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Bioethanol Production From *Brassica Napus* Biomass And Pineapple Waste

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Abstract

Crude oil consumption has increased exponentially in last few years across the globe. Mostly the transportation services around the world contribute for rise of the petroleum usage and it accounts for around 60%. As a result, these natural oil resources are getting depleted and fear of running out of oil is a great problem for rapidly growing countries like India. The alternative energy source needs to be renewable and sustainable for several reasons. Firstly, to reduce the effect of the greenhouse gases including carbon dioxide. Secondly, to ensure that the energy source does not compete with production of food. Bioenergy which is derived from agricultural biomass that is available in very large quantities is gaining importance these days.85,000,000,000 liters of bioethanol was produced in 2011 shows the considerably increasing interest of the industries to produce bioethanol. Biofuels represents an alternative to petroleum based fuel. Now a day's many countries are producing biofuels, main producers are United States of America with approximately 18.3 billion liters followed by Brazil producing 17.5 billion liters and China with 1 billion liters annually. Today the raw material used for

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bioethanol is mostly sugar or starch based. These raw materials are referred to as first generation (1G) bioethanol. However, they are also used in the food sector and also it demands for use of farming land which is dedicated specifically for food crops which makes the material questionable for bioethanol production. There is also the 2nd generation (2G) bioethanol, which is generated from lignocellulosic material such as agricultural waste. Countries like United States, Brazil are scaling up the production of bioethanol by keeping the biomass needs in the account, so that the lignocellulosic biomass will be available in enough amounts for higher yields and production value.

Keywords: Fossil fuel, biofuel, frmentation, lignocellulosic biomass, agricultural waste.

Introduction

Crude oil consumption has increased exponentially in last few years across the globe. Mostly the transportation services around the world contribute for rise of the petroleum usage and it accounts for around 60%. As a result, these natural oil resources are getting depleted and fear of running out of oil is a great problem for rapidly growing countries like India. In addition, oil prices are also increasing, emission of greenhouse gases and resultant climate change demand for alternative source of fuel. The alternative energy source needs to be renewable and sustainable for several reasons. Firstly, to reduce the effect of the greenhouse gases including carbon dioxide. Secondly, to ensure that the energy source does not compete with production of food. [1].

Bioenergy which is derived from agricultural biomass that is available in very large quantities is gaining importance these days.85,000,000,000 liters of bioethanol was produced in 2011 shows the considerably increasing interest of the industries to produce bioethanol [2, 3]. Biofuels represents an alternative to petroleum based fuel. Now a days many countries are producing

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biofuels, main producers are United States of America with approximately 18.3 billion liters followed by Brazil producing 17.5 billion liters and China with 1 billion liters annually [4, 5,]

Bioethanol which is an ecofriendly renewable alternative to fossil fuel and is being used most widely as transportation fuel in E85 engines as 85% ethanol and 15% petrol blend in countries like Brazil and United States (U.S. Department of Energy). There are a lot of reasons that make industrialized and developing nations to think about biofuels and consider them as revolutionary techniques. [6, 7]. Bioethanol concept is very important because the world needs a source of clean energy which mean very less pollution by combustion [8, 9, 10]. It has high octane number and has potential to decrease emissions of gaseous pollutants such as carbon monoxide (CO) and cancer causing volatile organic compounds (VOCs). [11]. However, bioethanol has very low energy content when compared to traditional petroleum fuel, its higher oxygen content helps in cleaner combustion and hence lowers the release of hazardous and toxic substances [12]. Use of biofuels can reduce greenhouse gases releases by approximately 30 to 85 % if compared to fossil fuels mainly petroleum.

Today the raw material used for bioethanol is mostly sugar or starch based. These raw materials are referred to as first generation (1G) bioethanol [13]. However, they are also used in the food sector and also it demands for use of farming land which is dedicated specifically for food crops which makes the material questionable for bioethanol production [14]. There is also the 2nd generation (2G) bioethanol, which is generated from lignocellulosic material such as agricultural waste. One side effect with 2G bioethanol is the difficulty accessing the sugars in the biomass, making the process more challenging. [13]. Countries like United States, Brazil are scaling up the production of bioethanol by keeping the biomass needs in the account, so that the

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lignocellulosic biomass will be available in enough amounts for higher yields and production value.

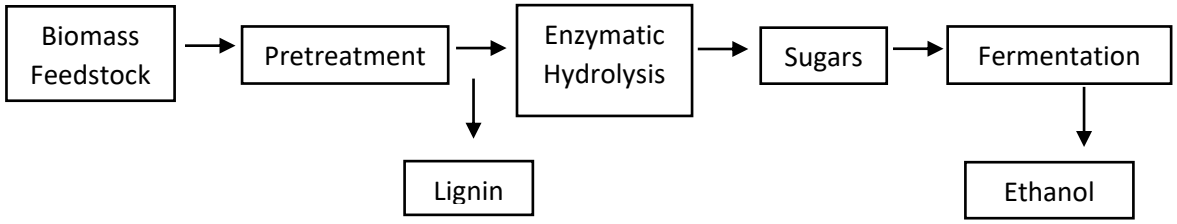


Figure 1: This flowchart represents the standard configuration of bioethanol production

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Now there are many companies that are looking into the large scale production of the bioethanol from lignocellulosic wastes from various resources. But there is problem with large scale or commercial productions, the industry cannot be dependent on one type of biomass because at particular time some biomass may not be available for the processing and consistency of the biomass is another factor. The industry must be able to utilize different types of the feedstock only then the process will be feasible. Availability of biomass near the bio-refinery is a must, because if the biomass is to be transported from a far area or agricultural land then it can add up the cost of the overall process which is not economically favorable. In the case of lignocellulosic feed or biomass the sugars content needed for bio-ethanol production is obtained mainly from hemicellulose and cellulose fibers. The type of sugars content in the cellulosic feedstock depends upon the structural properties of the biomass. Difference in chemical composition of the biomass can significantly affect the yield of substrate during pretreatment stage which translates into reduced yield in the final stage of fermentation. Different enzymes may be required in accordance with the biomass type so the pretreatment methods also vary from biomass to

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biomass and quality of the end product is also important in terms of purity. Enzymes such as ligninase, cellulase, xylanase etc. are very important for utilization of the lignocellulosic biomass to release simpler sugars. Delignification is very important part of pretreatment, if the biomass loaded for enzymatic saccharification has more solid residues it means more amounts of the lignin is present because it reduces the rate of hydrolysis by enzymes acts as a physiological barrier as it prevents the degradation of degradable portions of the substrate [15].

Bioethanol production from different feedstock

Agricultural Feedstock

The production of bioethanol from lignocellulosic feedstock such as agricultural waste is very good in terms of cost effectiveness, renewability and abundance. Lignocellulosic waste refers to plants waste and comprises the cellulose fibers, hemicellulose and lignin. These can be categorized as wood residues, grasses, paper industry waste, agricultural residues (which includes stalk and straw, tree peelings, wood cobs, nutshells, seedsand bagasse), food industry waste etc. There are some crops such as rice, maize, wheat and sugarcane are the crops having maximum product yield and have maximum area for cultivation as well. These yields are in charge of creating larger part of lignocellulosic biomass in rural segment and rest of the agricultural wastes are comprises just a minor extent of all out waste generation on the planet.

Sugarcane plays a great role in world's sugar production so it also produces enormous amounts of agro-waste in form of sugarcane bagasse, so it can also be used as source for lignocellulosic biomass for production of 2G bioethanol. Rice production generates an enormous amount of the waste such as leaf-blades, stem,leaf-sheaths and remaining part after threshing of panicle. Rice straw is the most plentiful lignocellulosic material on the planet. The yearly production of rice straw is 731,000,000 tons and Asian nations alone generate 667,700,000 tons.

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Mustard and Rapeseed (*Brassica napus*) crops are grown worldwide as per oil needs. About seventy percent of the complete plant is considered as agro-waste waste after extracting the oilseeds and is not being used as the feed for cattle also, either way it is abandoned in the field for natural degradation by microorganisms or burnt to ashes which results in environment pollution. Mustard agro-waste constitutes of cellulose (48.5%) and hemicellulose (29.6%) which is more than commonly used feedstock wastes such as rice straw (hemicellulose 24.7%, cellulose 28.5%) and wheat bran (hemicellulose 27.2%, cellulose 30%,) [16]. In India alone 7,977,000 tons of mustard and rapeseed oilseeds were produced in 2016-2017 (Department of Agriculture, Cooperation and Farmers Welfare, India).

Fruit Waste

The fruit wastes like pineapple peels, banana peels [17], mangoes [18], papaya [19] were used for bio-ethanol production. The fruits biomass peel can produce fuel grade ethanol. Banana and citrus fruits are important natural product harvests of the planet and Indians alone added to 24% and 8.5% of the aggregate production respectively. These both leave enormous amount of residues as peel, seed, pulp and so can be used as biomass for bioethanol production and disposal of these residues is a great problem and leads to environment pollution [20].

Pineapple waste is also a great source for bio-ethanol production. Pineapple waste is rich in sugars and lignocellulosic components. Pineapple (*Ananas*comosus) is most used and etable member of family Bromeliaceae. The 3rd most favored squeeze after orange and squeezed appleis pineapple juice. Pineapple byproducts consists of basically the remaining pulp, peel, leaves and stem. Increasing production of pineapple processed items, results in huge waste generation and this waste can be utilized as biomass for bioethanol production. [21]. Thailand has many pineapple industries and has turned into the fourth biggest maker and exporter of

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pineapple on the planet [22]. There yearly production is 179,000,000 tons [23]. Thailand has seventy-five companies that generates two hundred tons of waste in 24 hours [24].

Upadhyay *et al*. carried out the compositional analysis of pineapple wastes [25]. The cellulose, hemicellulose, lignin, reducing sugars, non-reducing sugars, moisture content and pH. The pineapple peel waste reported to have a moisture content (92.2%), cellulose (19.8%), and hemicellulose (11%).

Steam explosion of orange strips results in significant removal of limonene and hydrolysis of cellulose and hemicellulose fibers. The hydrolysate generatedby the blasting the steam is valuable for yieldingthe bioethanol from *Saccharomyces cerevisiae*. Amount of the bio-ethanol produced was 4.1% w/v [26]. Santi *et al.* (2012) utilized the orange peel waste, a solid residue of orange juice production for bioethanol production [27].

Bioethanol production from agricultural feedstock is a great utilization of waste but there are some challenges and limitations for example, dealing withand transport of the lot of biomass to the plant where it will be pretreated and finally fermented to produce bioethanol. Proficient pretreatment strategies should be implemented for delignification of lignocellulosic biomass. Amount of fermentable sugars can be enhanced using a proper pretreatment strategy and will result in better and efficient process [28].

Characterization of feedstock

Agricultural feed stock mainly consists of organic materials such as carbohydrates, fats, proteins and other polysaccharides such as cellulose fiber, hemicellulose and lignin etc. The measure of these components changes with the type of waste or feedstock. Biomass such as hardwoods, wood blocks, peels of the higher trees has much more complex packaging of the cellulose fibers in the matrix. On the other hand soft wood of the plant biomass such as dried and dead leaves

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have less crystalline structure. Sugars content varies in fruit wastes such as fruit peel, plant residues etc. as compared to non-fruit plants.

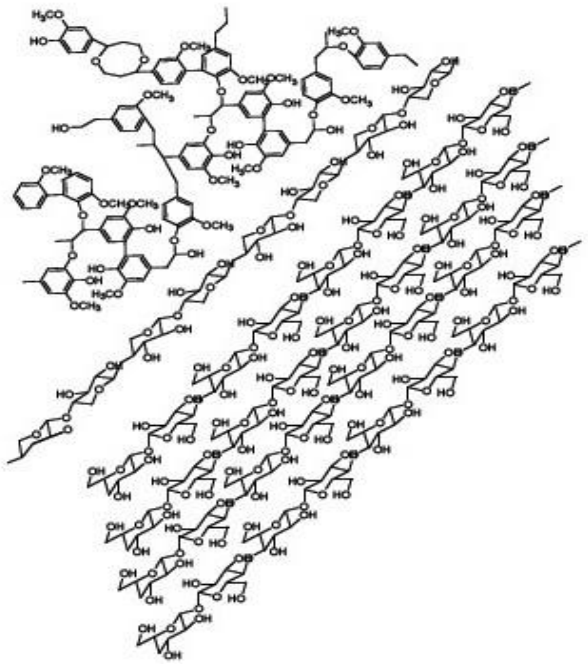


Figure 2: The lignocellulosic biomass contains lignin ranging from 11-25%, hemicellulose 8-40%, cellulose 30-57% respectively as shown in fig. above.

Source: https://biorrefineria.blogspot.com/2016/04/biorefinery-models-lignocellulosic.html

Pretreatment of the feedstock

There is a process called pretreatment which is very essentially required to utilize the lignocellulosic biomass by degrading available polymers. Pretreatment of biomass waste is most common, yet the most difficult and time-consuming task in whole procedure of biofuel generation from feedstock waste and comprises over forty percent of total production cost. The biomass composition is mainly the fibers or polysaccharides such as cellulose, lignin and hemicellulose. Pretreatment involves procedures for example, solubilization and partition of at least one of the components from the waste biomass so that rest of components will be more

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effectively and efficiently pretreated by following other biological and chemical treatments. Two or three methods or combination of different methods can be used to make process more efficient and reliable. For example initially physical and after that chemical or biological, depending on the material of choice and demand of the experiments.

In lignocellulosic biomass hemicellulose and lignin fills in as a framework and cellulose makes the skeleton. During pretreatment, this rigid framework should be broken-to make it less crystalline from regular cellulose to produce amorphous cellulose, which is the most reasonable structure for enzyme to act upon. Delignification is very important for the further process of saccharification and fermentation. The saccharification of cellulose yield after pretreating the biomass normally surpasses 90 % theoretically which when compared to the biomass without pretreatment is only 20% [29]. After pretreatment of the biomass the simpler sugars become available for easy conversion to bioethanol by microorganisms. More the amount of sugars will be released more will be the ethanol yield and results as an efficient process. Many techniques are developed around new and old concepts of biomass pretreatment which are based on physical methods, using various chemicals and biological agents such as microorganisms and enzymes which can digest the most prone sites off the treated components of the biomass. Let's take a look at the treatment effect on the structure of biomass in diagrammatic representation below:

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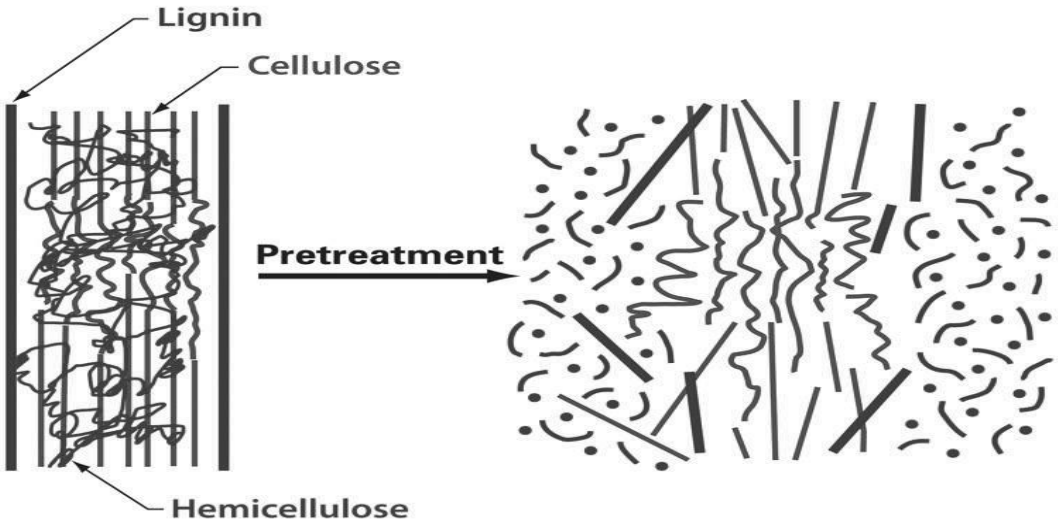


Figure 3 . Structural Changes in the biomass after pretreatment, the lignin breaks results in release of hemicellulose and cellulose fibers.

Physical pretreatment

The biomass can be reduced to fine particles by milling, grinding, shearing, chipping which increases surface area and more mass transfer that enhances the cellulase enzyme function to convert cellulose to monomeric or simpler sugars. Physical methods are more energy demanding even more than theoretical energy content available, so are not considered in large scale productions.

a) Milling

Granulating of the biomass can be achieved by utilizing the mechanical treatment method called milling which loosen the tightly bounded fibers in rigid framework. It for most part incorporates chipping, grounding andor mechanical milling. Biomass size can be diminished to 10–30 mm using chipping technique while milling and grounding can lessen the size of biomass upto 0.2 mm. However, researchers found out that 0.4 mm particle size has no noteworthy impact on speeding up the process of saccharification by enzymes [30]. These techniques can be utilized

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for improvement of heat transfer and mass transfer because these physical methods exert great shearing force to reduce rigidity. The increase in specific surface zone, last level of polymerization, and the net decrease in cellulose rigidity can be accomplished by using different sorts of milling processes example, colloid milling, hammer processing, two-roll milling and vibratory processing are utilized to make improvements to degradability of the lignocellulosic biomass. Compared to normal processing, the milling process which works using vibratory ball is observed to progressively compelling in decreasing rigidity of polysaccharides and improving the edibility of spruce and aspen chips for enzymes [31].

b) Microwave

Irradiation process can be used to pretreat the agro wastes using microwave. It has some advantages for example procedure is easy to carry out, it requires less energy and has high heat capacity all of it takes very less time. It generates very minimal amounts of inhibitor substances and efficiently degrades the rigid structure of biomass. Besides, the use of diluted alkali reagents is favored for more proficient breakdown. The yield of sugars can be increased using this method in combination with alkali pretreatment. According to a study done on switch grass the alkali-microwave treatment resulted in nearly 70 to 90 percent sugars yield [32].

c) Ultrasound

A new strategy which is based on use of ultrasonic waves for pretreating the biomass. However, researchers discovered that sonicationprocessis an achievable pretreatment choice. Ultrasonic waves from the instrument produce physicochemical impacts which alter the lignocellulosic framework. Ultrasonic treatment bursts cellulose and hemicellulose divisions by making bubble cavities and ads up to the effectiveness of cellulase enzymes for breakdown into monomeric sugars.

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Chemical pretreatment methods

a) Alkali pretreatment

Alkali pretreatment is commonly used method because it have some advantages like less sugras degradation. Basic pretreatment includes the utilization of bases for example NAOH, for biomass pretreatment. The utilization of a dilute base introduces breaks in ester linkage and glycosidic side chains achieving structural change of lignin and removal of lignin. It causes cellulose units to swell and results in degradation of rigid fibers and improves the saccharification.

Sun et al. experimented on wheat straw and studied the use and effectiveness of different bases and analyzed delignification and dissolution of hemicellulose and observed that ideal operational requirement was using 1.5% sodium hydroxide, time period of 6 days at a temperature of 20°C which brought about 60% lignin discharge and 80% hemicellulose discharge.

b) Acid treatment

In this pretreatment method pure (100%) and dilute acids are used to treat biomass. At commercial level, diluted Sulfuric acid, H_2SO_4 is most commonly used for wide variety of biomass pretreatment such as switch-grass, corn stover [33] and spruce (softwood). Furfural manufacturers use this technique for saccharification process of polysaccharides, cellulose to glucose and hemicellulose to xylose monomeric sugars utilizing various enzyme systems. Some other acids have also been studied, such as HCl by Wang et al. in 2010, H_3PO_4 by Zhang et al. in 2007. Acid pretreatments have capacity of removing lignin and releasing cellulose and hemicellulose units from toughest of the biomasses. If alkali is used on acid treated biomass it will result in more amount of cellulose.

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c) Wet oxidation

O_2 acts as an oxidizer on the componentsmixed in aqueous solution. Two responses happen amid this procedure. One at low temperature and the other at high temperature which are hydrolysis and oxidation respectively. Wet oxidation Process includes drying and processing by grounding the biomass to get 2 mm sized fine particles and the water is added in proportions of 1 Liter to 6 grams of the biomass. To reduce byproducts generation, Na_2CO_3 is used. Air is made available in the vessel using pressure level of 12 bars. Oxidation pretreatment process is carried out for10-20 min. at 195°C.

d) Ozonolysis

Ozonolysis is the process mainly used for delignification of lignocellulosic raw material. Ozone treatment does not affect the hemicellulose and cellulose structure [34]. Various types of biomass can be pretreated by this method. The pretreatment of the wheat straw using this method showed 60% lignin removal which brought about 5 fold increments in the enzymatic saccharification. In the case of poplar saw-dust, the sugars yield was increased by 57%. This pretreatment has an advantage over the other methods because it is carried at surrounding temperature as well as pressure. Ozonolysisdoes not leads to any toxic releases. The major factors affecting this procedure are moisture, if the moisture is higher than lower will be the oxidation of lignin. Although it is a powerful and proficient pretreatment strategy, the use of ozone gas in higher amounts, makes it a costly process.

Physicochemical pretreatment methods

Physicochemical methods are more efficient than the more energy demanding physical methods of pretreatment. Different chemical agents used for this purpose are acids, alkali, peroxides,

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ozone and organic solvents. There are several physicochemical methods which are used for the pretreatment are discussed below [35].

a) Steam Explosion

Steam explosion is very popular and most commonly utilized hydrothermal pretreatment method to break the complex lignocellulosic matrix into a simpler structure. This treatment subjects the material to high pressure and temperatures for brief span of time and then depressurizes the system, resulting in breakage in the structure of fibrils. Temperatures of 190^{o} C for 10 minutes and 270^{o} C for 1 minute are used for this process. The exposure time and temperature decides, how much of the defibrillation will be there. If the material is exposed to steam for less time and lower temperature it results in smaller cracks in the wood and if the time and temperature it can results in complete degradation of the wood fibers. This method is used to pretreat very hard and complex biomass such as wood blocks which have very high density and mechanical strength. So they resist the degradation action by hydrolyzing enzymes and microorganisms. In these higher plant woods the cellulose is tightly embedded in the lignin and hemicellulose matrix

b) Liquid Hot Water

LHW is alike the steam explosion strategy however utilizes water at very high temperature. To maintain water's fluid state, it exerts high pressure and hence results in breakage of rigid biomass. Temperatures ranging from 160°C to 240°C and of time ranging from a couple of minutes up to as long as 60 mins are required. The slurry rich in solubilized hemicellulose and the solid fraction rich in cellulose are two products formed after this process (Pérez et al. 2008). The solubilized product consists of oligosaccharides released from hemicellulose and lignin (35–60% of absolute beginning material) and cellulose in minor amounts (4–15%). The solid fractions will be subjected to enzymatic degradation after separation.

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c) Ammonia Fiber Explosion

In this treatment method the biomass is exposed to liquid ammonia under very high pressures and temperatures ranging between 60°C and 100°Cand depressurized rapidly. This works almost same in terms of breaking the lignin-carbohydrate linkage but at lower temperature range. [36].

Biological pretreatment methods

In biological methods microorganisms and enzymes are used. The brown rot, white rot, soft rot fungus such as *Phanerochaetechrysosporium, Trametes versicolor, Ceriporiopsissubvermispora, and Pleurotusostreatus*is very commonly used for pretreatment methods.

Du et al. (2011) used white rot fungus *Iprexlacteus*for corn stalk pre-treatment and had capacity to produce many enzymes which do hydrolysis and oxidation, mostly peroxidases and laccases are those enzymes [33, 34] 82% of the hydrolysis yield was noted by this method.

Potumarthi *et al.* (2013) examined rice husk biomass by using biological pretreatmentultilizing*Phanerochetechrysosporium* [37]. Fungi was grownon the rice husk biomass andthe pretreated biomass were exposed to enzymes for saccharification.

Other than these different basidiomycetes species were additionally used for different biomass types. Among these *Bjerkanderaadusta, Fomesfomentarius, Ganoderma resinaceum* and *Lepista nuda* are most documented. The species written below have been accounted to result high lignin removal potential [34].

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Micro-organism	Biomass	Major effects	References
Iprex lacteus	Corn stalks	82% of hydrolysis yield	Du et al. (2011)
P.chrysosporium	Rice husk	_	Potumarthi et al. (2013)
Fungal consortium	Corn stover	43.8% lignin removal	Song et al. (2013)
Fungal consortium	Plant biomass	Complete elimination of hazardous chemicals	Dhiman et al. (2015)
P.ostreatus/P.pulmonarius	Eucylaptus grandis saw dust	20-Fold increase in hydrolysis	Castoldi et al. (2014)

Enzymatic saccharification of pretreated biomass

After a successful pretreatment process, there are still very large amounts of undigested polysaccharides which needs to be degraded to monomeric sugars before fermentation process. This is achieved by using different enzymes in a process called enzymatic hydrolysis. In this process, cellulose and hemicelluloses fibers are converted into simpler sugars such as glucose. The cellulase enzyme mainly exocelluases breaks the 1, 4-β-glycosidic bonds after every two to four glucose units between the cellulose chains of the biomass. Cellobiases and Beta-glucosidases further breaks the bonds to form monomeric sugars. Hemicellulase enzymes are very complex set of enzymes such as xylanase, are very important for this process to degrade hemicellulose [38]. Enzymatic hydrolysis is very budget friendly method for 2Gbio-ethanol production. It has significant yields and maintenance expenses are also low in comparison to acid or alkaline treatment [29]. But there are some issues related to enzymatic hydrolysis, It is has higher incubation times to complete the saccharification. Enzyme cost is also a factor here if we are not using a crude enzyme, temperature and presence of the phenols during the reaction can inhibit the enzyme action. However the enzymatic saccharification has benefits over the

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chemical process because it generates less amounts of toxic compounds, higher yield, less energy requirements etc.

Fermentation

Hydrolyzed biomass contains fermentable sugars in liquid phase which can be fermented into ethanol using yeast or bacteria via anaerobic metabolism. When lignocellulosic material is hydrolyzed, the hydrolysate contains pentose (C5) - and hexose (C6) sugars, as well as lignin and- lignin derivatives; in addition, also inhibitory compounds. Glucose (C6) and xylose (C5) are the main sugars fermented from the hydrolysate. Content of the sugars depends upon the biomass type and also on pretreatment method chosen. Fermentation can be carried out as simultaneous saccharification and fermentation (SSF), separate hydrolysis and fermentation (SHF) and prehydrolysis and simultaneous saccharification and fermentation (PSSF). All these fermentation types can vary in the yield of the bioethanol produced. Commonly SHF shows the most yield when compared to other configurations. In SHF process the enzymatic hydrolysis is carried out first and the liquids obtained from that are subjected to centrifugation at high speeds and then fermentation is done. In SSF, enzymatic saccharification and microbial fermentation is done in single step: the sugars product from enzymatic hydrolysis is immediately utilized by the microorganism that carry out bioethanol production. But there are some negative aspects of the SSF when compared to SHF process. Enzymatic hydrolysis is mostly favored at the temperatures higher than the fermentation process, having yeast as inoculum. In separate hydrolysis and fermentation the temperatures can be independently controlled. And another issue is that in SSF the yeast cells are very difficult to separate from lignin and residues, so it is not re-useable. Most of the current researches are focused for finding optimal conditions in terms of new technologies

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for the fermentation process and new combinations of the process for making the process less time consuming and to complement the yield of the final products.

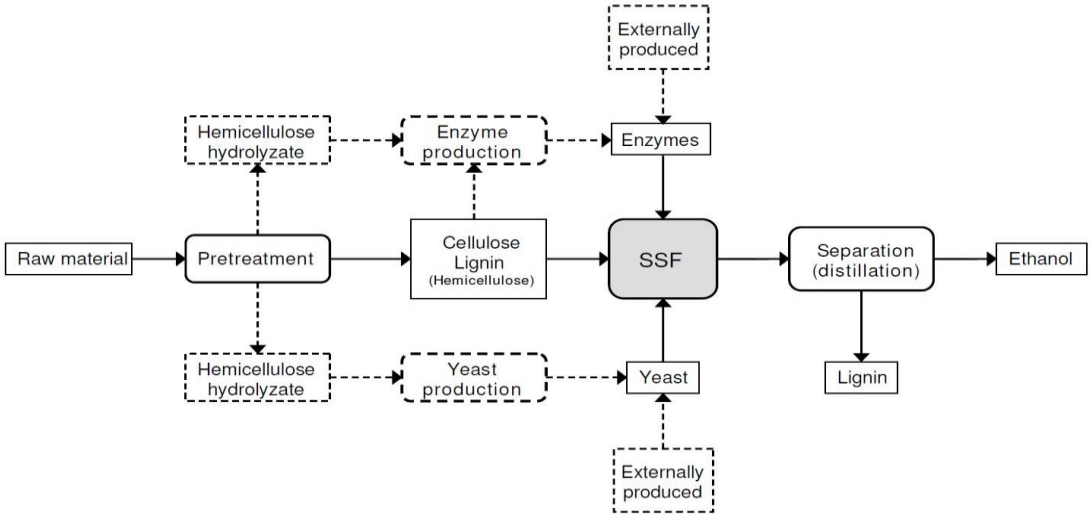


Figure 4: Steps involved in a Simultaneous saccharification and fermentation (SSF) process.

Most commonly and widely used microorganism for alcohol production is the yeast, *Saccharomyces cerevisiae* or simply known as baker's yeast. It is preferred over the bacteria because *S. cerevisiae* can work by tolerate wide range of pH but acidic environment is optimum which minimizes the chances of bacterial contamination. One more upside of using yeast is that it can tolerate higher amounts of ethanol when compared to other microorganisms [39]. In past years the researchers made significant efforts to engineer the recombinant yeast which can ferment xylose and arabinose. New strains can be developed by addition of new genes encoding for xylose isomerase from bacteria and fungus. Xylose reductase (XR) and (XDH) xylose dehydrogenase from fungus [40].

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