and process optimization can further improve the economic and environmental sustainability of this first-generation biofuel (Prasad, 2007). Integrating bioethanol production with other value-added co-products can also contribute to the overall viability of the biorefinery concept (Kwan *et al.*, 2018).

Conclusion

The microbial production of bioethanol from starch-rich materials presents a viable and sustainable alternative to fossil fuels, offering the potential to address the challenges of energy security, environmental protection, climate change mitigation and waste valorization. The production and purification processes involve several key steps like enzymatic hydrolysis, fermentation, distillation, dehydration, and purification, each of which offers opportunities for optimization and process integration. The research and development in this field have the potential to further improve the efficiency, cost-effectiveness, and environmental sustainability of bioethanol production.

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