STM JOURNALS

Recent Trends in
Infectious Diseases

Volume 1, Issue 2, 2024
July-December
DOI (Journals): 10.37591/RTID

https://journals.stmjournals.com/rtid

Review RTID

# Bioethanol and Beyond: A Comprehensive Review on Sustainable Biofuels for Global Energy Demands and Environmental Conservation

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***Abstract***

*This comprehensive review explores the concept of "biofuels," specifically focusing on energy-enriched chemicals generated through biological processes or derived from the biomass of living organisms, including microalgae, plants, and bacteria. As the global population continues to increase, there is a growing demand for more energy supplies to enhance the quality of life. Biofuels, harnessed from various sources such as sugar, starch, and organic waste, have emerged as potential solutions to meet the escalating global energy demand. The paper emphasizes bioethanol, a renewable fuel produced through fermentation, as a significant biofuel that can be blended with gasoline to reduce vehicle emissions and reliance on foreign oil. Highlighting the importance of bioethanol in substituting petrol fuel for road transport vehicles, the paper discusses the top five countries in bioethanol production, emphasizing the need for alternative biomass sources to meet the rising demand. Additionally, the study delves into the environmental benefits of bioethanol, which produces lower emissions of carbon monoxide, nitrogen oxides, and CO2 compared to fossil fuels, contributing to efforts to mitigate global warming. The review concludes by addressing the sustainability challenges posed by the fermentation of economically important cultivars and underscores the potential of plant leftovers and agro-energy crops as low-cost, long-term biomaterials for biofuel production, offering a promising avenue for sustainable energy resources and addressing environmental concerns associated with fossil fuel combustion.*

**Keywords:** Biofuels, environmental issues, fermentation, bioethanol, biomass

## INTRODUCTION

The “biofuels” in this review are referred to the energyenriched chemicals generated through the biological processes or derived from the biomass of living organisms, such as microalgae, plants and bacteria. The world's population is increasing in order to improve life. This requires high voltage. Biofuels can be one source to meet the world’s energy needs. For many years, fossil fuels have been the primary source of energy; However, this process is not sustainable and causes environmental problems due to the burning of fossil fuels. [1].

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Received Date: December 16, 2023
Accepted Date: December 18, 2023
Published Date: December 30, 2023

**Citation:** Kaushiky Sharma. Bioethanol and Beyond: A Comprehensive Review on Sustainable Biofuels for Global Energy Demands and Environmental Conservation. Recent Trends in Infectious Diseases. 2024; 1(2): 1–9p.

Bioethanol is ethanol that is produced by fermentation plant material such as sugar and starch. It is produced from the agricultural products like as beetroot, potatoes, rice, sugarcane, dates and other wastes. Humans have been using biofuels as an energy source.. Ethanol is an important renewable fuel source that can be produced from a variety of feedstock’s, including sugarcane waste. Gasoline can be blended with ethanol to reduce emissions and improve air quality. It is also a

 

domestically- produced alternative to petroleum- based fuels, which can help reduce our dependence on foreign oil [2].

Bioethanol is a biofuel commonly used to replace gasoline in road vehicles. The most popular fuel alternative in the world. The top five countries of the largest production of the bioethanol Brazil 16500 billion litres, The United State 16270 billion litres, China 2000 billion litres, The European Union 950 billion litres, India 300 billion litres. At present, bioethanol for the fuel market is produced from corn in the United States and from Sugarcane in Brazil. These feedstocks will not be sufficient to meet the growing demand for bioethanol fuel. On the other hand, bioethanol can also be produced from abounding and continuous biomass sources such as organic waste of agriculture crops, forests, and other crop [3].

## METHODOLOGY

### Bioethanol Production Method

There are three main stages in the biological conversion of biomass to bioethanol: enzymatic hydrolysis, fermentation, and pretreatment. Pretreatment is the most important part of the process because it has the biggest impact total bioconversion. Glucose, hemicellulose, and lignin are tightly packed together in lignocellulosic biomass for a variety of plant purposes, including resistance to enzymatic hydrolysis. To provide more cellulose for the enzymes that convert carbohydrate polymers into volatile sugars, the cellulosic biomass must first be processed. The pretreatment technology, circumstances, and severity all affect how much lignin and hemicellulose are removed.

#### *Pretreatment of Biomass*

From a long time, effect of pre-treatment on lignocellulosic material have been reorganized. The main purpose of pre-treatment is to reduce cellulosic Crystallinity and increase the porosity off biomass to facilitate hydrolytic enzymatic action on cellulosic biomass [4].

As the lignocellulosic biomass in its natural form is very recalcitrant pre- treatment is required. Pre-treatment must meet the following requirements: Avoid loss off sugars (pentose) during degradation. To reduce formation of by products which can impede subsequent process. To improve the production of sugars. To improve yield of sugar during enzymatic hydrolysis. Preliminary treatment should be reasonably charged. They use very low capital and operating costs. There are different approaches for pre-treatment which are follows: Physical, physicochemical, chemical and biological pre-treatment [5].

#### *Physical method*

Mechanical comminution is the most widely used method under physical approach. In this method combination of milling, grinding and chipping is performed to lignocellulosic material for comminution of particles from 10-30mm 00.2-2mm. Especially in the context of collateral, in the form of milling milling crystallinity of biomass is decreased and particle size gets reduced. This improve the digestibility of biomass than other method power requirement depends on the type of material and final size of the Particle [5].

*Pyrolysis*

It is another physical method which consumes less energy. In this method biomass is treated at very high temperature, generally above 300°C so that cellulosic material degrades rapidly to produce gases like CO. H2 and residual char. At lower temperature rate of decomposition of cellulosic biomass is very slow which leads to formation of volatile products and the rate of decomposition in this process can be enhanced in presence of oxygen [5].

*Microwave*

Microwave method is one of the most promising one for increasing enzymatic susceptibility of the cellulosic material by degrading the native structure of lignin and hemicellulose. Operational approach

 

of this method is very simple when compared to other. Electron beam irradiation and high energy gamma rays for deeper penetration are some of the alternative method which comes under physical pretreatment of lignocellulosic material [5].

***Physico Chemical Method***

*Steam Explosion*

Another name for this process is autohydrolysis. During steam blasting, the lignocellulosic biomass is first treated at high pressure and saturated steam, followed by a rapid reduction in pressure.Due to high temperature and steam the cell wall of lignocellulosic biomass gets expanded which results in breakdown of lignin and hemicellulose structure and which further exposes the internal surface of the biomass for access of enzymes [6].

Optimal conditions which are required for this method are: temperature220°c residual time30second, ratio of water and solid 1:2 and 1% H2SO4. Addition of acid catalyst like sulphuric acid or phosphoric acid can decrease the production of inhibitory compounds and can reduce temperature and time substantially. Some of the advantages of the steam explosion method are, low requirement of energy cost effective has less impact on environment as less chemicals are used in the process [7].

*AFEX (Ammonia Fiber Explosion)*

It is a type of physicochemical Pre treatment method in which the lignocellulosic biomass is first exposed to liquid ammonia at temperature range of 90-100° and pressure is reduced swiftly. Partial de crystallisation and fractionation of lignocellulosic material takes place in this process. Lignocellulosic biomass is processed with liquid ammonia, low temperature, and high pressure for a predetermined period of time before rapid pressure reduction in a physicochemical machine called "ammonia fiber blasting" (AFEX) Steam blast pretreatment and AFEX methods are fairly comparable. The chemical action of ammonia under pressure causes the biomass to swell, increasing surface accessibility and simultaneously reducing cellulose crystallization [4].

AFEX/ARP pretreatment has some drawbacks, such as high energy consumption and production costs associated with ammonia recycling. Onsite handling and storage of ammonia in industrial laboratories raises safety and environmental concerns. This technique has been studied to reduce its cost. [8].

***Chemical Method***

*Acid Pretreatment*

It is the most effective and widely accepted method to enhance the yield of sugars in lignocellulosic biomass. One of the oldest and most useful for solubilisation of lignocellulosic material at higher temperatures with acids like sulphuric acid and hydrochloric acid. Optimal conditions required for this process are: temperature 120-180° and residual time 15-60 minutes. High temperature with low acid concentration and same for low temperature with high acid concentration. Diluted sulphuric acid has high reaction rate which promotes breakdown of hemicellulose material into xylose and other sugars. This method is very appreciated as cost of maintenance is low and high yield can be obtained. One drawback of this approach is formation of inhibitory by products.

*Type of acid & Pre-treatment conditions*

1.5% $H_2SO_4$ at 170°C for 15 min
0.5% $H_2SO_4$ at 120°C for 120 min
2.0%$H_2SO_4$ at 155°C for 10 min

*Sulfuric acid*

0.5% $H_2SO_4$ at 180°C for 15 min
1.25% $H_2SO_4$ at 121°C for 2 h

 

0.5% $H_2SO_4$ at 121°C for 60 min
2.5% $H_2SO_4$ at 140°C for30 min

*Hydrochloric acid*
1.2% HCI at 121°C for 4 h

*Phosphoric acid*
3.5% $H_3PO_4$ at 130°C for 180min
4%$H_3PO_4$ at 122°C for 300 min

*Nitric acid*
6% $HNO_3$ at 122°C for 9.3 min

*Alkali Pretreatment*

The efficacy of this method is determined on the amount of lignin present, as well as the material's physical and chemical composition. Alkali pre-treatment involves the saponification process of intermolecular cater bond and linking xylan hemi cellulosic components Various bases like potassium hydroxide, hydrogen peroxide, sodium hydroxide [9] and calcium hydroxide (lime) [10] can be used. Alkali pre- treatment requires low temperature and pressure when compared to other approaches of pre-treatment. When lignocellulosic material is treated with dilute sodium hydroxide it results in swelling which can leads to increase the internal surface area of the cell and disrupt it's lignin structure and decrease in degree of crystallization and polymerization of the biomass [10].

*Ozonolysis*

In this approach ozone is used for degradation of lignocellulosic material like cotton straw, poplar sawdust, Bagasse and wheat straw etc. Ozone acts as a officiant oxidant and it is also soluble in water which makes it more reactive towards functional group with high electron density and compounds with double bond conjugate. This method is very efficient for lignin degradation and produces fewer toxic residues during downstream process. The reaction could proceed at pressure and at room temperature.Drawback associated with this approach is that pretty large amount of ozone is required which makes this whole process very expensive and less environment friendly [11].

*Organosolv*

In this pro-treatment approach strong inorganic acids which works as catalyst is mixed with organic solvent to curry out the degradation of lignin-lignin and lignin-carbohydrate bond within the biomass. In order to reduce the cost of the process solvent must be drained, evaporated and condensed which can also improve the efficacy of this technique Organosoly method was less chemicals for neutralization and waste generated in the process is also low [11].

***Biological pre-treatment***

Among all the pre-treatment approaches this is considered to be most environmental friendly as niemoorganisms are used to carry out the degradation of lignocellulosic biomass various cellulolytic and hemicellolytic microorganisms such noc white rot fungi and soft rot fungi are commonly used. White rot fungi like pycnopous-cinnabarinus ceriporialacerata and pleuritic ostreatus have been used successfully for treating lignocellulosic biomass [12]. Brown rot fungi attack cellulose only where as other microorganisms attack both cellulose and lignin. A number of enzymes like manganese per oxidase, polyphenol oxidase, hydrogen peroxide and laccase plays important role in this approach. mild Conditions required for this process, overall energy requirement is low and no added chemicals make it more environment friendly [12].

Some of the disadvantages of this technique include: a longer residence time as compared to other methods, poorer efficiency, a sluggish rate of hydrolysis, the need for a controlled environment for

 

microbe development, and a significant loss of carbohydrates. Slow, brown, and white microbial decomposition Biological pretreatment uses fungi to break down lignin and hemicellulose in waste products White and coarse rot attack both cellulose and lignin , whereas brown decay targets only cellulose. White-rot fungi are the most effective natural basidiomycetes for the pretreatment of lignocellulosic materials. Pleurotusostratus transformed 35 percent of wheat straw to reducing sugars in five weeks, according to researchers who evaluated the pretreatment of wheat straw by white-rot fungus. Pretreatment with Phanerochaete Sordid and Pycnoporus cinnabarinus yielded similar results in four weeks, A cellulase-free mutant of Sporotrichum pulverulentam was created to prevent the loss of cellulose. The lignin-degrading enzymes lignin peroxidase and manganese dependent peroxidase are metabolized by the white-rot fungus P. aeruginosa during secondary metabolism in response to carbon or nitrogen limitation. Chrysosporine is produced [13].

***Hydrolysis***

It is the process in which long chain of glucose molecules present in cellulosic biomass are broken down into simple, free or monomeric sugars before fermentation. Chemical or enzymatic hydrolysis are two of the most common method used for hydrolysis of lignocellulosic biomass [14].

***Chemical hydrolysis***

Acid hydrolysis, another name for chemical dissolution, is a very practical method for the removal of hemicellulosic materials from biomass.

In this method cellulosic material is treated with water in presence of an acid for conversion of lignocellulosic biomass into fermentable sugars, Sulfuric acid, hydrochloric acid and $H_3PO_4$ are some of the acids which are used for hydrolysis Treatment of biomass with dilute sulphuric acid has proven to be beneficial for providing xylose and other sugar constituents. Acid hydrolysis can be carried out in two ways: dilute acid hydrolysis and concentrated acid hydrolysis.In dilute acid hydrolysis temperature is low with reaction time is long and high temperature with less reaction time. It is step by step process. In chemical hydrolysis there is minimum degradation of sugar and maximum sugar yield up to 100% can be achieved. Factors like pH, temperature acid concentration and reaction time can help to achieve better results [15].

**ENZYMATIC HYDROLYSIS**

Enzymatic hydrolysis of cellulosic material is carried out by highly specificenzymes like cellulase and hemicellulase. Cellulase which catalyse the hydrolysis of cellulosic biomass into monomeric sugars are mainly mixture of endonuclease, exonucleases cellobiohydrolases and B. glucosidases Exonucleases cleave end chain of cellulose to release soluble cellobiose [3]. Endonuclease attacks on internal region of cellulose, break B: 1,4 glycosidic linkage to produce new chain end which depolymerized the structure of cellulose. B- glucosidases and xylenesattack hemicellulosic backbone results in production of xylose monomers. As this process is performed at mild conditions like (pH 4.8 and temperature 40-50°C) it doesn't have corrosive problem hence utility cost of this method is very leas compared to other hydrolysis approaches. Microorganisms such as bacteria and fungi can be used for cellulase production. Microorganisms used in this can be aerobic anaerobic, mesophilic and thermophile. Some of the microorganisms which are used for the production of enzymes are aspergillus Niger, clostridium streptomyces penicillium spp., Aspergillus nidulane, gracibacillus species and Tricoderma reesei. This general procedure of pre-treatment of biomass for degrading lignin and hemicellulose and further enzymatic saccharification leads to production of simple unit of sugar. Among all the microorganisms tricoderma is most widely accepted for production of hydrolytic enzymes like cellulase and hemicellulase, pH, temperature, substrate concentration, and cellulase activity are all factors that influence the enzymatic hydrolysis process [3] (Figure 1).

***Fermentation***

Ethanol fermentation is also known as alcoholic fermentation, it is a biological process which involves variety of microorganisms, either bacteria, yeast fungi which converts simple sugar like

 

glucose, fructose xylose into cellular energy providing ethanol, lactic acid and carbon dioxide as by products. Overall chemical reaction involved in the process of fermentation is: **C6H12O62C2H5OH + 2CO2** [16].

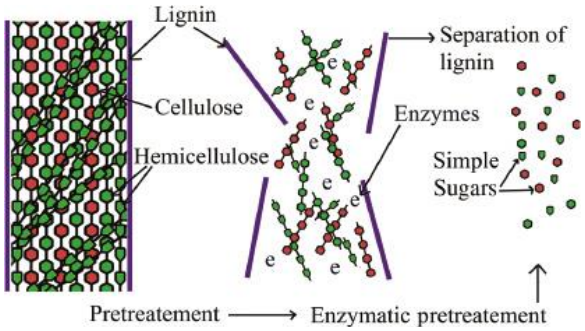


**Figure 1.** Enzymatic hydrolysis.

In alcohol fermentation, one mole of glucose yields two moles of ethanol, two moles of CO2 and two moles of ATP.Co sugar fermentation method were known to people for about 6000 years ago. Egyptians, Sumerians used this process for fermentation for making beer from grain starch. In the end of 19 th century, it becomes possible for conversion of C5 sugars into ethanol Among all the microorganisms *saccharomyces cerevisiae* is the most accepted microbe as it provides high ethanol yield and has high tolerance limits [17].

***Methods used for fermentation***

*Submerged Fermentation*

Submerged fermentation is an efficient technique of producing biomolecules that involves submerging enzymes and other reactive substances in a liquid which can be alcohol, oil, or any nutritional broth. Submerged Fermentation (SmF/Liquid Fermentation (LF) uses free- flowing liquid substrates like broth and molasses for fermentation [18].

Submerged fermentation occurs in many commercial media when a microbe grows as a suspension in a liquid medium containing various different nutrients that are either dissolved or suspended as particulate particles [19]. The growth and development of microorganism in a liquid broth is referred as submerged fermentation. This liquid broth is rich in nutrients and is used to make industrial enzymes, antibiotics, and other goods. A specialised microbe, such as fungus, is placed in a very small closed flask containing the rich nutrition broth in this method. The process also necessitates a large amount of oxygen [20].

*SHF (Separate Hydrolysis and Fermentation)*

In this type of fermentation saccharification and fermentation and saccharification occur in individual vessel, increasing number of vessels make the process uneconomical In SHF, carbohydrates like cellulose or cellobiose have impact on inhibition of enzyme such as cellulose cellobiose, therefore prevent end product inhibition B-glucosidase is added during commercial cellulose preparation. Addition of B glucosidase plays a major role in end product inhibition and have negative impact on saccharification [21].

*SSF (Simultaneous Saccharification and Fermentation)*

It is a stage process in which saccharification and fermentation of sugar is carried out in same reactor vessel. Residual sugar and cellulose are produced simultaneously in the same reactor. [22]. Saccharification involves enzymatic reaction which is performed around tamp of 50 C, where as in fermentation temperature is maintained between 28 37°C. Some advantages of this method are: lower requirement of enzyme, less reactor volume, processing time isles, high yield of ethanol, potential to enzymatic inhibition by simultaneous removal of end product. Thermo tolerant and ethanol genic yeast

 

Can effect the efficiency of the SSP. Sugarcane bagasse is used commonly as protein enriched animal feed by SSF using yeast and fungi. SSCF (simultaneous saccharification and co fermentation) is a process under SSF. In SSCP no feedback inhibition takes place [22].

*(c) SSCF (Simultaneous saccharification and co-fermentation)*
This unit involves the synthesis of pentose sugars during hydrolysis and saccharification. Since conventional strains of Saccharomyces cerevisiae are unable to degrade pentose sugars, a genetic modification of the fungus capable of synthesizing xylose is commonly used [23] Like SSF, SSCF reduces cost, increases ethanol production is increased, and shorter reaction time Furthermore, since most bacteria consume xylose, SSCF increases the concentration of xylose and glucose and reduces the inhibition of the enzymatic hydrolytic process of sugars [24].

*CBP (Consolidated Bioprocessing) CBP connect different stages of Bioethanol production like enzyme production*
Enzyme saccharification and fermentation of sugar. Some of the advantages of using CBP process are: reduction in capital investment, higher hydrolysis efficiency, risk of contamination is very low, minimum toxic compounds are formed, tolerance for high level end product, co- fermentation of sugar with minimal carbohydrates degradation Cardona [25].

***Fermentative microorsanisms***
Microorganisms which have high fermentative activity and show the protuberance are considered to be most appreciated for Bioethanol production. Fermentative microorganisms should be resistant towards environmental stressors, should provide high yield of ethanol they should have the potential to grow on different media so can be utilised effectively in xylose metabolic pathway. They can be easily obtained from sources such as distillery waste and can be recultured. Some researchers have focused specifically on isolation, identification and further evaluation of different strains that can produce ethanol from xylose, glucose and other sugar. Suitable microbial strains are selected on the basis of behaviour in media, in presence of inhibitory product and after pre-treatment methods [26].

*Saccharomyces cerevisiae*
*Saccharomyces* is derived from a Latin word saccharon means sugar and mice's means fungus so combined sugar- mold or sugar- fungus All the strains of *Saccharomyces cerevisiae* can easily grow in aerobic conditions on sugars like glucose, fructose, maltose but failed to grow on lactose and cellobiose. *Saccharomyces Cerevisiae* unable to use pentose sugar as it doesn't generate Specific enzyme for its metabolism and it's pentose phosphate pathway is not able to work properly [27].

*Zymomonas mobilis*
*Zymomonas mobilis* is a gram-negative bacterium which facilitates on anaerobic conditions. It uses enter doudoroff pathway for degradation of sugar into pyruvate which is fermented further for production of Bioethanol. Glucose, fructose, and sucrose are simple carbon sources for fermentation of Zymomonas mobiliz.It has more potential than yeast in some aspects: It has higher intake of sugar and yield is high. Less biomass is produced.High tolerance for ethanol It does not require addition of oxygen during fermentation. Xylose and arabinose, two C5 sugars in lignocellulose biomass, are not fermented by wild type Zymomonas mobilis . Poisonous inhibitors phenols, found in lignocellulosic material, such as acetic acid and are toxic to it.

*Tricoderma reesei*
*T. reesei* is a mesophilic filamentous fungus. It's a fungal anamorphic of Hypocrea jecorina. *T. Reesei*may produce a variety of cellulolytic enzymes (celluloses and hemi cellulases). Technologically, microbial cellulases are used to convert cellulose—an essential component of plant biomass—into glucose. During 2nd world war *T. reesei* isolate QM6a was isolated from the Solomon Islands due to its deterioration of US army canvas and clothes. Because of its ability to produce cellulase, it is an essential

 

commercial and industrial microbe Since its discovery many strains of *T.reesei* have been created with a focus on enhancing cellulase production [26].

*Klebsiella oxytoca*

Like Klebsiella pneumoniae , Klebsiella oxytoca is an indole-positive rod-shaped, Gram-negative bacterium. In addition, it has unique growth properties; For example, it is increased in melezitose but not in 3-hydroxybutyrate. The term Bacillus oxytocus perniciosus (from the Greek oxus meaning acid, and tokos meaning creative) was first coined in 1886 when the bacterium was isolated from sour milk [28]. *Klebsiella oxytoca* has showed potential in the manufacture of industrial ethanol fuels, and it is mentioned in patents filed by Nanologix, Inc. As being utilised to make hydrogen [29].

## CONCLUSION

One of the big challenges in engineering is to transition the transportation sector away from petroleum and gasoline and toward more environmentally friendly, renewable and sustainable energy sources like second generation bioethanol. Advancements in the pretreatment. Enzymatic hydrolysis, and fermentation phases are necessary to enhance the cost-effectiveness of bioethanol production and to make transition from the laboratory to the industrial or commercial level. One of the most essential objectives is to improve the fermentation process efficiency, so that all sugars (pentoses and hexoses) generated during the pretreatment and hydrolysis stages are fermented into ethanol. Technical obstacles to second-generation biofuel production include biomass composition. Inhibitor formation during presscharification treatment ethanol accumulation, osmotic and oxidative stress, and end-product inhibition. Progress is underway, and the resolution of these technical barriers is imminent, paving the way for the optimization of the biochemical method used in synthesizing second-generation liquid bioethanol.

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