



Biofuel production by using ionic liquids

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Abstract

The development of large-scale production of non-petroleum-based powering fuels or alternative fuels such as bioethanol and biodiesel are due to growing concerns related to environmental pollution, energy, security, and future oil supplies. Bioethanol is made from cheap and abundant resources such as lignocellulosic biomass. Biodiesel is produced by transesterification of vegetable oils and animal fats. For efficient production of bioethanol and biodiesel from lignocellulosic biomass and vegetable oils, the pretreatment and transesterification are generally required. Environmentally harmful chemicals like alkalis, acids, or organic solvents are used in these processes. Ionic liquids are becoming popular as solvents and catalysts for biofuel productions as these are environment friendly chemicals. The present review critically describes important properties and uses of ionic liquids in biofuel productions.

Keywords: Bioethanol, Biodiesel, Ionic liquids, Transesterification, Lignocellulosic biomass

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1. Introduction

Environmental pollution and climatic changes forced scientists to look for clean energy forms including bioethanol, and biodiesel. The main source of organic carbon is plant biomass. The organic material that is obtained from sustainable sources is called as biomass. It covers mill leftovers, wood and wood wastes, animal wastes, water-based plants, trees, and industrial wastes. The only class of liquid fuel is the biofuel which is obtained from plant biomass and animal fats. It covers bio alcohols which may be ethanol, butanol, biodiesel, bio oil or syngas. The first-generation biofuels mainly uses plants that are enriched in sugar and starch. First generation feed stocks put constrains on food crop prices. The second-generation biofuels are obtained from non-food plant materials such as lignocellulose. They can be obtained from organic waste crop straw, dumped trash etc. Now a days lignocellulosic materials are of great demand for biofuel production as these

are available in large amounts at low prices. The use of biomass and fossil fuels based paths for energy production in industry is wide spread currently. The alcohols which are used as biofuels include 1-butanol, 1-propanol, ethanol, and methanol etc. Ethanol is used in bio feed stock production. It is also used as a solvent and as a starting material in chemical industries. Similarly, 1-butanol is also used in bio feed stock production and as a solvent. 1-butanol can also be used as biofuel. It has remarkably high density in comparison to ethanol. It is more water repellent than ethanol.

Ionic liquids (IL's) have incredibly unique, and ultra-efficient solvents . These are used for biomass breakdown and biomass pretreatment. Ionic liquids have very unique properties like inflammable, high chemical stability, non-volatile. They have high thermal stability, high ionic conductivity, and are recyclable [1]. Ionic liquids solvents have basic role in the pretreatment of lignocellulose because it can mix with and become the part of a liquid.

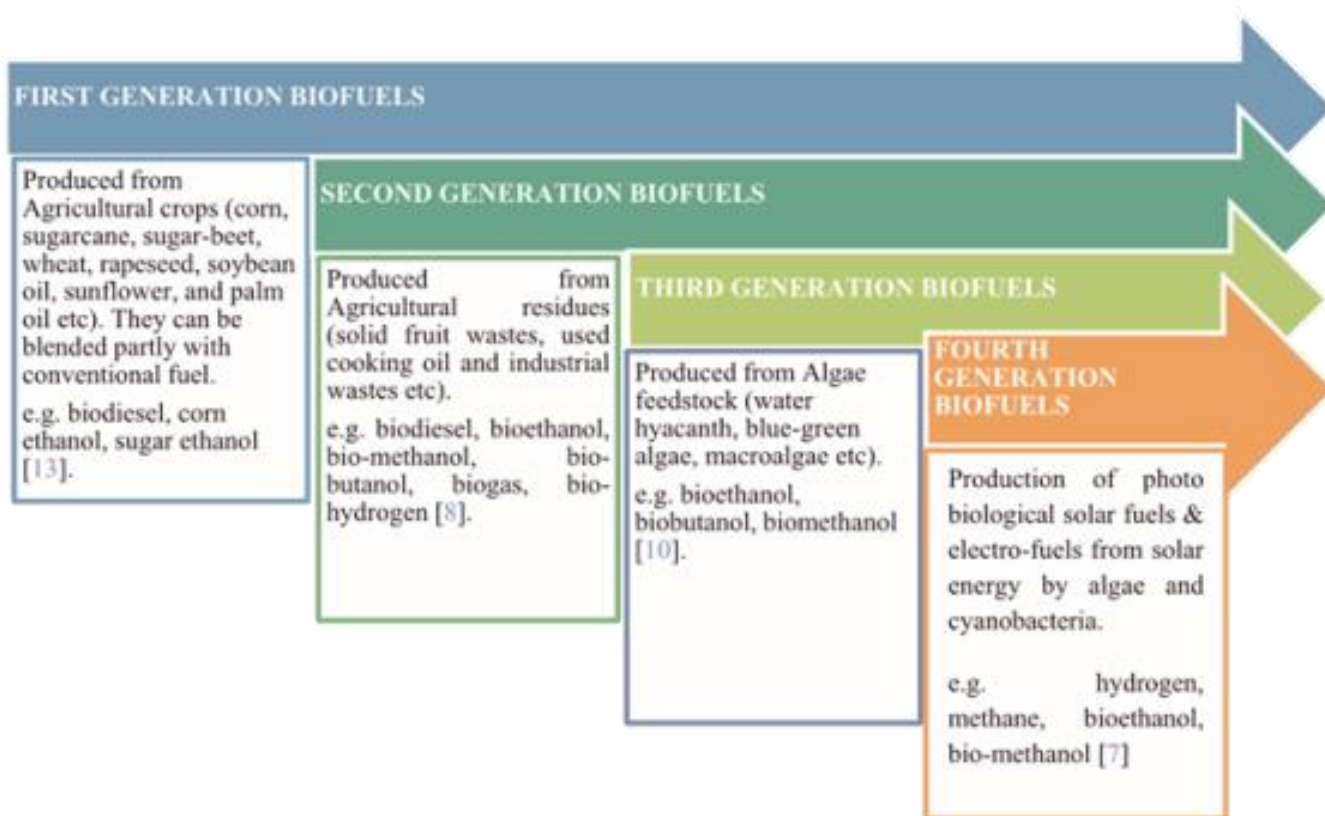


Figure 1: Generations of Biofuels

A great quantity of cellulose can be absorbed by it at normal conditions and almost 100% efficiency is obtained. Ionic liquids are classified as cations and anions. Cations are usually organic while anions are inorganic in nature. The sodium chloride which is a salt is an ionic liquid at relatively high temperature. The ionic liquids are in liquid form at room temperature at relatively low temperature. [EtNH₃][NO₃] was the first ionic liquid observed in 1914. There were only a few ILs based patent applications that increased up to 100 in 2000 and up to 800 in 2004. Ionic liquids have very unique chemical properties. They are environment friendly molecular solvents. They are used in the catalytic and organic reactions. Ionic liquids are considered as the "Green Solvents".

The liquid, solid and gaseous fuels are collectively called as biofuel. The biofuels are produced from biomass. The thermochemical and biological pathways are used to convert biomass into liquid and gaseous fuels. Biofuels are environmentally friendly, easily available, cheap and are obtained from recyclable and renewable sources. Ionic liquids are categorized as bio alcohols, vegetable oils and biodiesels, bio-crudes and biosynthetic oils. Biomass is largely used in feedstock due to the three reasons (i) biomass is a source that is renewable and easy to maintain in the future (ii) it is environmentally friendly. It has relatively low sulfur contents and no release of carbon dioxide takes place from it (iii) it has economic potential because biomass is very cheap while fossil fuel price will rise in future.

1.1. Bio-alcohols

Bio-alcohol is produced from biomass. It can be used in pure or blended form. The 100 % pure biofuels are getting popular for use in many vehicles. Blended forms of biofuels are already in use. The alcohols which are used as biofuels include 1-butanol, 1-propanol, ethanol, and methanol etc.

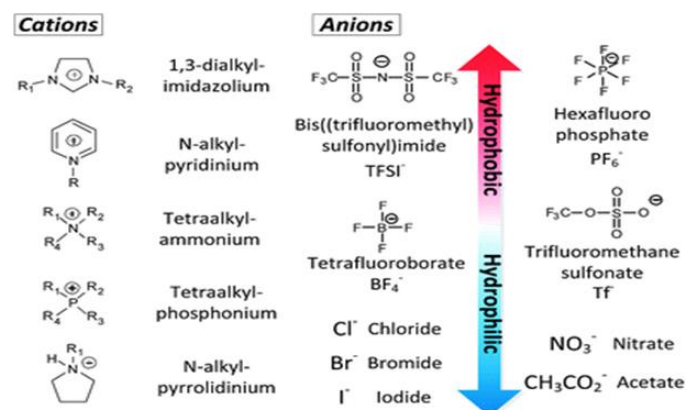


Figure 2: Characterization of Ionic liquids

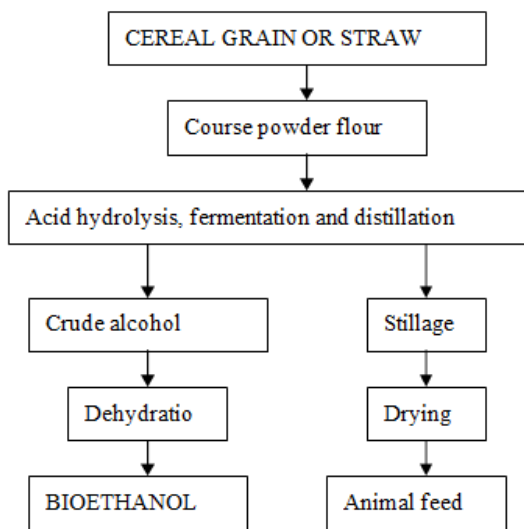


Figure 3: Production of bioethanol

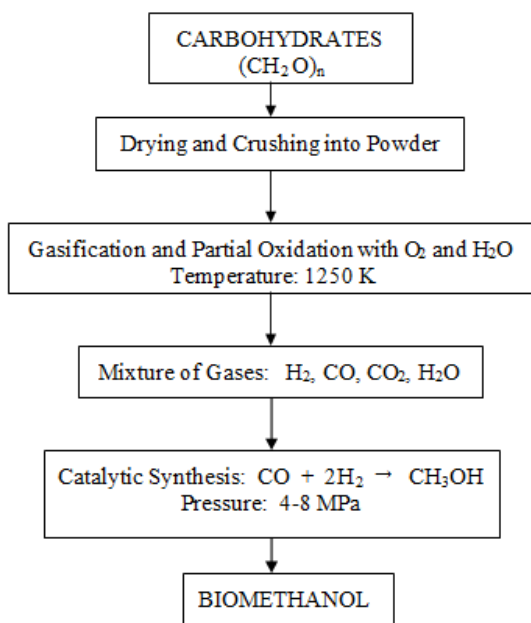


Figure 4: Biomethanol production

1.2. Biodiesel

For diesel engines, vegetable oils can be used as fuels, but the viscosities of them are very high as compared to diesel. To lessen the viscosities of vegetable oils, different methods have been proposed such as dilution, micro emulsion, pyrolysis, catalytic cracking and transesterification. Pyrolysis has more advantages than transesterification. The chemical components of liquid fuel and diesel fuel are almost same. The conversion of vegetable oil to liquid and gaseous fuels can be done by pyrolysis, decarboxylation, deoxygenation, and catalytic processes.

1.3. Bio-oils

Bio-oils are liquid fuels that are produced by biochemical or thermo-chemical processes. The bio-oils can be used in the vehicles.

1.4. Biogas

The organic material of biomass is broken down through anaerobic digestion. The products obtained are methane and carbon dioxide. This mixture is called as biogas. The organic material includes plants, animals, and industrial wastes. Biogas is environmentally friendly, cheap, and clean fuel. It is considered as a resourceful fuel. The digesters are filled up with feedstock and the digestion time of biogas takes from 10 days to few weeks. The digester helps in the breakdown of waste to simple organic substances and biogas as a product is obtained. The digesters need proper care, orientation, and operational techniques. The floating drum, fixed dome, plastic tube, plug flow etc. are the types of bio-digesters.

2. Biomass resources

A large amount of biomass is used for biofuel production. On dry weight basis, the annual formation of biomass is 220 billion tones which is equal to 4500 EJ. The annual bioenergy obtained from this biomass is 270 EJ.

Table 1: Biomass resources

Biomass Resources	Biomass Energy Yield
Wood and wood waste	64%
Municipal waste	24%
Agricultural waste	5%
Landfill gases	5%

The resources of biomass could be wood and wood waste, crops, waste by-product, water-based plant and algae. Poplar, willow, or eucalyptus has high growth rate. 10-13 tons of dry material can be harvested. The waste wood is used for energy production. The waste wood includes bark, sawdust etc. Algal biomass is used for third generation biofuel production [2]. The agricultural biomass consists of food based and non-food-based portions, grasses and animal wastes. Cellulosic wastes are the part of fruit wastes. The crops that are worldwide cultivated are wheat, maize, rice, and sugarcane. The lignocellulosic biomass is produced from them. The crops yield is 5358.54 million tons. Some of its part is used in animal feedstock.

3. Chemical composition of biomass

Cellulose, hemicellulose, lignin, protein, and ash are included in agricultural wastes. It may consist of lignocellulosic materials. It is a polymer of cellulose, hemicellulose and lignin [3].

Table 2: Chemical composition of biomass

Organic Molecules	Percentage Composition
Cellulose	35-50%
Hemicellulose	20-35%
Lignin	15-20%

Lignocellulose	15-20%
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3.1. Cellulose

Cellulose is formed of repeating units of glucose. It is linear and crystalline in nature. It has β -1, 4 glycosidic bonds. The longer chains are linked by hydrogen bonds and van der Waals forces. The linear chain consists of 10,000 glucose units. The chains arrange themselves in parallel form.

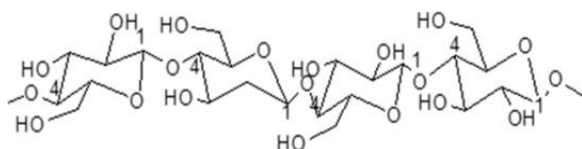


Figure 5: Structure of cellulose

3.2. Hemicellulose

It is shorter and compactly packed polymer. It is heterogeneous in nature. It is a polymer of D-Xylose, D-arabinose, D-glucose, D-galactose and D-mannose. It may be [4] homopolymer or heteropolymer. If it has same glucose subunits, it is homopolymer, and if it has different subunits it is a heteropolymer. There is a difference in hemicellulose and cellulose. The difference is due to size, branching and ability to resist chemicals.

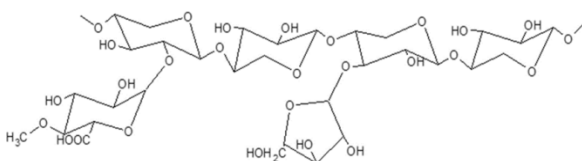


Figure 6: Structure of hemicellulose

3.3. Lignin

Lignin is formed by phenyl propane units linked together. It is a complex three-dimensional structure. The monomers of lignin are three phenyl propionic alcohols which could be coumaryl A, coniferyl B, and sinapyl C. Lignin is closely related to cellulose and hemicellulose. It provides strength and cohesion to water. It is hydrophobic in nature. It is tightly bound to cellulose and hemicellulose. So, it gives them protection from the attack of microbial organisms.

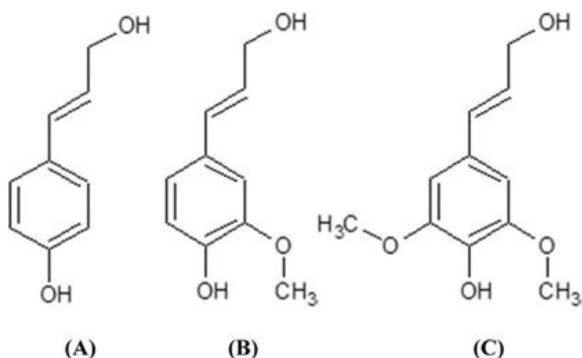


Figure 7: Structure of lignin

4. Materials and methods

4.1. Pretreatment of lignocellulosic biomass

Cellulose, lignocellulose, and lignin are the parts of lignocellulosic biomass. It is the process in which the transformation of lignocellulose into its components like cellulose, hemicellulose and lignin takes place. By this process, the lignin is removed, hemicellulose is preserved, the crystallinity of cellulose is reduced and the porous nature of the material is increased. It is a cheap treatment that gives better efficiency as it enhances the sugar formation, the breakdown of the carbohydrates is lessened, and for hydrolysis and fermentation, the formation of inhibitors takes place in it [5].

The effective pretreatment can take place by following means: (i) Direct formation of sugars by hydrolysis (ii) Sugars formed are avoided from degradation (iii) The inhibitory product formation is inhibited (iv) Energy demands are reduced (v) Biofuel production cost is minimized.

4.2. Methods of pretreatment of lignocellulosic biomass

Pretreatment methods are classified as:

- Physical pretreatment.
- Chemical pretreatment.
- Physio-chemical pretreatment.
- Biological pretreatment.
- Combined pretreatment.

4.2.1. Physical pretreatment

The lignocellulosic waste is pretreated by different physical pretreatment methods as chipping, grinding, milling, radiation and freezing. By these methods, the size of the particle becomes small and the surface area of lignocellulosic material is increased. These pretreatment methods work with other methodologies also.

Mechanical size reduction or mechanical comminution or milling

Milling is the very first step of pretreatment. There are many types of milling such as ball milling, hammer milling, two rolling milling, disk milling and colloid milling. The required size of feedstock is obtained by milling. For example, after chipping, milling, and grinding, the size reduces to 10-30 mm and 0.2-2 mm. Milling uses a high amount of energy. From an economic point of view, wet milling is regarded as the best one. If the size is reduced beyond limits, then, it can reduce the formation of biofuel. As a result of this, volatile fatty acids are formed that inhibit methane formation. The biogas formation takes place from rice straw by a combination of milling, grinding, heating and after 2% ammonia treatment. The ethanol fermentation inhibitors like furfural and hydroxyl methyl furfural are not formed in milling.

Extrusion

Extrusion is a very demanding and novel physical pretreatment used to convert biomass into ethanol. In this process, the materials are heated, and using the extruder physical and chemical changes take place in material by mixing it. Lignocellulosic structure is broken down by high speed and high temperature. It causes the shortening of fibers. The ability of enzymes to attack the carbohydrates is increased. The highest efficiency could be achieved by different parameters. Extrusion is largely used for ethanol formation.

Pyrolysis

It is an endothermic process. It consumes less energy. In this process the temperature of lignocellulosic biomass is set at 300 °C. The breakdown of cellulosic biomass into H₂, CO and bio char takes place in this process. Mild acid and water is added in the leftover char. The water drained contains glucose. This glucose is used as a carbon source in biofuel production [6]. Microwave assisted pyrolysis is also used for biofuel formation. It is deduced that there are several improvements when some catalysts are added in the process including activated carbon, silicon carbide and char. The improvements are in the form of heating rate, and biomass conversion rate etc.

4.2.2. Chemical pretreatment

Acid pretreatment

Acid pretreatment is a well-known process for the lignocellulosic biomass treatment. In this process, the polysaccharides are solubilized into monomers by acid and the enzymatic activity to increase the biofuel production. Low acid concentration with high temperature or high acid concentration with low temperature is required for acid pretreatment [7]. Its drawback is that the different types of inhibitors are formed in it like acetic acid, furfural, and 5-hydroxymethyl furfural etc. These inhibitors inhibit the growth of microbes. For dilute acidic pretreatment, the organic and inorganic acids can be used like hydrochloric acid, sulphuric acid, nitrous acid, maleic acid, formic acid, and acetic acid. Sulphuric acid is most used [8]. For cellulose hydrolysis, the concentrated acid treatment is highly effective. Concentrated acids have several drawbacks like these can cause corrosiveness, and toxicity. These require non-metallic containers, and are very expensive.

The neutralization is an important step in pretreatment. However, the neutralization of pH requires large quantity of gypsum and as a result the disposal issues also arise. [9]. If the concentration of furfural and 5-hydroxymethyl furfural is in the range of 15-20 Mm, then it does not inhibit microbial growth. Acids like HNO₃, CH₃COOH and HCl can be effective in lignin removal [10]. Phenolic compounds can show inhibitory effect due to their physio-chemical nature like solubility, and hydrophobicity etc. [11]. So, the formation of inhibitors must be controlled
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in acid pretreatment, and conditions must be monitored. The selection of acid can also reduce the inhibitor formation.

Alkaline pretreatment

Alkaline pretreatment removes inhibitors like acetyl group, lignin, and uronic acid substituents. It breaks the cell walls of polysaccharides like cellulose, hemicellulose, lignin, and silica by the enzymatic hydrolysis. The swelling of fibers of cellulose takes place during this process. It covers saponification and salvation reactions. It increases the porosity of biomass and breakdown the cross links of hemicellulose. Its drawbacks are that it needs neutralization and long recovery time [12]. The commonly used alkalies are sodium hydroxide, calcium hydroxide, ammonia, and potassium hydroxide.

The commonly used alkali in alkali pretreatment is sodium hydroxide. It is more effective than biofuel that are produced from untreated biomass [13]. Ammonium hydroxide is used in aqueous ammonia soaking in switch grass pretreatment. It solubilizes 35 % lignin and 41 % hemicellulose. By this pretreatment methane yield is increased up to 65 %. Its dry weight is 1.4 g, the temperature is provided for 5 days. Sodium hydroxide pretreatment is more efficient. It is used for pretreatment of biomass in biofuel production. The excessive use of sodium hydroxide can cause anaerobic digestion inhibition. It can cause soil salination and water pollution. A new procedure has been designed to improve the biogas yield from grape pomace and rice straw, and to recycle sodium hydroxide. This process is used to reduce cost and environmental pollution.

Organo-solvation

Ozone is an oxidant. Its efficiency for removal of lignin is very high. The removal of lignin increases the yield by enzymatic hydrolysis. The pretreatment takes place at room temperature and normal pressure. It doesn't form the inhibitory compounds that effect hydrolysis and fermentation. It is applied on agricultural leftovers as wheat straw and rye straw. In both cases, it increases the yield. The research is being carried out to form ethanol by pretreatment of lignocellulose with ozone. It makes the process economically suitable. Organo-solvation is an excellent methodology. Its drawback is that a large amount of ozone is needed for this purpose. To solubilize the lignin, different organic or aqueous mixtures such as methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol can be used. It provides cellulose for enzymatic hydrolysis. Organosolv is used for the separation of lignin. Sometimes acid catalysts are also used. The acid catalysts could be HCl, H₂SO₄, oxalic acid or salicylic acid. These breaks and weakens the hemicellulose bonds. As a result of this, high amount of xylose is obtained. Hemicellulose and lignin are separated by acid hydrolysis. As a result of this, about 70 % lignin and 20 % cellulose is removed. It is a necessary process and done by extraction, evaporation, and condensation etc. The price of solvents is very high, so they

are industrially very significant. Those solvents are favored that have low molecular weight and low boiling points such as ethanol and methanol.

Ionic liquids (ILs) pretreatment

Ionic liquids are largely used for cellulosic biomass pretreatment as solvents. These are the salts. These could be cations or anions. The cations are organic while anions are inorganic in nature. The solvent properties depend on anions and alkyl substituents of cations. Ionic liquids have the given properties and chemical and thermal stability, non-flammability and have low vapor pressure. Ionic liquids forms no toxic gases. Hence, these ionic liquids are called as green solvents. The carbohydrates and lignin are dissolved in ionic liquids. The ionic liquids form hydrogen bonds between chloride portion of ionic liquid and hydroxyl part of sugar. To prevent the decrease of sugars, and to improve the economics, ionic liquids are largely used. Due to the advanced research in the field, ionic liquids can play a vital role in reducing the cost. Ionic liquids provide pretreatment for future lignocellulosic biorefining processes.

4.2.3 Physico-chemical pretreatment

Ammonia fiber explosion pretreatment

Liquid ammonia and steam explosion methods are applied in the pretreatment of biomass. AFExp is alkaline thermal pretreatment and it is monitored by water loading, ammonia loading and temperature of reaction [14]. There is no need of size reduction in this process and inhibitors are not formed. It has some drawbacks, as it is less efficient in high lignin containing biomass. For example, newspaper and softwood. AFExp is an excellent technique for biomass pretreatment. Ammonia can be recovered after pretreatment method.

Liquid hot water pretreatment (LHW)

To keep water in liquid form, high temperature and high pressure is used in this process. The used temperature is the range of 160-220 °C is used. The xylose yield is 80 %. Because of the removal of hemicellulose, the cellulose solubilization is increased. Expansion or decompression is not required in LHW. Water is maintained by pressure utilization in liquid state by avoiding evaporation. Different types of lignocellulosic materials are treated by this method. For example, sugarcane bagasse, wheat straw and rye straw. Less inhibitors are produced and more solubilization of hemicellulose at 190 °C for 15 minutes takes place in LHW. It does not require corrosion resistant materials. On controlling pH in LHW pretreatment a minute quantity of inhibitors is produced and it undergoes anaerobic digestion process smoothly.

Wet oxidation

It is an oxidative pretreatment method. It utilizes oxygen or air as catalyst. The reactions take place at low temperatures and require very less reaction time. The oxidation takes place for 10-15 minutes at 170 to 200 °C temperature and at 10-12 bar oxygen pressure. If oxygen is added at 170 °C, then the reaction becomes exothermic and it lessens the total energy demand. The formation of acid from hydrolytic processes and oxidative reactions takes place by wet oxidation. It is an accurate method for dissolving hemicellulose and cellulose. It is largely used in ethanol formation. During wet oxidation, these compounds further breakdown to carboxylic acids. Due to the oxidation inhibitors like furfural and HMF are not produced. Sodium bicarbonate decreases the formation of inhibitors, and they maintain the pH in neutral to alkaline range. Almost 96 % cellulose and 70% hemicellulose are recovered by pretreating the wheat straw with sodium bicarbonate. Higher yields can also be obtained by changing the biomass. In wet oxidation normally less inhibitor are produced. But its drawback is that, oxygen and catalyst are more costly.

Microwave pretreatment

It is a physicochemical process. It covers thermal and non-thermal effects. It is done by dissolving biomass in dilute chemical agents and then the mixture is subjected to microwave radiation for 5-20 minutes. Alkalies as sodium hydroxide are regarded as best chemical agents for microwave pretreatment.

Ultrasound pretreatment

The ultrasound pretreatment is applied for the extraction of hemicellulose, cellulose, and lignin from lignocellulosic biomass. But hydrolysis of lignocellulosic material is in research. There is less research on ultrasound pretreatment on the lignocellulosic material. But it has been discovered that saccharification is increased by ultrasound pretreatment. After this treatment, high enzymatic hydrolysis occurs as the mechanical effects are highly enhanced by this technique as the formation of cavitation takes place by adding the ultrasound field in the enzymatic processes. When the cavitation bubbles breakdown or collapsed, the surface area of solid substrate is increased for the enzymes to attack. At 50 °C maximum cavitation effects are observed, as this is the optimum temperature for the enzymes to work.

CO₂ explosion

For the lignocellulosic biomass pretreatment, CO₂ explosion is also used. CO₂ is used as supercritical fluid. It is a liquid which is gaseous in form but is converted to the liquid form by compressing at suitable temperatures. It gives a liquid like appearance. By this process, the lignin is efficiently removed and it increases the substrate solubilization. The add up of co-solvents can help in the

efficient removal of lignin. Super critical CO₂ has many advantages as it is used as an extraction solvent. CO₂ forms carbonic acid in aqueous solution, and it helps in the formation of polymers by hydrolysis. The size of CO₂ is equivalent to water and ammonia, so due to the same sizes, they can easily enter to the small pores of lignocellulose. This process is done under high pressure conditions. When the explosion occurs, the release of CO₂ pressure takes place. This pressure causes the breakdown of cellulose and hemicellulose structure, so it is available for the enzymes to attack. At low temperature, it cannot breakdown monosaccharide, but yield of sugar is high as compared to steam and ammonia explosion. CO₂ is more efficient as compared to ammonia explosion and less inhibitors are formed as compared to steam explosion in it. The enzymatic hydrolysis is increased by supercritical CO₂. There are no efforts to study its economic status. It needs high pressure. The use of CO₂ reduces the cost of this procedure. It is non-toxic, non-flammable and CO₂ recovery is very easy.

4.2.4 Biological pretreatment

It is safe and environment friendly method for removal of lignin from lignocellulosic biomass. Biological pretreatments include fungal pretreatment, enzymatic pretreatment, and microbial consortium

Fungal pretreatment (FP)

The famous white rot fungi belongs to basidiomycetes. Its growth rate is very high, and it can easily cause the breakdown of lignin [15]. There is a difference of fungal pretreatment with chemical and physicochemical pretreatment method. It is based on moisture ratio, size of particle, temperature, yield, and time taken by the reaction for pretreatment. *Ceriporiopsis subvermispora* is used for ethanol production by 31.59 % lignin breakdown and 94 % cellulose. It causes low energy consumption. It has some drawbacks also.. It requires large space, long time for carrying out the reaction and there is a need of microorganism growth recording.

Enzymatic pretreatment

Cellulases and hemicellulases are used in the pretreatment of lignocellulosic mass. In anaerobic digestion, the hydrolysis of cellulose and hemicellulose is a limiting step. The enzyme pretreatment is a limited process because its cost is very high and this process has very less effect in biogas production. For butanol formation, different feed stocks are treated like sugarcane bagasse, cassava pulp and water etc. Enzymatic pretreatment is not considered fully accurate as it has many short comings like it is costly, highly selective, and the retention time of enzymatic pretreatment is high. Research is continued to overcome these short comings.

4.3. Advantages and disadvantages of various pretreatment processes for lignocellulosic material

Table 3: Advantages and disadvantages of various pretreatment methods for biomass

Pretreatment Process	Advantages	Limitations and Disadvantages
Mechanical communication	Reduces cellulose crystallinity	Power consumption generally higher than inherent biomass energy
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of inhibitory compounds to micro-organisms
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	Not efficient for biomass with high lignin content
CO ₂ explosion	Increases accessible surface area; cost-effectiveness; does not cause formation of inhibitory compounds	Does not modify lignin and hemicellulose
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence time required; irrecoverable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	Produces gas and liquid products	High temperature; ash production
Pulsed electrical field	Ambient conditions; disrupts plant cells	Process needs more research
Biological	Simple equipment degrades lignin, hemicelluloses; low energy requirements	-

4.4. Biofuel production

Ethanol, butanol, and biodiesel are the transport powering fuels. If they are produced from non-food biomass then they act as carbon neutral transport powering fuels. The large amount of energy is produced from lignocellulosic materials. There is a strong covalent and hydrogen bond between carbohydrate polymer of cellulose and hemicellulose and phenolic polymer lignin. The lignocellulose is broken down into its components to convert it into biofuel. Many chemical treatments are required for this process. Cellulose and lignocellulose can be dissolved in those ionic liquids having anions with good

hydrogen bond accepting properties as chloride ion, carboxylates and oxanions.

So wood and other plant materials are dissolved in it. By the addition of water in the ionic liquid we can get cellulose or lignocellulose. It is done as cellulose-anion hydrogen bonds are disrupted by water, and hence the enzymes can easily ferment sugars into alcohol by precipitating the cellulose. For example, the formation of ethanol and butanol work is continued for ionic liquid pretreatment of biomass. To take butanol from dilute aqueous fermentation, ionic liquids can be used.

Despite of cellulose, ionic liquid can also be used to dissolve other polymers as silk, wool keratin nano particles and carbon nanotubes. Ionic liquids have been replaced as cheap, and recyclable biopolymers that are extensively used in biofuel formation.

Biodiesel is another powering fuel because it is best suited for powering structure and automobiles. To transform triglycerides to glycerol and biodiesel, ionic liquids are extensively used. After this for biodiesel formation transesterification is done by lipase, or by homogenous catalysis.

4.5. Lignocellulosic bioethanol production by using biomass

4.5.1. Physical Destruction of (Ligno-) Cellulose

Cellulose fibers are crystalline and strongly packed. The surface of cellulose is so tightly packed that there is no space for cellulose or water to hydrolyze. The surface are can be increased by different pretreatment methods. These methods are significant in transforming cellulose into glucose, they improve the kinetics of hydrolysis. Cellulose dissolution based pretreatment is most significant beside all the pretreatment techniques. Ionic liquids are solubilized in cellulose and cellulose is recovered by adding anti-solvent. The anti-solvent could be water or alcohol. So the crystalline structure of cellulose was deformed by adding ionic liquid which is [BMIM] Cl.

The number of adsorption site increased for enzymes to attack on cellulose. The rate of hydrolysis of enzyme in initial was 50% higher than the reformed cellulose. The solubility of untreated is 15% and 30% w/w, it does not break the structure of cellulose. A decrease in crystalline structure of cellulose was observed when imidazolium ionic liquid containing alkyloxyalkyl-chain ([Me(OEt)₂-Et-Im]OAc) is used for cellulose pretreatment. The size decreased from 58-75%. Its hydrolyzing time is 6h. Ionic liquids are used for cellulose pretreatment, before doing hydrolysis, all the left-over ionic liquid must be removed, because it can deactivate cellulose.

4.5.2. Hydrolysis of (Ligno-) Cellulose

Ionic liquids are the efficient solvents for the solubilization of cellulose. In [BMIM]Cl, wood can partially

be dissolved. It is completely dissolved in [EMIM]OAc. A solution mixture was formed in which proton is more convenient to attack on glycosidic bonds. The hydrolysis of cellulose and lignocellulose can be done with catalyst and without catalyst in ionic liquids. The most commonly and widely used ionic liquid is [BMIM]Cl. At low temperature and atmospheric pressure, a method was formed in which the yield of reduced sugars and glucose is 64 % and 37 %, respectively. For the hydrolysis of lignocellulose such as corn stalk, rice straw, pine wood and bagasse ionic liquid [BMIM]Cl can also be used. The acid hydrolysis of cellulose and hemicellulose in [BMIM]Cl occurs by first order reaction. In the depolymerization of lignocellulose in ionic liquids certain features play a very vital role including temperature, water content, acid strength and concentration. There is a drawback of water in making carbohydrates soluble, but it is good for cellulose hydrolysis. Hence equivalency must be between water content and rate of hydrolysis of cellobiose and pinewood with imidazolium ionic liquid. A new process has been discovered that gives high yield of chemical reaction of lignocellulosic hydrolysis. By acid hydrolysis with [EMIM]Cl almost 90 % yield of glucose is achieved. Besides this, for the recovery of ionic liquid, ion-exclusion chromatography can also be used. It allows the transformation of sugar feedstock and it has no negative effect on growth of ethanologenic microbes. For the hydrolysis of cellulose in acidic ionic liquids, metal chlorides can also be used as polar catalysts. Besides it, they contain dilute acid. Cellulose can be converted to reduced sugar or 5-hydroxymethyl furfural (HMF) by using ionic liquid which contain 1-alkyl (R)-3 methylimidazolium chloride salt with chromium chloride. The yield is 97 % and 89 %, respectively. In other example, HMF and furfural was produced by the hydrolysis of lignocellulosic biomass. In the presence of chromium chlorate, their yield was 52 % and 31 %, respectively. But chromium chloride, chromium chlorate and ferrous chloride are extremely toxic and have negative environmental impacts. By adding ferrous chloride, the yield of HMF and furfural is 34 % and 19 %, respectively.

Ionic liquids can be used as solvents and co-solvents. These are good for enzymatic hydrolysis. 1,3-dimethylimidazolium dimethyl phosphate ([MMIM]DMP) produces less inhibitors in the cellulose enzymatic activity. The enzyme maintains 30 % of its activity at 10 % (v/v). The increase in viscosity and ionic strength causes a fundamental decrease in enzymatic activity. For example, the activity of cellulose is high in 1-methylimidazolium chloride [MIM]Cl with respect to [BMIM]Cl. Because the viscosity of [MIM]Cl is very low while imidazolium ionic liquid has very long alkyl chain. The anions greatly affect the stability of enzymes. Those ionic liquids are highly stable that have low hydrogen bond basicity and nucleophilicity.

Stability and temperature tolerance are increased by tris-(2-hydroxyethyl) methylammonium methylsulfate (HEMA). At 100 °C temperature. The cellulose solubility can be decreased by low hydrogen bonding basicity. So cellulose activity and lignocellulose solubility are the requirements for the suitable ionic liquid. Corncoobs were converted to sugars by saccharification using [MMIM]DMP by hydrolysis yielding 70 %. The cellulose activity reduces about 20 % (v/v) in the presence of [MMIM]DMP. Heterogeneous catalysis is used to separate fermentable sugars from cellulose. So, for this process, cello oligomers are formed by depolymerizing cellulose in the presence of ([BMIM]Cl). Sugars can be formed from cellulose by ionic liquids in the absence of catalyst. Ionic liquids are the excellent source for hydrolysis of cellulose and lignocellulose.

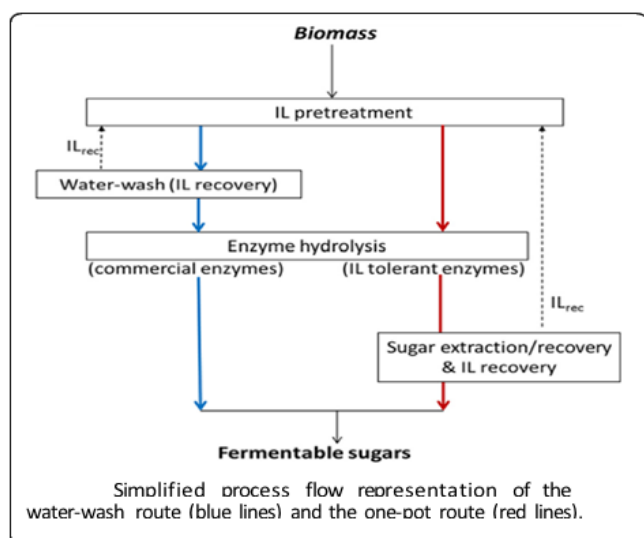


Figure 8: Production of Fermentable Sugars by Biomass

4.5.3. Biomass dissolution in ionic liquids

Due to compact network of inter and intra molecular hydrogen bonds and Vander Waals forces, it is very difficult to dissolve cellulose. It is investigated that by using [BMIM]Cl as ionic liquid, the cellulose dissolve due to chloride anion. Chloride anion has strong hydrogen bonding basicity. In this process, firstly the hydrogen bond formation takes place between the chloride portion of ionic liquid and hydroxyl portion of the sugar molecule. Those biomass samples whose solubility is greater than 25 %, for them [BMIM]Cl is favorable. Those ionic liquids that contain coordinating anions are not suitable as a solvent for cellulose and lignin. Longer chain ionic liquids are has less efficiency. At 100 °C, only 5 % wt, of cellulose is dissolved by [HMIM]Cl whereas at same temperature 10 % of cellulose is dissolved by [BMIM]Cl.

High chloride anion concentration is regarded as the main source of hydrogen bonding in [BMIM]Cl. The dissolution of cellulose is increased as the number of chlorides in the ionic liquids is increased. The anions show poor performance due to the weak hydrogen bonding

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basicity. The chloride concentration is decreased due to the longer chain substituted ionic liquids as [HMIM]Cl and [OMIM]Cl.

4.5.4. Cellulose regeneration and hydrolysis

By adding ionic solvents, such as acetone, water or alcohol, cellulose can be regenerated. Anti-solvent cannot change the nature of cellulose formed. By using Fourier Transforms Infrared Spectroscopy, X-ray diffraction, Differential Scanning Calorimetry (DSC), Size Exclusion Chromatography (SEC), Scanning Electron Microscope (SEM), Nuclear Magnetic Resonance (NMR), and Thermogravimetric Analysis (TGA) the difference between the actual cellulose and recovered cellulose can be obtained. The enzymatic hydrolysis is increased by pretreatment of cellulose and lignocellulose with ionic liquids. If the anions are larger in concentration in ionic liquids, then it leads to enzyme inactivation. The cellulose can be regenerated if small amount of ionic liquid is added in the glucose solution. The cellulose recovered must be rinsed or washed off with water before enzymatic saccharification.

4.5.5. Ionic liquid recycling

When the cellulose is recovered from ionic liquid solution then anti-solvent is evaporated and ionic liquid is reused. Ionic liquid can be reused up to 45 times and yield of sugar is not affected [16]. Solubilized lignin is formed because of cellulose regeneration in recycling of ionic liquids. This lignin is a raw material for polymeric materials, or can be used as a starting material in liquid hydrocarbons.

4.5.6. Enzyme compatible ionic liquids

One of the drawbacks of ionic liquid is that it can denature the enzymes. The recovered cellulose must be washed off before enzymatic saccharification and ionic liquid must be recovered. To minimize the need to recover the cellulose, the 'in situ' enzyme saccharification of cellulose in enzyme compatible ionic liquid (1-ethyl-3-methylimidazolium diethyl phosphate) is done. In aqueous ionic liquid mixture of cellulose, cellulose is added at 40 °C. The cellulase activity is greatly affected by the ratio of ionic liquid and water. Cellulase activity is observed when the ionic liquid to water ratio is 3:2. But the cellulase activity is enhanced by decreasing the ionic liquid to volume ratio to 1:4. It results in the conversion of 70 % cellulose into glucose and cellobiose. The decline in enzymatic activity is caused when the viscosity and ionic strength is increased.

4.5.7. Acid catalyzed hydrolysis of cellulose and lignocellulose in ionic liquids

The acid catalyzed hydrolysis of cellulose and lignocellulose in ionic liquid has been observed. It is opposite to enzymatic hydrolysis. By using ionic liquid [BMIM]Cl in the presence of 7 % wt. of hydrogen chloride

at mild condition of 100°C and normal atmospheric pressure the sugar yield was 66 %, 74 %, 81 % and 68 % for hydrolysis of corn stalk, rice straw, pine wood and bagasse, respectively. The reactivity of other minerals was also observed. The reactivity order was hydrochloric acid > nitric acid > sulfuric acid > maleic acid > phosphoric acid. Acidic ionic liquids such as 1-(4-sulfobutyl)-3-methylimidazolium bisulfate [SBMIM]HSO₄ and 1-butyl-3-methylimidazolium bisulfate [BMIM]HSO₄ act as a catalyst in place of HCl. The corn stalk dissolved very quickly in ionic liquid. It forms a solution which have low viscosity here, The polysaccharide depolymerization occurs readily. The reducing sugars yield is 15-23 %. The rate of sugar breakdown was increased and the depolymerization reaction was also enhanced. The corn stalk is hydrolyzed in [BMIM]Cl and it is then added in acidic ionic liquids [BMIM]HSO₄ and [SBMIM]HSO₄. The yield was 68 % and 71 %, respectively.

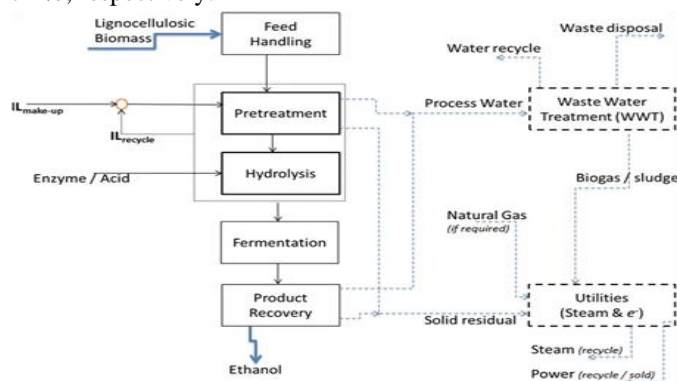


Figure 9: Biofuel production pathways

5. Biodiesel production

The most common biofuel is biodiesel. It is used as transport powering fuel [17]. The greenhouse gases are lessened up to 78 % using biodiesel. The unburnt hydrocarbons are also reduced in environment. The percentage of non-pollution causing vehicles is increasing day by day in Japan by using biodiesel. And it has reduced the greenhouse gases by more than 80 % [18]. The diesel production is affected by feedstocks. Microalgae are largely existed in the ecosystem. These are eukaryotes. Microalgae, by using carbon dioxide cause the formation of biofuel by photosynthetic metabolism [19]. It causes the emission of CO₂. There are many microorganisms that produce remarkably high yield of oil and biodiesel. More than 10,000 gallons of oil per acre is produced by microalgae. Microalgae photo reactors are present on water surfaces and non-arable land. They do not interfere with feedstock. There are many hindrances in the biodiesel formation. These hindrances could be economical, environmental, and technological [20]. If viewed according to technological point, it is necessary to combat the two issues, first one is by means of synthetic biology and improved light supply system to increase the productivity of oil per acre [21]. And

the second one is to lessen the negative environmental impact. Simple environmental techniques lead to the efficient extraction of oil from microorganisms and other materials [22]. The production of biodiesel depends on different factors such as feedstock, supply chain and distribution. The profit is increased, and ecological footprints are lessened by means of this. Hence, the reduction of greenhouse gases emission takes place. The use of ionic liquids is very interesting for getting high and efficient extraction and low environmental impact as a solvent [23]. Ionic liquids can improve the catalytic activity for the transesterification reaction [24]. Ionic liquids can help in intensification by increasing the integration stages in the biodiesel production processes and lessens energy and other material tasks. The melting point of ionic liquids is not greater than room temperature. These are organic salts present in liquid phase. The salt is known as amphoteric i.e. it is made up of organic cations and inorganic anions [25]. When ionic liquids come in contact with cells, it breaks the walls of cells and membranes and as a result of this, oil is released. Ionic liquids can act as biocatalyst in other reactions of biodiesel production [26]. These are interesting solvents for lignocellulosic treatment. Liquid liquid demixing occurs under suitable conditions by water ionic liquid mixtures. It allows easy, low energy cosolvent recovery of ionic liquid. Different oil sources are used for the enzymatic production of biodiesel by using ionic liquid as solvent and catalyst. The effectiveness in other feedstocks have been observed by lipid extraction and lack of knowledge of industrial applications has severe draw backs on its activity. In this way, by using ionic liquids, bio-oil extraction in algal biodiesel production was studied. It is used to check, the effect of temperature on extraction of oil by using butyl-3-methylimidazolium chlorides (BMIMCl) as ionic liquid. It computes and analyzes the different properties by thermodynamic models. This process describes the efficiency of extraction of oils with respect to different traditional organic bio solvents, to verify the efficiency of selected solvent.

5.1. Production methodologies

In this process for getting complete efficiency, simulation is provided. The process of simulation is done by commercial software and it shows how ionic liquids properties have been estimated.

5.1.1 Process simulation

Process simulation software Aspen Hysys V7.3® was used for bio-oil extraction process. It covered different stages of the formation of biodiesel from microalgae. It involved harvesting, dewatering, oil extraction and recovery of ionic liquid.

5.1.2 Microalgae harvesting

With more carbon dioxide, microalgae are grown in a photo bioreactor. In the first harvesting step, the algae solution is thickened by lamella settler, to obtain 2 % dry weight after bio flocculation.

5.1.3 Microalgae dewatering

After bioflocculation, mechanical dewatering or centrifugation is done for down streaming the process to 20 % threshold. The cyclization of water is done by microalgae harvesting system.

5.1.4 Oil extraction (wet route)

The working conditions of lysis reaction are 80 °C temperature and atmospheric pressure. The microalgae slurry coming from mechanical dehydration step is mixed with the ionic liquid. The debris is removed by using a filter. The debris includes animal feedstock. While the oil is separated by three-phase decanter from the top. And the aqueous solution is separated from the bottom.

5.1.5 Ionic liquid recovery

The water is evaporated to recover the ionic liquid. As water is more volatile than ionic liquid. The mixture is preheated until the bubble formation takes place. It is done before the flash stage. The feed is preheated by feed that is enriched with liquid phase rich in ionic liquid. It is then moved to lysis reactor. The vapor phase which is mainly the water is condensed and moved back to the microalgae harvesting section.

5.1.6 Ionic liquid and biomass properties estimation

Ionic liquids need different requirements for the efficient work. It needs high yield, low toxicity, high biodegradability, high catalytic activity towards transesterification, and the use co-solvents. BMIMCl is used in different bioprocesses. Because it has high temperature bearing ability, and even the temperature closed to normal boiling point can be tolerated by this solvent. So, it's very helpful in measuring the boiling points also.

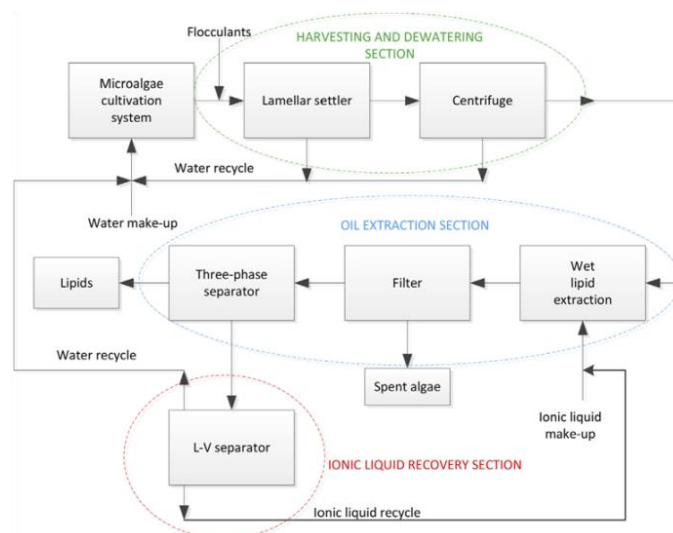


Figure 10: Biodiesel production pathways

6. Biodiesel production processes using ionic liquids

Different ionic liquids are being used for biodiesel production. A two-phase catalytic system is used for the breakdown of alcohol of soybean oil by dissolving Sn(3-hydroxy-2-methyl-4-pyrone)₂(H₂O)₂ in ionic liquids. It is found that when ionic liquid is changed from [BMIM]PF₆ to [BMIM]InCl₄, the biodiesel yield enhances from 55 % to 83 %. The systems was poor in terms of reusability and after three reuse cycles, the yield of biodiesel is decreased to zero. Besides this, a high biodiesel yield was obtained from vegetable oil (93-98.5 %), when an organic acid or base and Lewis acid is combined such as [ET₃NH]Cl-AlCl₃ and H₂SO₄ and immobilization in [BMIM]NTf₂. And small change was observed after six reuse cycles. The active and stable catalysts are produced when enzymes are combined with imidazolium in ionic liquids. Ionic liquids can save lipase from deactivation produced by methanol. The biocatalytic synthesis of biodiesel in ionic liquids is dependent on short-chain 1,3-dialkylimidazolium cation e.g., [BMIM]PF₆ or [BMIM]NTf₂. The reaction takes place in two phase system that needs a suitable amount of water. [BMIM]NTf₂ is used with lipase for biodiesel formation. It is reported that it produced about 96.3 % of biodiesel from soybean oil. When salt hydrate is added directly to the non-aqueous system, it is not suited to meet water need in lipase catalyzed reaction in [BMIM]PF₆. On enzymatic activity, salt hydrate has dual effect. The first effect is water buffering effect and the second one is specific ion effect. Specific ion effect predominates in ionic liquids [27]. The third phase can be made by adding water to the two phasic system after transesterification. It allows the settling of by-products and acyl acceptors. It facilitates the recycling of ionic liquids. The lipase activity is reduced after the sixth reuse. For one phase system of lipase catalyzed biodiesel production, imidazolium ionic liquids with long alkyl chains were produced e.g., [C₁₆MIM]NTf₂ and [C₁₈MIM]NTf₂. The direct interaction between enzyme and pure methanol is

avoided and reuse of lipase in ionic liquids was allowed. For oil transesterification, non-aqueous system was created by long chain and lipid loving ionic liquids. Biodiesel extraction was facilitated by three phase system. When the viscosity of ionic liquid was increased with [CnMIM] cation, then a negative effect on lipase-catalyzed transesterification was produced. By using anion, the reaction remains consistent with increasing viscosity: [PF₆]⁻>[BF₄]⁻>[NTf₂]⁻ [28]. For oil transesterification, some ionic liquids can be used as catalyst. The properties of pyridinium ionic liquids include non-corrosive, highly active and water stable organic solvents. It is good for catalytic reactions. It is also suitable for the separation of products from the system of catalyst. About 92 % of biodiesel yield is produced from cottonseed oil by using the ionic liquid 1-(4-sulfonic acid) butyl pyridinium hydrogensulfate. And its reusability was demonstrated. In the transesterification process of jatropha oil, after seven complete cycles consistent activity of ionic liquid was shown. Ionic liquids gave more than 90 % yield and its reusability was also being demonstrated [29]. The ionic liquids are more acidic, and it shows efficient catalyst. Anions play an important role in the acidic intensity of ionic liquids. Cations have significant role in making accessible the acid active sites. For the separation of biodiesel and removal of glycerol by-product, ionic liquids are used. But there is lack of continuous biodiesel formation, the accumulations and recovery of ionic liquids are future challenges.

7. Conclusions

Ionic liquids as "green" solvents are producing many innovations by producing the biofuels. The current processes have disadvantages like high water usage, pollution, odor, high pressure operations, use of toxic flammable solvents etc. besides this, ionic liquids have many advantages.

The solvent properties of ionic liquids depend on suitable cations and anions. So ionic liquids are substrate specific. Besides this, ionic liquids are used as catalyst in transesterification of vegetable oil in the formation of diesel. In ionic liquids, lipase makes the reaction stable and recovery of catalyst becomes very easy. Ionic liquids can be used for lignocellulosic bioethanol production and biodiesel production.

References

- [1] Nargotra, P., V. Sharma, M. Gupta, S. Kour and B. K. Bajaj. 2018. Application of ionic liquid and alkali pretreatment for enhancing saccharification of sunflower stalk biomass for potential biofuel-ethanol production. *Bioresource technology*, 267: 560-568.
- [2] Chandra, R., H. Takeuchi and T. Hasegaw. 2012. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews*, 16(3): 1462-1476.
- [3] Mood, S. H., A. H. Golfeshan, M. Tabatabaei, G. S. Jouzani, G. H. Najafi, M. Gholami and M. Ardjmand. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews*, 27: 77-93.
- [4] Bai, X., P. A. Lant, P. D. Jensen, S. Astals and S. Pratt. 2016. Enhanced methane production from algal digestion using free nitrous acid pretreatment. *Renewable energy*, 88: 383-390.
- [5] Chiaramonti, D., M. Prussi, S. Ferrero, L. Oriani, P. Ottonello, P. Torre and F. Cherchi. 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. *Biomass and bioenergy*, 46: 25-35.
- [6] Bu, Q., H. Morgan Jr, J. Liang, H. Lei and R. Ruan. 2016. Catalytic microwave pyrolysis of lignocellulosic biomass for fuels and chemicals. In: *Advances in bioenergy*. Elsevier: pp: 69-123.
- [7] Sen, B., Y. P. Chou, S. Y. Wu and C. M. Liu. 2016. Pretreatment conditions of rice straw for simultaneous hydrogen and ethanol fermentation by mixed culture. *international journal of hydrogen energy*, 41(7): 4421-4428.
- [8] Ranjan, A., S. Khanna and V. Moholkar. 2013. Feasibility of rice straw as alternate substrate for biobutanol production. *Applied energy*, 103: 32-38.
- [9] Wen, Z., M. Wu, Y. Lin, L. Yang, J. Lin and P. Cen. 2014. Artificial symbiosis for acetone-butanol-ethanol (abe) fermentation from alkali extracted deshelled corn cobs by co-culture of *clostridium beijerinckii* and *clostridium cellulovorans*. *Microbial cell factories*, 13(1): 92.
- [10] Antonopoulou, G., D. Vayenas and G. Lyberatos. 2016. Ethanol and hydrogen production from sunflower straw: The effect of pretreatment on the whole slurry fermentation. *Biochemical engineering journal*, 116: 65-74.
- [11] Barakat, A., F. Monlau, J. P. Steyer and H. Carrere. 2012. Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. *Bioresource technology*, 104: 90-99.
- [12] Wan, C., Y. Zhou and Y. Li. 2011. Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility. *Bioresource technology*, 102(10): 6254-6259.
- [13] García-Torreiro, M., M. Á. Pallín, M. López-Abelairas, T. A. Lu-Chau and J. M. Lema. 2016. Alkali treatment of fungal pretreated wheat straw for bioethanol production. *Bioethanol*, 2(1).

- [14] Bals, B., C. Wedding, V. Balan, E. Sendich and B. Dale. 2011. Evaluating the impact of ammonia fiber expansion (afex) pretreatment conditions on the cost of ethanol production. *Bioresource technology*, 102(2): 1277-1283.
- [15] Sindhu, R., P. Binod and A. Pandey. 2016. Biological pretreatment of lignocellulosic biomass overview. *Bioresource technology*, 199: 76-82.
- [16] Shill, K., S. Padmanabhan, Q. Xin, J. M. Prausnitz, D. S. Clark and H. W. Blanch. 2011. Ionic liquid pretreatment of cellulosic biomass: Enzymatic hydrolysis and ionic liquid recycle. *Biotechnology and bioengineering*, 108(3): 511-520.
- [17] Ahmed, S., M. H. Hassan, M. A. Kalam, S. A. Rahman, M. J. Abedin and A. Shahir. 2014. An experimental investigation of biodiesel production, characterization, engine performance, emission and noise of *Brassica juncea* methyl ester and its blends. *Journal of cleaner production*, 79: 74-81.
- [18] Eguchi, S., S. Kagawa and S. Okamoto. 2015. Environmental and economic performance of a biodiesel plant using waste cooking oil. *Journal of Cleaner Production*, 101: 245-250.
- [19] Oncel, S. S., 2013. Microalgae for a macroenergy world. *Renewable and Sustainable Energy Reviews*, 26: 241-264.
- [20] Oltra, C., 2011. Stakeholder perceptions of biofuels from microalgae. *Energy Policy*, 39(3): 1774-1781.
- [21] Arudchelvam, Y. and N. Nirmalakhandan. 2013. Energetic optimization of microalgal cultivation in photobioreactors for biodiesel production. *Renewable energy*, 56: 77-84.
- [22] Kim, D. G., H. J. La, C. Y. Ahn, Y. H. Park and H. M. Oh. 2011. Harvest of *scenedesmus* sp. With bioflocculant and reuse of culture medium for subsequent high-density cultures. *Bioresource technology*, 102(3): 3163-3168.
- [23] Fang, Z., R. L. Smith and X. Qi. 2014. Production of biofuels and chemicals with ionic liquids. Springer.
- [24] Elsheikh, Y., Z. Man, M. A. Bustam, S. Yusup and C. Wilfred. 2011. Brønsted imidazolium ionic liquids: Synthesis and comparison of their catalytic activities as pre-catalyst for biodiesel production through two stage process. *Energy Conversion and Management*, 52(2): 804-809.
- [25] Liu, C. Z., F. Wang, A. R. Stiles and C. Guo. 2012. Ionic liquids for biofuel production: Opportunities and challenges. *Applied energy*, 92: 406-414.
- [26] Lai, J. Q., Z. L. Hu, P. W. Wang and Z. Yang. 2012. Enzymatic production of microalgal biodiesel in ionic liquid [bmim][pf6]. *Fuel*, 95: 329-333.
- [27] Zhang, K. P., J. Q. Lai, Z. L. Huang and Z. Yang. 2011. *Penicillium expansum* lipase-catalyzed production of biodiesel in ionic liquids. *Bioresource technology*, 102(3): 2767-2772.
- [28] De Diego, T., A. Manjón, P. Lozano, M. Vaultier and J. L. Iborra. 2011. An efficient activity ionic liquid-enzyme system for biodiesel production. *Green Chemistry*, 13(2): 444-451.
- [29] Ghiaci, M., B. Aghabarari, S. Habibollahi and A. Gil. 2011. Highly efficient brønsted acidic ionic liquid-based catalysts for biodiesel synthesis from vegetable oils. *Bioresource technology*, 102(2): 1200-1204.