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Plant Based Bioethanol Production: A Review

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ABSTRACT

Demand of fossil fuels is increasing every year as it dominates global utilization of energy and contributes to many environmental challenges such as emissions of carbon dioxide, methane, nitrous oxide and global warming. These problems associated with conventional fuels can be resolved by replacing them with bioethanol produced by microbial fermentation process. A range of plant-based substrates, together with starch, cellulose, hemicellulose, and pectin, can be used to produce this unconventional fuel, making it a longstanding and lucrative resolution with little environmental influence. As non-food biomass sources are being used for production of second-generation bioethanol, it is considered a sustainable energy solution. This approach decreases greenhouse gas productions as well as competition for food production. As algae can thrive in a variety of conditions, including wastewater and ocean, without demanding freshwater resources or arable land, third-generation bioethanol production by means of algae as has gained popularity and became more ecologically favorable and justifiable choice. In inference, bioethanol production by microbial fermentation of carbohydrates is an environmentally friendly and practical approach for lowering dependence on fossil fuels while minimalizing their environmental consequences.

Keywords: *Bioethanol, Biomass utilization, second generation biofuel, third generation biofuel, environmental sustainability, hemicellulose*

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INTRODUCTION

Fossil fuels are non-renewable energy sources derived from the remnants of ancient creatures and plants spanning millions of years. They include: Petroleum (crude oil), Natural Gas, Coal and Tar Sands (Oil Sands). These fossil fuels served as the important energy sources that drove industrialization and contemporary financial prudence. Fossil fuels provide for around 86% of global primary energy consumption, which is increasing progressively [1]. Traditional fuels have many problems such as: Environmental Pollution - The utilization of fossil fuels releases greenhouse gases such as carbon dioxide, methane, nitrous oxide, which gives rise to greenhouse gas effect as well as global warming. This causes increase in temperatures of earth's surface and environmental deterioration, The depletion of Fossil Fuels - Fossil fuel supplies can take thousands of years to recreate if depleted as they are limited and quickly diminishing. This emphasizes the importance of sustainable alternatives, Energy Shortages - As global energy consumption is predicted to quadruple in the next decades, there is increasing concern about energy shortage [2, 3]. Considering upon these issues, a shift toward sustainable and renewable energy sources, such as second-generation biofuels is necessary, which use non-food feedstocks or materials from agricultural waste to improve energy security, decrease environmental impact and encourage circular economies. Bioethanol is considered as one of the utmost possible substitutes for fossil fuels, but its application in reality as a bulk commodity depends heavily on the process technology [4]. Microbial fermentation of various sugars results in formation of a type of alcohol called as Bioethanol [5]. These microbes can be homofermentative or heterofermentative. Though first-generation bioethanol which is made of substrates from food crops which are rich in sugar such as sugarcane, maize, sorghum, sugar beet, oats, barley, rye etc. provides low manufacturing costs and well adopted technology, it competes for human food and water supplies [6, 7]. The solution for problem associated with first generation ethanol production is second generation ethanol. The second-generation bioethanol, which is mentioned to as advanced bioethanol, is made from lignocellulosic biomass together with forestry waste, agricultural leftovers and energy crops such as switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus giganteus*), reed canary grass (*Phalaris* *arundinacea*), kenaf (*Hibiscus cannabinus*), etc. This kind of bioethanol is not at all competing with food supply, making it a more sustainable alternative for bioenergy production. Second-generation bioethanol every so often needs more complex processing and more capital investments, this increases its production expenses than the first-generation bioethanol [6, 8]. Lignocellulosic biomass, such as agricultural leftovers, can be used to synthesize second generation ethanol at an economical cost and with high availability. Cellulose, hemicellulose, starch, pectin and lignin are the primary elements found in this waste from agriculture [9]. Using these waste items for second-generation bioethanol gives various benefits:

1. Agricultural waste is often accessible at little to no cost which lowers the total expenses of bioethanol production [7].
2. Substrates for second generation ethanol are numerous, renewable, and do not interfere with the cultivation of food [7, 10].
3. As several feedstocks are available bioethanol production is flexible [7].
4. Emissions of greenhouse gases is reduced as less garbage is delivered to landfills or incinerated [10].

Therefore, it is advantageous in terms of sustainability, affordability, environmental effect, and feedstock diversity, using plant waste to produce bioethanol. Additional study is required for scalability and economic feasibility.

MICROORGANISMS PRODUCING BIOETHANOL

Bioethanol generation through fermentation involves a variety of microorganisms. Some common microorganisms utilized in bioethanol synthesis are:

Yeast: Recent research on the yeast *Saccharomyces cerevisiae* for the bioethanol production has primarily focused on maximizing the use of various feedstocks and metabolic processes, with the goal of developing renewable and affordable alternatives to fossil fuels [11, 12, 13]. Ethanol synthesis from the starch of cassava using a very prolific strain of *Saccharomyces uvarum* has been investigated [14]. *Saccharomyces carlsbergensis*, when combined with other yeasts, produces a higher ethanol yield. It has also been utilized as an efficient fermenting organism in the context of mass synthesis of bioethanol from cashew apple juice [15, 16]. Studies are looking at *Spirogyra peipingensis* algae as a source of substrate for generation of bioethanol with *Pichia kudriavzevii*, *Saccharomyces cerevisiae* and *Kluyveromyces thermotolerans*. Simultaneously cultured *Pichia stipitis* and *Saccharomyces cerevisiae* has been demonstrated an increase bioethanol production from *Prosopis juliflora* hydrolysate, showing the effectiveness of these yeast strains in bioethanol synthesis. Furthermore, *Pichia kudriavzevii* has demonstrated greater stress tolerance than other yeast strains by producing high gravity bioethanol, [17, 18, 19]. By using non-food substrates, such as cassava peel *Kluyveromyces marxianus* was effectively used for bioethanol synthesis, proving its viability for sustainable bioenergy production [20]. Research on bioethanol production utilizing *Kluyveromyces lactis* and *Kluyveromyces fragilis* involves investigations on increasing ethanol production efficiency through genetic engineering and environmental adaptability [21, 22]. Several research have explored *Candida tropicalis*, proved that it has the ability to produce bioethanol. Furthermore, a transcriptional profile study of *Candida tropicalis* revealed gene expression requirements for bioethanol synthesis. Although there has been little particular study on *Candida brasiliensis* and *Candida utilis* for bioethanol production, related research suggests that these species may be promising candidates due to fermentative and transcriptional profiles that resemble industrially important yeast strains such as *Saccharomyces cerevisiae* [23, 24].

Molds: *Aspergillus niger* has been genetically transformed using multiple genes to promote ethanol synthesis, with the goal of engineering integrated bioprocessing inside the fungus for higher bioethanol yields [25]. Research on bioethanol synthesis employing *Penicillium* species, notably *Penicillium janthinellum* and *Penicillium echinulatum*, has been widely conducted. These fungi have the capacity to produce cellulase and break down lignocellulosic biomass, both of which are required for bioethanol synthesis [26, 27]. Several research have looked at the usage of *Trichoderma reesei* for the production of bioethanol. For example, a dual method incorporating the utilization of *Trichoderma reesei* for the conversion of the elephant grass cellulose into fermentable sugars for bioethanol production was proposed [28]. One research looked at the utilization of *Chaetomium globosum* for making bioethanol from *Delonix regia* pods [29]. Lignocellulose in bagasse is made up of cellulose, hemicellulose, and lignin, which is converted into bioethanol by crude cellulase enzymes generated by the fungus *Phanerochaete chysosporium* [30].

Bacteria: The study Azilah et al. (2017) examines bioethanol synthesis with *Zymomonas mobilis* in high-gravity extraction fermentations [31]. *Zymomonas mobilis* has been identified as a promising microbial culture for producing alcohol as well as additional biochemicals using biomass, making it a unique for future biorefineries [32]. Each of the three subspecies, *Z. mobilis* subsp. *pomaceae*, *Z. mobilis* subsp. *mobilis* and *Z. mobilis* subsp. *francensis*, demonstrated the ability to ferment hexose sugar to ethanol [33]. Soleimani et al. (2017) used paper, corn, corncobs, and pine cones as carbon sources to create bio-alcohol using *Lactobacillus sakei*, *Pediococcus acidilactici* and *Weissella viridescens* bacteria [34]. Studies shows that *Clostridium ljungdahlii* and associated bacteria can use CO (carbon monoxide) as a carbon and energy source for ethanol synthesis, highlighting the possibility of using it in syngas fermentation for bioethanol production [35]. *Escherichia coli* has shown the capacity to metabolize pentose sugars often present in lignocellulosic hydrolysates, producing biohydrogen and bioethanol [36]. Bioethanol production research employing *Thermoanaerobacterium thermosaccharolyticum* involves efforts to increase ethanol production efficiency and to investigate novel ethanol synthesis routes. Researchers have sought to adapt *T. thermosaccharolyticum* to produce more ethanol from seaweed hydrolysis product by eliminating competing routes like as lactate and acetate synthesis. In addition, attempts have been made to introduce additional ethanol production mechanisms, including the pyruvate decarboxylase pathway, that has proven effective in other thermophilic species. Overall, study of *T. thermosaccharolyticum* for the production of bioethanol aims to enhance known routes while also introducing new ones to increase overall process efficiency [37, 38, 39]. *Bacillus coagulans* offers potential for bioethanol production because to its thermophilic characteristics, resistance to inhibitors, and capacity to use a variety of carbohydrate sources [40]. Studies have investigated ethanol synthesis by *Sporomusa ovata*, an electrosynthetic bacterium, showing its potential in bioethanol production from lignocellulose [41]. *Cellulomonas* sp. obtained from termite gut has the capability of the saccharification process and fermentation for agricultural biomass, which aids in bioethanol production [42]. Crude glycerol, an outcome of biodiesel manufacturing, might possibly be transformed to valuable commodities like ethanol by *Kluyvera cryocrescens* [43].

SUBSTRATES FOR BIOETHANOL PRODUCTION

The primary elements found in plant-based materials are cellulose, hemicellulose, starch and pectin. These polysaccharides are converted into simple sugars and the saccharified material is fermented to ethanol.

Starch: Starch is made up of amylose and amylopectin. Various enzymes are required for the transformation of starch to bioethanol. Alpha Amylase: Alpha Amylase is essential for starch hydrolysis; it acts on alpha 1-4 linkages and converts starch to shorter oligosaccharides such as maltose and glucose. Alpha amylase breaks amylose and amylopectin and convert into glucose syrup. Glucoamylase: Glucoamylase acts on short oligosaccharides generated by α-amylase into glucose. This enzyme breaks α-1,4 and α-1,6 linkages and convert oligosaccharides into fermentable sugars. Zymase: Zymase is an enzyme complex found in yeast, namely *Saccharomyces cerevisiae*, this complex do the conversion of glucose to ethanol. This enzyme catalyzes transformation of glucose into ethanol and CO_2 during the fermentation process [44, 45]. Researchers have investigated starch-based food waste materials focusing on the utilization of both fermentation and saccharification processes for synthesis of bioethanol. Different technologies have been used, such as microwave radiation and enzyme treatments, to demonstrate the potential of food waste for ethanol synthesis from it [46]. Recent developments in bioethanol synthesis from renewable raw materials such cassava, avocado waste, etc. emphasizes the necessity of starch hydrolysis in ethanol generation. The study stresses the sustainable and economic elements of bioethanol synthesis from various feedstocks [44]. A small-scale bioethanol production method using the starch recovered from avocado seeds displays good yields as well as efficacy in transforming avocado seed starch to ethanol. This indicates the opportunity of using avocado seed in place of a substrate for bioethanol production [47]. Sustainability research looks at bioethanol production from tuber starch feedstocks such as cassava, maize, sweet potato and sorghum. The study evaluates economic, environmental, and energy elements to improve the sustainability of bioethanol manufacturing processes, proposing options for enhanced efficiency of energy and environmental performance [48]. Emad et al. (2019) investigated bioethanol synthesis from *Pseudomonas poae* by using castor bean cake as a substrate. This study demonstrates the possibility for using agricultural waste for biofuel generation [49]. These studies help to further understanding about converting starch into bioethanol by using novel techniques, sustainability issues, and new raw materials for effective bioethanol manufacturing procedures.

Cellulose: Cellulases are the enzymes responsible for breaking cellulose into simple sugars and ultimately to bioethanol. Cellulases are a complex set of hydrolytic enzymes that function together to convert. Cellulases are of following major groups: Endoglucanases function randomly in the amorphous area of cellulose and initiates the hydrolysis process by breaking down cellulose chains. Exoglucanases work on both the reducing as well as non-reducing ends of cellulose chains, producing cellobiose and further breaking down the chains. β-Glucosidases convert cellobiose to glucose, releasing sugars that can be used to produce ethanol. These enzymes work together to degrade the intricate structure of cellulose into simpler sugars such as glucose, cellobiose, and cellooligosaccharides, which may subsequently be fermented to produce bioethanol [50, 51].

The most recent studies on transforming cellulose to bioethanol works on increasing the efficacy of the three processes 1) conversion process using diverse pretreatment processes, 2) hydrolysis and 3) fermentation methods. Pretreatment procedures include chemical, physical and biological treatments improve biomass accessibility for enzymatic activity. Steam explosion, alkali, acids, and biological preparation with microorganisms are among the most promising pretreatment approaches. Cellulolytic enzymes hydrolyze cellulose into glucose and oligosaccharides. Fermentation is the conversion of glucose to bioethanol utilizing yeast or bacteria. Recent advances in nanotechnology have showed promise in boosting bioconversion efficiency by increasing lignocellulosic biomass accessibility to enzymes and minimizing inhibitor production. The application of nanoparticles in pretreatment was demonstrated to boost [50, 52, 53, 54, 55].

Hemicellulose: Hemicellulase plays critical role in the breaking down the biomass from plants and carbon transport back into nature. Enzymes such as β-mannanases, xylanases, α-l-arabinofuranosidases, β-xylosidases, α-d-glucuronidases and hemicellulolytic esterases break down hemicelluloses, complex carbohydrates present in plant cell walls. Hemicellulases are used for conversion of hemicellulose into fermentable sugars such as xylose, galactose, mannose and arabinose, which are used to synthesize bioethanol from lignocellulosic biomass [56]. Hemicellulase converts hemicellulose into ethanol by breaking the links between glucose and polymers found in fibers from plants with water molecules, resulting in hemicellulose hydrolysis [57]. Wheat straw as a source of hemicellulose have been mentioned in many literatures. Detroy et al. (1982) reported conversion of straw from wheat cellulose/hemicellulose into the ethanol by *Pachysolen tannophilus* and *Saccharomyces uvarum*, with a yield of 70-82% for cellulosic pulp but only 40-60% for ethanol due to treatment-induced inhibition [58]. Nigam (2001) investigated the production of bioethanol from acid hydrolyzed hemicellulose of wheat straw utilizing *Pichia stipitis* strains, resulting in increased yields as well as productivity [59]. Koti et al. in 2016, demonstrated increased production of bioethanol from wheat straw hemicellulose utilizing mutant *Candida shehatae* and *Pichia stipitis* strains [60]. Tsegaye et al. (2019, 2020) evaluated several wheat straw pretreatment strategies to optimize parameters for ethanol production [61, 62]. Chen et al. (2021) employed sub-critical water pretreatment along with high solid hydrolysis to increase ethanol conversion rate from wheat straw [63]. Tabañag et al. in 2018 used genetically modified *Saccharomyces cerevisiae* to produce ethanol using hemicellulose. They enhanced hydrolytic activity by showing hemicellulase onto the yeast surface [64]. Scordia et al. (2012) used *Scheffersomyces stipitis* to produce 8.20 g L^{-1} bioethanol at 6.0 pH, after 48 hours of hydrolysis of gigantic reeds [65]. Mihiretu et al. (2017) studied microwave-induced pressured hot water conditions in xylan-based biopolymers and ethanol from biomass co-production using aspen wood sawdust and sugarcane waste, yielding highest xylan yields of 66% and 50%, correspondingly [66]. Batog et al. (2020) studied bioethanol production in several sorghum varieties and recommended Sucrosorgo 506 for both main and secondary crop farming [67]. Sharma et al. (2019) used rice straw to generate ethanol with yield of 2-4 g L^{-1} [68].

Pectin: Various enzymes are involved in the transformation of pectin to bioethanol, which breaks up pectin to fermentable sugars. Polygalacturonase (PG): Polygalacturonases hydrolyze alpha 1,4-glycosidic linkage to polygalacturonic acid chains, resulting in units of D-galacturonate. These enzymes are required for the breakdown of pectin to simpler sugars used in bioethanol synthesis. Pectin Lyases: Pectin lyases eliminate alpha 1,4 glycosidic linkages in polymers of polygalacturonic acid, resulting in unsaturated C-C bonds within the cleaved pectin polysaccharide. They help break down pectin to smaller parts for use in bioconversion processes. Pectin Esterases (PE): Pectin esterases, also referred to as pectin methylesterases (PME), catalyse the de-esterification of methyl ester links in galacturonate units, resulting in pectate and methanol. Enzymes like these target methyl ester groups found in galacturonate units, allowing pectin to be broken down into fermentable sugars. Protopectinases transform insoluble protopectin to soluble pectin, which aids in the early degradation of pectic compounds before additional enzymatic conversion activities [69, 70]. Biomass rich in pectin, including sugar beet pulp, citrus waste, and apple pomace, are ideal feedstocks for ethanol generation. These commodities, which are frequently regarded as waste products within the sugar and juice industries, can be used to supplement ethanol supply through leveraging their current feedstock value. According to Zhou, Widmer, and Grohmann (2007), co-products from citrus peels like pectin and pectin residues have significant economic value in bioethanol generation. A ton peel of orange may yield 44 lb of galacturonic acid through the conversion of peels to bioethanol [71]. The industrial fruit processing generates waste rich in pectin, creates a suitable biomass for production of ethanol. Firstly, biomass is willingly stacked in fairly large quantities in processing plants, significantly lowers the expenses required for gathering and transferring it [72]. Himmel et al. (2007) present a sophisticated method for lignocellulosic degradation, including thermochemical pretreatment and digestion with the help of enzyme to produce simple sugars for the production of bioethanol [73]. Highly resistant biomass, such grasses and forests, requires treatments that involve dilute sulfuric acid or ammonia fiber expansion pretreatment [74] or pretreatment for disturbing the structure of biomass or to eliminate chemicals antagonistic to fermentation, such as limonene present in waste derived from citrus [75].

THIRD GENERATION BIOETHANOL

Third-generation bioethanol is synthesized by using non-food biomass sources like algae, which are thought to be more sustainable and ecologically less harmful than first- and second-generation bioethanol raw materials like maize and sugarcane [76]. Various algae have been studied for their ability to make ethanol such as *Spirulina*, *Chlorella, Chlorococcum* sp., *Gelidium amansii*, *Prymnesium parvum, Gracilaria* sp., *Sargassum* sp., *Laminaria* sp. and *Spirogyra* sp. These algae make ethanol through various processes such as anaerobic digestion and fermentation [77]. Lot of research is being going on third generation of bioethanol which concludes that algae are regarded as a viable resource for bioethanol production as they can create ethanol at two to five times lower rate than that of sugarcane and corn while using less energy [78, 79]. Growing, harvesting, drying, oil extraction, and bioethanol fermentation are the steps of current algae-based biofuel production methods [79]. The cost, water, CO_2, and energy footprints of algal biomass derived third-generation biofuels were studied. It depicts that this biofuel derived from algal biomass have the potential to significantly increase the global biofuel share, but there are still challenges to commercialization to overcome, such as cost, investments, policy and regulations [79]. The problems and projections for third-generation ethanol generation have been studied, including enhancing algal carbohydrate content, biomass output, and sugar extraction efficiency [80].

CONCLUSION

Fossil fuels provide for around 86% of global primary energy consumption, which is increasing progressively. These fuels have a huge impact on environment like emission of greenhouse gases and change in climate. Bioethanol is a renewable and sustainable option which is synthesized from a different substrate, includes non-food biomass sources such as starch, cellulose, hemicellulose, and pectin. Third-generation bioethanol synthesized using algal biomass is also a lucrative choice as algae can survive in variety of conditions together with waste water and sea water without competing for arable land and freshwater. Switching over bioethanol is practically workable choice to reduce the dependence on fossil fuel.

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DECLARTION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest or any affiliation or involvement in any organization whether it is academic, commercial, financial, personal and professional relevant to the work under consideration to avoid the potential of bias and accept responsibility for what is said in the manuscript.

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