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FEEDSTOCKS AND PRETREATMENT METHODS FOR BIOETHANOL PRODUCTION

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Abstract

The increasing global energy demand and depleting fossil fuels lead to the search for fuel alternatives. Biofuels produced from renewable biomass sources have received great attention in recent years. Bioethanol is a fuel alternative or fuel blend that can be produced by the fermentation of sugars. Cellulosic and lignocellulosic biomasses are considered as important feedstock for the production of biofuel. Lignocellulosic biomass materials are widely available in nature which may include forest residues, rice straw, barley straw, sugarcane baggase etc. The recalcitrant nature of these renewable resources is the primary bottleneck in bioenergy production. This has to be overcome by suitable pretreatment methods. Prevalent pretreatment strategies involve physical, chemical and biological methods. Pretreatment methods are followed by hydrolysis, fermentation and distillation. This paper reviews mainly on different biomass sources and pretreatment methods that can be used for the production of bioethanol.

Keywords: bioethanol, pretreatment, biomass, lignocellulose, fermentation

Introduction

Energy crisis is one of the major problems facing the world and fossil fuels contributes over 85% of the world's energy supply. Fossil fuels like petroleum, coal and natural gas are being utilized at a rapid rate. They are gradually depleting and will be unavailable in the near future. It causes adverse effects on the environment such as release of carbon dioxide which results in global warming. It also causes release of gases such as sulphur dioxide, nitrogen oxides, methane etc which causes environmental damage directly or indirectly. These make a great concern about environmental pollution and lead to the search for alternative sources of fuels.

Biofuels have been found to be one promising solution to the declining reserves of fossil fuels [22]. Biofuel can be defined as fuels that are made from biological origin such as plants, which can be used as a substitute for petroleum-based fuels. Bioethanol has been trusted as the most promising biomass-based fuel. According to Renewable Fuel Association, the global bioethanol production is approximately 26 billion gallons in 2020, among which US and Brazil contributes over 80% [6]. Bioethanol is a renewable energy resource, environmental friendly and it significantly contributes to reducing pollution. Liquid biofuels like bioethanol can be used as an excellent transportation fuel as well as a blending agent [15]. So, they are considered as sustainable alternative to improve the energy security.

Microorganisms are good candidates that are capable of converting different types of feedstocks into bioethanol through the process of fermentation. There are two types of fermentation. One is

separate hydrolysis and fermentation and the other is simultaneous saccharification and fermentation. SSF processes are preferable because of their low operation cost, low enzyme requirement and higher productivity [14].

Feedstocks and microorganisms

Bioethanol production is divided in to four generations based on the feedstock used. First generation biofuel is mainly from food crops such as corn, wheat, cassava, soybean, sugarcane, etc. They have the limitation of compatibility with food supply. They contain high level of starch. Most common feedstock in United States is corn, in Canada both corn and wheat and in Brazil, sugar cane. First generation feedstocks were criticized as it is used as food material for human as well as animals. Second generation biofuel focus on lignocellulosic biomasses which is obtained from agricultural and forestry waste. It reduces the conflict with feeding purposes [6]. Third generation biofuels include algal biomass. Algae are high energy renewable resources, having a higher growth yield which relies on the lipid content of the microorganisms. There are some geographical and technical challenges associated with algal biomass [32]. Fourth generation biofuels utilize genetically modified algae for producing bioethanol.

The sugars present in the feedstocks for bioethanol production may be starch, cellulose, hemicellulose or lignocellulose. These sugars can be hydrolysed and further transformed into bioethanol through various processes [27]. Starch is a major storage form of carbohydrates in plants, which is made up of amylose and amylopectin. The starch based raw materials for bioethanol production include maize, cassava, potato etc [15,21]. *Saccharomyces cerevisiae* and *Zymomonas mobilis* possess a high potential and production of bioethanol from cassava peels using these microorganisms have been reported [20].

Lignocellulosic biomass is one promising renewable resource which may include agricultural residues, forestry wastes, ground nutshell, wood chips, wheat, rice straw, sugarcane baggase, bark residues, grasses etc. [12,34]. Lignocellulosic biomass is mostly composed of cellulose, hemicellulose and lignin [31,37]. Cellulose is a structural linear polysaccharide which is the primary component of lignocellulose. Cellulose accounts for around 30-50% dry weight of lignocellulose [8]. The framework of cellulose is formed by D-glucose subunits linked by glycosidic bonds [12]. Cellulose is highly bio compatible and hydrophilic [39]. The hydroxyl groups are cross linked by hydrogen bonds and Vander Waals forces, and they form microfibrils which give high tensile strength. Hemicellulose is an amorphous heteropolysaccharide composed of pentoses and hexoses. Due to its non-crystalline nature, hemicelluloses are readily degradable. Another component in lignocellulose is lignin. Lignin is a complex, amorphous, aromatic, heteropolymer which is insoluble in water [31,40]. Lignin gives strength and rigidity to plant cell wall. The presence of lignin makes the lignocellulosic materials more recalcitrant in nature.

The production of bioethanol from lignocellulosic biomass involves pretreatment, hydrolysis, fermentation and distillation. The recalcitrance of lignocellulose has to be changed to enhance the sugar yield. This can be facilitated by pretreatment of the raw material. This also helps to reduce the crystallinity of cellulose [31]. Hydrolysis is the process of conversion of cellulose to fermentable sugars. Microorganisms ferment these sugars into alcohol. These are then recovered by distillation [20].

Bioethanol was produced from sugar cane leaf waste and also studied the effect of various pretreatment methods and fermentation kinetics [28]. Plantain peel extract was used as the carbon source and *Chlorella vulgaricus* was used for the synthesis of bioethanol [25]. Stems of sweet sorghum were used as the feed stock for production of bioethanol and sweet sorghum gave high sugar yield per stem than grain sorghum [29]. Kenaf or *Hibiscus cannabinus* was evaluated for bioethanol production by using dilute sulphuric acid for pretreatment followed by simultaneous saccharification and fermentation [23]. Water -hyacinth can be used as a suitable substrate for biofuel production [10]. Brewer's spent grain is a low cost feedstock that can be fermented by *Fusarium oxysporum* [35].

Most yeast cells have the ability to convert hexose sugars to ethanol. Among these, Saccharomyces cerevisiae is the most widely used microorganism for fermentation. It is a facultative anaerobic organism and possess high ethanol tolerance [9]. Bioethanol production was experimented with *Bacillus stearothermophilus* and *Saccharomyces cerevisiae* using ground nutshell waste as substrate. An ethanol yield of 16.11% was obtained with 2% ground nutshell waste at 50 degrees

Celsius and simultaneous saccharification and fermentation was performed. The microorganisms were isolated from the soil of rice field at Lovely professional university campus, Jalandhar [5]. Twelve yeast isolates from different rotten fruits were isolated and conducted pre-screening using yeast glucose chloramphenicol agar. Experiments were conducted mainly to explore the various sources of yeasts [17].

Studies have been done on maximising the value-added products and minimising the waste emission using lignocellulosic biomass [6]. Bioethanol production was optimized by modifying culture conditions of yeast used. Batch culture fermentation was carried out for 72 hrs. The level of ethanol produced by alcohol yeast and commercial baker's yeast was compared using sugarcane molasses as media under aerated culture conditions. The level of ethanol production by alcohol yeast was 74.8g/L and commercial dry yeast produced 102.854g/L without aeration and 120.917g/L with aeration. It is shown that Baker's instant dry yeast produced high ethanol compared to alcohol yeast [16]. The performance of *Saccahromyces cerevisiae*, *Pichia stipites*, *Zymomonas mobilis* using coconut fibre as the raw material was analysed and compared and evaluated the raw material using simultaneous saccharification and fermentation (SSF) and semi-simultaneous saccharification and fermentation (SSF) [3].

Pretreatment methods

These lignocellulosic biomasses have to be first pretreated before undergoing fermentation. Pretreatment is carried out to make all the fermentable sugars available for metabolism. There are different pretreatment methods available for the degradation of lignocellulose. They include physical, chemical, physico chemical and biological methods.

Physical methods

Physical pretreatment methods include milling, ultrasonication, extrusion, microwave assisted size reduction, freeze pretreatment etc.[36]. Particle size of the lignocellulosic biomass can be reduced up to 0.2mm by milling. Reduction in particle size leads to high surface area which enhances the further reactions. The principle of ultrasonication is based on the generation of ultrasound waves which result in cavitation. This pretreatment depends on the duration of sonication, ultrasound frequency etc. Extrusion is a physical pretreatment method in which disruption occurs due to high temperature and shear forces [39].

Physico-chemical methods

Physicochemical pretreatment methods are steam explosion, liquid hot water method, ammonia fiber explosion etc. Steam explosion uses less chemicals and has low environmental impacts. Lignocellulosic material is exposed to liquid ammonia under high temperature and pressure in ammonia fiber explosion method [31].

Chemical methods

Acid pretreatment, alkali pretreatment, pretreatment with ionic liquids, pretreatment with deep eutectic solvents etc are some of the chemical pretreatment methods. Alkali treatment is the widely used chemical pretreatment method in which the reagents used are sodium hydroxide, potassium hydroxide, calcium hydroxide etc. [39]. The basis of alkali treatment is the saponification reaction and there by the cleavage of linkage between the polymer molecules.

In acid pretreatments, both organic acids (eg: Oxalic acid, citric acid,) and inorganic acid (eg.sulphuric acid, phosphoric acid) may be used. Formic acid was used for chemical pretreatment of various biomasses and lignin was extracted [38]. Acid hydrolysis was carried out as the first pretreatment method for bioethanol production from okra (Hibiscus esculentus) stalk, using yeast (*Saccharomyces cerevisiae*) [22]. The raw material peanut was pretreated with dilute sulphuric acid and also in combination with steam and was optimized through Box-Behnken design[13]. Rice hulls were pretreated with deep eutectic solvents using either conductive heating or microwave heating [1].Pretreatment with deep eutectic solvents was found effective for the degradation of lignocellulose from pineapple leaves[26].

Biological methods

Biological pretreatment methods are environmental friendly methods which uses micoorganisms or enzymes. white rot fungi, brown rot fungi, soft rot fungi etc have been found capable for the pretreatment of lignocellulosic biomasses. Lignin can be degraded using enzymes such as laccase, manganese peroxidase, lignin peroxidase. The fermentation using *Saccharomyces cerevisiae* under anaerobic condition was conducted using banana peel as the substrate. The banana peels were pre-treated with sulphuric acid, ionic liquids, cellulase enzymes and all the methods have been found efficient to hydrolyse cellulose and hemicellulose [19]. A preliminary study on pre-treatment and fermentation on areca nut husk was conducted. Fungal pre-treatment by cellulolytic fungi was found to be more effective for ethanol production and areca nut husk was found to be a suitable substrate. The areca nut husk fibers consist of cellulose, hemicellulose and lignin [2]. Water hyacinth can be used as a substrate for bioethanol using simultaneous saccharification and fermentation process. Acid pre-treatment and enzymatic hydrolysis were preferred due to effective production of sugars at optimal conditions [30]. Microbial pretreatment using *Aspergillus flavus*, *Phanerochaete chrysosporium* and coculture has been carried out on areca nut husk. Fermentation was carried out using *Zymomonas mobilis* NCIM 2915. Overall yield of bioethanol was found to be very high with *Aspergillus flavus* and *Phanerochaete chrysosporium* NCIM 1197[2]. The ability of white rot fungi *Phanerochaete chrysosporium* to produce lignocellulolytic enzyme for the degradation of wheat straw was also studied [4].

Pretreatment of coconut fibre mature was carried out by hydrothermal pretreatment catalyzed with sodium hydroxide followed by fermentation using *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Pichia stipites* [3]. Two cellulase producing strains *Aspergillus niger* and *Aspergillus flavus* were identified which can hydrolyze lignocellulosic materials to sugar units and bioethanol was produced using immobilized *S.cerevisiae* [7].

SI. No:	Feedstock	Microorganism	Pretreatment	Reference
1	Areca nut husk	Yeasts and <i>Zymomonas mobilis</i> NCIM 2915	Aspergillus flavus, Phanerochaete chrysosporium NCIM 1197 and co- culture	[2]
2	Delignified coconut fibre mature	Saccharomyces cerevisiae, Pichia stipitis and Zymomonas mobilis	Hydrothermal pretreatment catalysed with sodium hydroxide	[3]
3	Groundnut Shell	Bacillus stearothermophilus, and Saccharomyces cerevisiae	Steam explosion, inorganic chemicals, organic chemicals	[5]
4	Empty Fruit bunch	Dry yeast	Sodium hydroxide	[14]
5	Banana peels	Yeast culture	Sulphuric acid, ionic liquids, cellulase enzyme	[19]
6	Cassava Peels	Saccharomyces cerevisiae and Zymomonas mobilis	Pasteurization in a hot water bath	[20]
7	Hibiscus cannabinus	Yeast	Dilute sulphuric acid	[23]
8	Sugarcane leaf waste	Saccharomyces cerevisiae BY4743	Steam-salt-alkali method, microwave assisted salt alkali method	[28]
9	Water hyacinth	Saccharomyces cerevisiae	Sulphuric acid, sodium hydroxide, microwave-alkaline combined pretreatment	[30]
10	Wheat straw	Pichia fermentansMTCC189	White rot fungi	[4]

Tab1: Feedstock and pretreatment

Conclusion

Lignocellulosic biomass represents an important feedstock for biofuel production. They are cheap, highly available and offer possibilities for industrial scale production of bioethanol. In order to increase the cost effectiveness pretreatment, hydrolysis and fermentation stages requires improvement. Bioethanol production by yeast fermentation is found to be a realistic approach. It is expected that technical barriers could be overcome leading to economic production of second generation biofuel.

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COMPUTATIONAL ANALYSIS, SCREENING, AND EXTRACTION OF BIOACTIVE FROM PLANT SOURCES TO COMBAT PLAQUE FORMATION

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Abstract

Periodontal disease causes infection of the tissues that hold our teeth in place. It is caused by poor brushing and flossing habits that allow plaque (a sticky film of bacteria) to build up on the tooth surface and harden further. The currently available treatment regimens use synthetic drugs, toothpaste, and mouthwashes containing artificial compounds and sweeteners which could cause side effects such as teeth staining, dry mouth, and tooth sensitivity on prolonged usage. Hence the current work is an attempt to screen for alternatives from herbal sources by screening natural compounds from selected plants like banana, garlic, and guava for the development of a natural product for Periodontal disease in the near future. The aim of this work was to understand the interaction between the bacterial community responsible for the formation of the plaque or the tartar on the tooth surface and bioactive compounds from natural plant-based sources. The natural analogs were screened using the Autodock Vina tool to identify the best binding energy against the target compounds like allicin, guercetin, and guaijaverin and exhibited good binding affinity with a value of -6.6 kcal/mol,-4.4 kcal/mol and -7.4 kcal/mol respectively. The results were further validated for the amino acid interactions using the Discovery Studio tool. Further, the crude extraction of these bioactive compounds was subjected to antimicrobial studies. Antimicrobial studies revealed a clear zone of inhibition of 13mm of guaijaverin on exposure to these compounds which shows that these compounds can serve as an oral formulation for periodontitis treatment.

Keywords: Autodock Vina, Discovery Studio tool, Gtf, bioactive compounds

Introduction

As per Global Oral Health Status Report (2022) published by WHO, severe periodontal diseases are estimated to affect around 19% of the global adult population, representing more than 1 billion cases worldwide. The main reason for these diseases is dental plaque. Dental plaque is the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin. Once a tooth surface is cleaned, a conditioning film of proteins and glycoproteins is adsorbed rapidly to the tooth surface. As the biofilm develops, gradients in biologically significant factors develop, and these permit the co-existence of species that would be incompatible with each other in a homogenous environment. Dental plaque develops naturally, but it is also associated with two of the most prevalent diseases (caries and periodontal diseases) affecting industrialised