MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 1 (Jan-Mar 2024)

Review Article

Production of Bio-Ethanol from Agricultural Waste Using Microbes: An Overview

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ARTICLE INFO

Keywords:
Biofuel, Bioethanol, Pretreatment, Biocatalyst, Fermentation

How to cite:
Mazhar, S., Yasmeen, R., Hafeez, F., & Iqbal, A. M. (2024). Production of Bio-Ethanol from Agricultural Waste Using Microbes: An Overview : Production of Bio-Ethanol from Agricultural Waste. MARKHOR (The Journal of Zoology), 5(01). https://doi.org/10.54393/mjz.v5i01.75

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Received Date: 9th August, 2023
Acceptance Date: 19th February, 2024
Published Date: 31st March, 2024

ABSTRACT

Ethanol produced through the fermentation of plant biomass is considered an environment friendly alternate to fossil fuels. Bioethanol and biodiesel, commonly known as second-generation biofuels, are produced through biological processes using agro-industrial waste and are considered sustainable, safe, and ecofriendly. These biofuels can minimize the emission of carbon dioxide and reduced the world's dependence on fossil fuel. This review article focuses on three generations of biofuels, particularly the production of biofuel using fungal biocatalysts specifically *Aspergillus niger* and Saccharomyces cerevisiae and the mechanism by which they convert biomass into biofuel. *A. niger* is known for releasing cellulolytic and pectolytic enzymes to hydrolyze biomass and survive against toxins, while *S. cerevisiae* produces invertase and zymase enzymes to convert sucrose into fructose and glucose sugars, and then further convert fructose and glucose into ethanol. The main purpose of this review is to explore alternative techniques for generating biofuels, as well as to investigate the role of diverse microbial species and their fermentation capabilities in biofuel production.

INTRODUCTION

Advancements in technology have transformed the world and become essential for economic and social development. But all these advancements need energy to work, and these energy requirements are in large part fulfilled by fossil fuels, i.e., coal, petroleum, and natural gas [1]. Fossil fuels have become a staple of the modern lifestyle and are used to produce steam and electricity, and to power transportation systems that make possible the production of thousands of tons of commercial goods. Fossil fuels are non-renewable organic compounds that are formed from the remains of plants and animals that have been buried in the earth's crust over hundreds of millions of years. The world contains huge reserves of solid, liquid, and gaseous fossil fuels that now support the world's economy. The economies of industrialized countries are fully dependent on the availability of fuels at relatively low cost. For example, more than 70 countries have coal reserves, and currently 40% of power is generated from coal. Deposits of energy sources are unevenly distributed around the world, and over the past two decades, these deposits are rapidly depleting. This depletion is causing an increase in fuel prices, and large increases in price could adversely affect the world's economy. Rising population growth will always remain the key factor of energy demand [2]. According to an estimate 82% of fossil fuels were extracted worldwide in 2011, but by 2050, deposits are projected to decrease to 76%. Apart from the risk of energy crises, fossil fuels have another major drawback: climate change. As fossil fuels burn, they emit carbon dioxide. Not only does carbon dioxide, along with other greenhouse

DOI: https://doi.org/10.54393/mjz.v5i01.75

gases such as methane and man-made halocarbons, lead toward global warming, but it will also induce a long-term impact on ozone. According to recent studies on climate change, accumulation of these gases has strongly contributed to the uptrend in the world's atmosphere temperature by approximately 0.6–0.7℃[3]. The rapid loss of glaciers and sea ice is a cause for international alarm[4]. In recent years, the increased accumulation of halogenated gases has resulted in increased ozone depletion [5]. According to a recent study, Asia, Europe, and the United States altogether emit 88% of total global emissions [6]. However, China is a well-known proponent of bioenergy, producing first-, second-, and third-generation biofuels with great energy potentials, and is expected to produce 8 billion liters of biofuel by 2019 [7]. Billions of tons of emitted greenhouse gases have accumulated in the atmosphere for decades, and the rate of increase in temperature has doubled in the past 50 years. This is likely to result in frequent heat waves and altered weather patterns in the future. Human activity is considered the main culprit for carbon dioxide emissions by way of fossil fuel burning in vehicles and industries. The Intergovernmental Panel on Climate Change (IPCC) estimates that these gasses will remain in the atmosphere for decades. According to NASA's recent record, half of the carbon dioxide emitted by burning fossil fuels is soaked up into ocean along with heat; this leads to increasing ocean acidification. IPCC predicts that by the end of the 21st century, the temperature of Earth's surface will rise by 2–6°C [8]. Global warming leads to climate change; recent studies show an increased rate of precipitation, flooding, and droughts between 1975 and 2002, with over 200,000 lives lost and about 2 billion affected. Marine ecosystems are also severely affected by the increase in ocean temperatures [9] and, as stated above, elevated atmospheric carbon dioxide concentrations have acidified the water to the point of irreversible impact on marine animals[10].

Biofuel as an Alternative

Renewable alternatives form a single solution to the above-stated problems with fossil fuels. Biofuels are an ecofriendly alternative option to reduce the emission of carbon dioxide [11]. Biofuel can be obtained from organic material present in the environment as waste; this would minimize the dependence of the world on fossil fuel [12]. Two commonly and extensively produced and used forms of biofuel are bioethanol and biodiesel [13]. Fermentation of sugar and the starch component of plant matter results in the production of bioethanol [14-15], and this production involves the use of diverse enzymes [16]. Ethanol is categorized into three major mixtures: E95, E85, and E10. E95 is 95% ethanol, and it must be blended with gasoline before use as fuel for vehicles. E85 is used in the U.S. as an alternative source of fuel, comprised of 85% ethanol mixed with 15% gasoline, while E10 is comprised of 10% ethanol and 90% gasoline [17]. The European Union and the United States began to produce biofuel in the 1980s after the enactment of the National Energy Conservation Policy Act of 1978 [18]. Brazil is considered highest in ethanol production [19]. With its tropical climate and thriving biodiversity, Brazil is considered the most suitable region in the world for biomass production and is already using sugarcane bagasse in the bioenergy sector [20-22]. Industrial waste and raw materials are also useful resources for energy generation [21, 23]. As it is produced on a large scale, Brazilian ethanol is thought to be more competitive with low cost and low greenhouse gas emissions [24]. Brazil does not import and export sugarcane byproducts; instead, these byproducts are being used in the same mills and distilleries for the same purpose [25]. Bioethanol can also be produced from grasses and wood to synthesize biodiesel, and this ethanol is known as cellulosic ethanol [26-28]. Biodiesel obtained from cellulosic ethanol reduces emissions of greenhouse gasses by 85% [29]. Cellulosic biomass as a means of obtaining ethanol could produce 60 billion gallons of ethanol per year—that is enough to replace 30% of gasoline consumption by 2030[30].

Classification of Biofuel

Biofuels are classified as first generation, second generation, and third generation on the basis of their source of biomass.

First-Generation Biofuel

Food crops such as corn and sugarcane are used as a source of biomass for first-generation biofuel [31-32]. Brazil produces sugarcane ethanol, the U.S. produces corn ethanol, Germany uses oilseed for biodiesel, and palm oil is the source of biomass for biodiesel in Malaysia [33-34]. The edible parts of corn and rice crops are used to produce the first-generation biofuels, but their stalks and inedible residue make up the ingredients for second-generation biofuel [35-36]. There is a drawback for the production of first-generation biofuels: high prices of food due to food crises make first-generation biofuel expensive [37-38].

Second-Generation Biofuel

Second-generation biofuels are produced from non-food biomass; this includes industrial, agricultural, and forestry residue such as sugarcane bagasse, straw, grasses, and wood [28, 35, 39]. These biofuels are synthesized by lignocellulosic, cellulosic, and hemicellulosic components [40]. Ten percent of the world's primary energy demand is covered by second-generation biofuels such as biomethanol, bioethanol, and biohydrogen [34, 41-43]. Forestry residue—e.g., hardwood, softwood, wood chips,

DOI: https://doi.org/10.54393/mjz.v5i01.75

and sawdust—is also a source of biomass for second-generation biofuels. These biopolymers are converted into sugar through pretreatment of biomass and then converted into ethanol through the process of fermentation[44].

Third-Generation Biofuel

Third-generation biofuels, also known as advanced or next-generation biofuels, are non-renewable fuels that derived from non-food sources such as algae, bacteria, industrial waste materials, agricultural residue, and municipal solid waste. These are advanced biofuels with several advantages: improved energy efficacy, reduced greenhouse gas emissions, minimal competition with food production, and the potential for sustainable and large-scale production. These biofuels are in the initial stages of development, but they hold significant promise in addressing the environment and economic challenges associated with conventional fuels[40, 45-46]. The detail are also summarized in table 1.

Table 1: Different generation and feed stocks and their products[47]

Microalgae Biofuel Production			
Food crops (1st generation)	**Lignocellulosic feed stock (2nd generation)**	**Microalgae (3rd generation)**	**Genetically engineered microorganisms (4th generation)**
Corn Wheat Oil seeds Different energy crops	Non-food crops Citrus peels Cotton stalk	*Chlorella vulgaris* *Chlamydomonas reinhardtii* Other microalgae species macroalgae	Genetically engineered feedstocks with Genomically synthesized microorganisms
Biodiesel Bioethanol Bioether solid biofuel	Cellulosic ethanol Biohydrogen Biomethane Myco-diesel	Biodiesel Bioethanol Biohydrogen Biomethane	Biodiesel Bioethanol Biohydrogen Biomethane

Methods for Pretreatment

Agricultural products such as sugarcane, corn, wheat, and rice are the starchy biomass that is converted into sugar through pretreatment and then into ethanol when those sugars are fermented [48]. The pretreatment process is applied to delignify or to remove the chemical components that would resist the fermentation process. Pretreatment is also useful for reducing these sugars so that biocatalysts can more quickly metabolize them(Figure 1)[49].

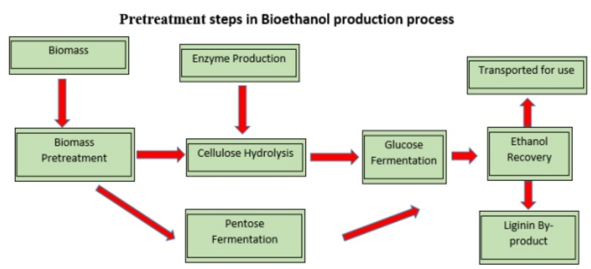

Figure 1: Use of pretreatment techniques in ethanol production

Two basic techniques are frequently applied for pretreatment: acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis is an efficient and cost-effective technique. Enzymatic hydrolysis, on the other hand, involves enzymes that not only hydrolyze the lignocellulosic components but also metabolize the toxic components, though this process is time-consuming and comparatively expensive [50—51]. Steam pretreatment is also used for delignification; it is referred to as a secondary pretreatment process, as it can be applied along with other pretreatment techniques[52].

Lignocellulosic Biomass

Azadirachta indica is an abundantly grown plant that is a rich source of lignocellulosic biomass[53]. Lignocellulosic materials are the dry, woody parts of a plant that are mostly inedible to humans; for example, the wide range of feedstock used as a substrate (e.g., sugarcane, molasses, grasses, etc.) are lignocellulosic [54]. Lignocellulosic biomass is required for second-generation biofuels, but it is difficult to ferment as it releases toxic components that resist yeast's fermentative activity, but the toxicity can be reduced by enzymatic pretreatment of the biomass. Peroxidase and laccase are used for detoxification purposes. However, the microorganisms themselves release toxic components through hydrolysis and reduction during pretreatment, such as furfural, hydroxymethylfurfural, carboxylic acids, phenolic compounds, and glycolaldehyde[55].

Use of Sugarcane for Bioethanol Production

The production of bioethanol through sugarcane was started for the first time in Brazil and the United States in the early 1970s [56]. Currently, in many countries, especially China and India, the levels of bioethanol production have been increasing significantly. In Brazil, bioethanol for fuel is derived from sugarcane and is used pure or blended with gasoline in a mixture called gasohol (24% bioethanol, 76% gasoline) as reported by [12]. Brazil produces the largest quantity of ethanol (15.10 billion liters per year) followed by the U.S. (13.38 billion liters per year), China (3.65 billion liters per year), and India (1.75 billion liters per year)[57].

Use of Crops for Bioethanol Production

Bioethanol can be produced from carbon-based feedstock. Using agricultural waste for the production of bioethanol is considered cost-effective and

DOI: https://doi.org/10.54393/mjz.v5i01.75

environmentally friendly, making it a promising avenue for bioethanol synthesis [58]. Some of the common sources that are used to produce bioethanol include sugarcane, corn, potatoes, wheat, sugar beets, wood chippings, and starchy grains. Salassi, reported that 60% of global ethanol is produced from sugar crops, and the remaining 40% is produced from starchy grains. Edible crops like corn and rice are used to produce first-generation biofuel, while their stalks and residue are used for the production of second-generation biofuel [59].

Use of Plants with Starch and Cellulose Content for Bioethanol Production

Starch and cellulose molecules are strands consisting of glucose molecules joined by acetyl linkages. Starch and cellulose are similar components that differ only in the number, location, and type of linkages. The chemical formula of the starch molecule is (C6H10O5)n. It is a polysaccharide molecule consisting of glucose monomers with amylose and amylopectin in variable percentages in linear and branched forms [60]. Cellulose, on the other hand, consists of only linear chains of thousands of D-glucose units [61]. It is a strong and rigid molecule that requires pretreatment to convert it into glucose, which can be further fermented to produce cellulosic ethanol. In the process of bioethanol fermentation, the sugars present in the biomass are converted into ethanol and carbon dioxide:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{heat}$$

Role of Microbes in Biofuel Formation

Microbes form the backbone of biofuel production, as they are responsible for transforming biomass into ethanol through fermentation [62], either in the presence or absence of oxygen. The synthesis of bioethanol by microbes is regulated through changes in substrate, pH, and temperature [63]. Microorganisms such as yeast *(S. cerevisiae)*, bacteria (*Bacillus thuringiensis*, *Escherichia coli*, and *Zymomonas mobilis*), and fungi (*A. niger*) are the most widely cultivated means of converting plant biomass-derived sugars into biofuel [64]. Cheap and timesaving, S. cerevisiae is a major biocatalyst that is currently used for the production of bioethanol on an industrial scale. It is thought to be superior to bacteria and other filamentous fungi due to its high tolerance to acidic pH levels—as biomass is introduced to strong acids during pretreatment—and it is resistant against toxins [65-66]. Alternatively, *A. niger* is a filamentous fungus with the ability to survive temperatures between 20–50°C and an acidic pH range of 2–6. It is ethanol tolerant and easily isolated from soil and rotten fruits, producing ethanol within seven days of fermentation [67]. According to a study, *A. niger* produces 0.279% ethanol per kg of sugarcane bagasse [68]. Bacteria and yeast can also be combined in a co-culture that more efficiently utilizes the complex substrate and gives a higher yield. In co-cultures, both species are usually cultured on artificial or synthetic media, particularly those which contain all the ingredients that could support the growth of both organisms. Co-cultures are preferred because of their efficiency, as ethanol production starts within 24 hours of co-culture inoculation [69]. *E. coli* is one of the well-studied fermentative bacteria, capable of fermenting sugars by glycolysis to produce lactic acid and ethanol. *E. coli* ferments hexose sugars via the Embden–Meyerhof–Parnas (EMP) pathway, and pentose sugars are metabolized via the pentose phosphate pathway (PPP) [70]. Some other bacteria are also viable alternatives for the fermentation process by having genetic similarities in their native enzymes and pathways. One such microorganism is *Z. mobilis*. It metabolizes sugars through the Entner–Doudoroff (ED) pathway. This pathway produces only one molecule of ATP from each glucose molecule and produces less biomass as compared to the EMP pathway, which gives two ATP molecules from each molecule of glucose and generates sufficient biomass. Genetically engineered Z. mobilis has the ability to ferment lignocellulosic biomass-derived sugars to synthesize ethanol. These strains are being genetically modified to resist the toxins produced during fermentation [71-72].

CONCLUSIONS

Different feed stocks or agro-industrial waste matter can be recycled for the production of lignocellulosic or second-generation biofuel through a diverse selection of microbes. These microbes act as biocatalysts in the conversion of complex structural materials to simple sugars, resulting in the formation of a product that can be used to overcome the energy crises that arise from depleting non-renewable sources of energy.

Authors Contribution

Conceptualization: SM, RY
Writing-review and editing: SM, RY, AMI, FH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

DOI: https://doi.org/10.54393/mjz.v5i01.75

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