
Pretreatment Empty Fruit Bunch of Oil Palm Tree for Improving Enzymatic Saccharification

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Abstract

Empty fruit bunch of oil palm tree (EFBOPT), solid waste of palm oil industries, is potential for raw materials of biofuel especially bioethanol production because of its cellulose and hemicellulose contents. There are four steps to produce bioethanol, called the second generation bioethanol, from EFBOPT or other lignocellulosic materials. The steps are (a) pretreatment of lignocellulose biomass into cellulose/hemicellulose, (b) hydrolysis of cellulose/hemicellulose into monosaccharides, (c) fermentation of monosaccharides into bioethanol, and (d) recovery of bioethanol from medium fermentation broth. Pretreatment steps are the key success factor to convert lignocellulosic materials into bioethanol. This paper will review EFBOPT and pretreatment steps, including physical pretreatments, physicochemical pretreatments, and biological pretreatments.

Keywords: bioethanol, biomass, empty fruit bunch of oil palm tree, lignocellulose, pretreatment

1. Introduction

Depletion of fossil fuels, increasing of climate changes, and improvement of world energy consumption [1] have directed towards the development of biofuel production [2]. Biofuels are able to replace petroleum-based fuels, decrease greenhouse gas emission [3], and have significant potential sustainability [4]. Biofuels can be as gaseous (methane or hydrogen) or liquid (biodiesel, biobutanol, or bioethanol) forms and are commonly generated from agricultural materials. Either agricultural commodities or agricultural waste materials can be used for developing biofuels; for example, vegetable oil [5, 6] is used for biodiesel and agricultural

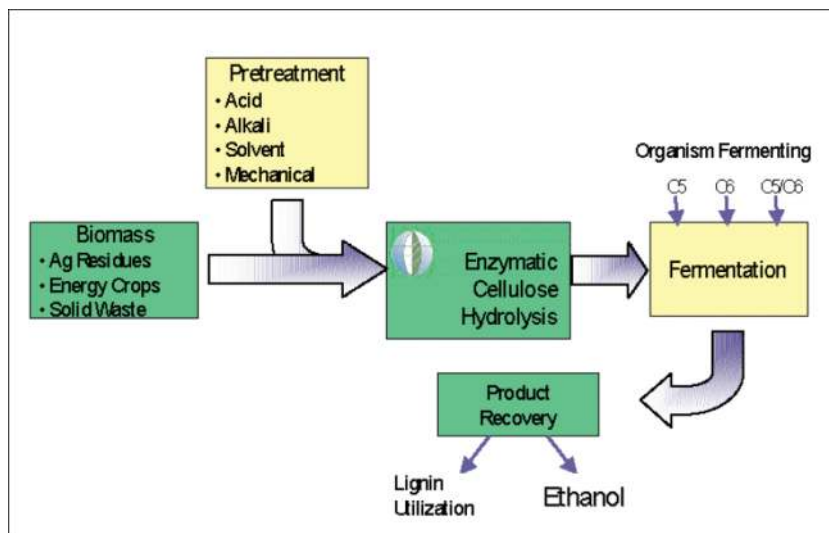


Figure 1. Steps of second generation bioethanol production from agricultural waste biomass. Source available from [30]: Knauf and Moniruzzaman.

wastes [7, 8] were processed for developing methane or hydrogen gases. Meanwhile, bioethanol can be produced either from agricultural high starch/sugar-containing commodities such as corn [9] and sugarcane [10] or from agricultural solid wastes such as non-edible parts of cassava [11], banana peels [12], cocoa-pod waste [13], rice straw [14, 15], sugarcane bagasse [16–19], sorghum bagasse [20], and empty fruit bunch of oil palm tree (EFBOPT) [21–24]. Bioethanol produced from starch/sugar containing commodities is called the first generation bioethanol, and one produced from agricultural solid wastes or lignocelluloses is called the second generation bioethanol.

Among agricultural materials, oil palm tree (*Elaeis guineensis* Jacq.) is considered as a major source of biofuel. In the world, oil palm tree is planted in about 43 developing countries and plantation area increased eight time in the past four decades to over 12 million ha in 2009 [25]. In a good condition, oil palm tree can yield about 4.5 ton oil and 7–8 ton EFBOPT/ha/year. Unlike palm oil which can be processed directly into biodiesel, EFBOPT has to be pretreated before converted into bioethanol [26].

There are four steps to produce bioethanol, called the second generation bioethanol, from lignocellulosic materials [27–29] such as EFBOPT. They are (a) pretreatment of lignocellulose biomass into cellulose/hemicellulose, (b) cellulose/hemicellulose hydrolysis into monosaccharides, (c) monosaccharides fermentation into bioethanol, and (d) bioethanol recovery (**Figure 1**) [30]. The key success factor to convert EFBOPT into bioethanol is pretreatment step [31]; therefore, this paper will review the EFBOPT pretreatment and discuss recent research results which significantly enhanced enzymatic saccharifications.

2. Empty fruit bunch of oil palm tree

EFBOPT is solid waste residue generated from palm oil factories. Fresh fruit bunches are harvested from oil palm tree (**Figure 2**) and then sterilized in a steam sterilizer for inactivating enzymes that present in pericarp and loosening fruits from bunches. The sterilized bunches are then fed into a rotary drum thresher to separate the sterilized fruit from bunches without fruit, which are called as empty fruit bunch of oil palm tree (EFBOPT). The sterilized fruits are further processed for palm oil production and the EFBOPT (**Figure 3**) are conveyed to the damping ground and left unused. It was reported that each 100 ton of fresh fruit bunches yielded 14 tons oil-rich fiber and 20–22 tons of EFBOPT [32]. EFBOPT is dry and brown; its shape is not uniform with low bulk density; and its length and width can vary from 17 to 30 cm long and 25 to 35 cm wide depending on the size of fresh fruit bunch [32].

Like other lignocellulose materials, native EFBOPT fiber is mainly constructed from a complex matrix of three main polymers (**Figure 4**) [33], namely cellulose, hemicellulose, and lignin. The EFBOPT fiber consists of 44.2–50.0% cellulose, 22.0–33.5% hemicellulose, and 20.4–21.0% lignin [34, 35].

Cellulose, a major constitutes of FEBOPT, is closely associated with hemicelluloses and lignin, and the separation of the cellulose from hemicelluloses and lignin requires intensive physical and chemical treatments. Cellulose is a linear polymer of D-glucopyranose units bound by β -1-4-glycosidic linkages (**Figure 5**). The successive glucose units are rotated by 180° relative to each other to form a cellobiose unit as a repeating unit of cellulose chains ended by a hydroxyl group. The hydroxyl (OH) groups in the cellulose act as functional groups which are able to interact each other or with O-, N-, and S-groups forming hydrogen bonds. Hydrogen bonds also occur between the OH-group of cellulose and water. Through hydrogen bond, cellulose chains are packed together to set up highly crystalline microfibrils. An individual microfibril contains ten of glucan chains in a parallel orientation [36]. This microfibril fraction can be hydrolyzed into glucose either by enzymatic or by chemical methods [37].



Figure 2. Oil palm tree (A) with fresh fruit bunches (B). Source available from: http://www.thestar.com.my/~media/online/2014/09/07/08/01/str2_ma_0809_p10a-lead-pic.ashx?w=620&h=413&crop=1&hash=B2A67ABD156961DCE9E42C52EA4D72C3F9503686 (accessed on February 25, 2018).



Figure 3. Empty fruit bunch of oil palm tree (EFBOPT) on damp ground. Source available from: <https://s3-ap-southeast-2.amazonaws.com/ecostore-static-assets/Page+images/Palm+Oil+Page/Empty-fruit-bunches.jpg> (accessed on February 25, 2018).

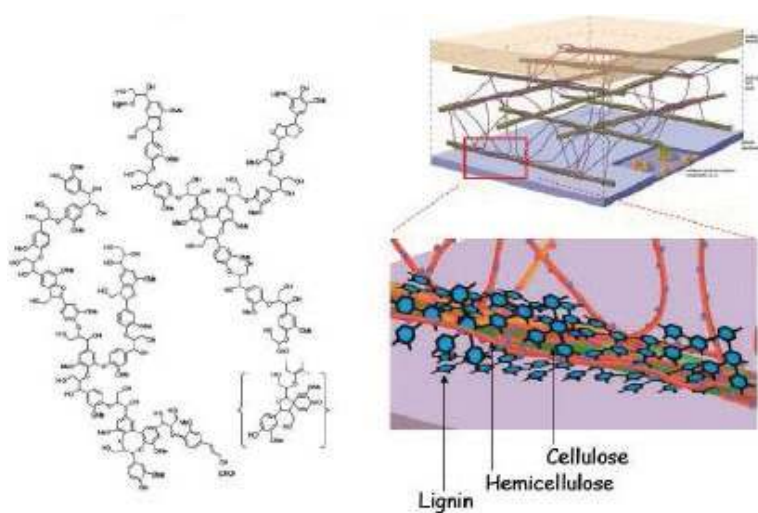


Figure 4. Polymer structure of lignocellulosic biomass. Source [33] Quintero-Ramirez.

Hemicellulose is a polymer consisting of heteropolymers of D-xylose, D-mannose, D-glucose, D-galactose, and L-arabinose, in the form of linear and branched (**Figure 6**). Its structure is not crystalline and is, therefore, easier to hydrolyze than cellulose [38]. Hemicelluloses usually form cross-linked to other polysaccharides, proteins, or lignin. Xylans are considered to be the main interface between lignin and other carbohydrates [39].

Lignin is a cross-linked aromatic, hydrophobic, and complex polymer consisting of three different phenyl-propane precursor monomer units which are very difficult to biodegrade (**Figure 7**). Lignin is mostly observed as an integral part of the plant cell wall, embedded in a polymer matrix of cellulose and hemicellulose. Thus, lignin is the most non-biodegradable component of the plant cell wall [36].

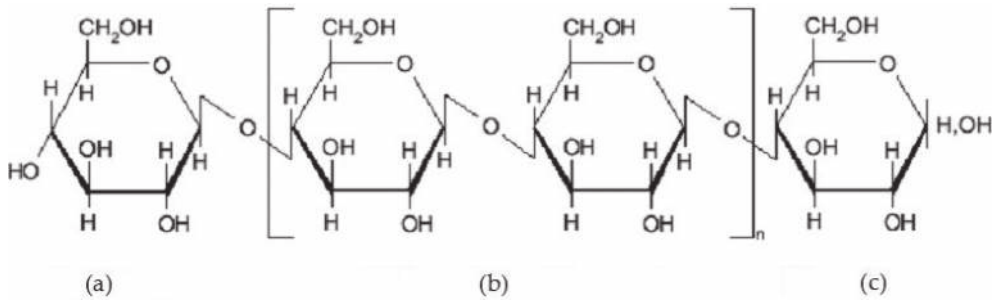


Figure 5. Chemical structure of cellulose. Source: available from <https://www.intechopen.com/books/cellulose-fundamental-aspects/cellulose-microfibril-angle-in-wood-and-its-dynamic-mechanical-significance> (accessed on February 26, 2018).

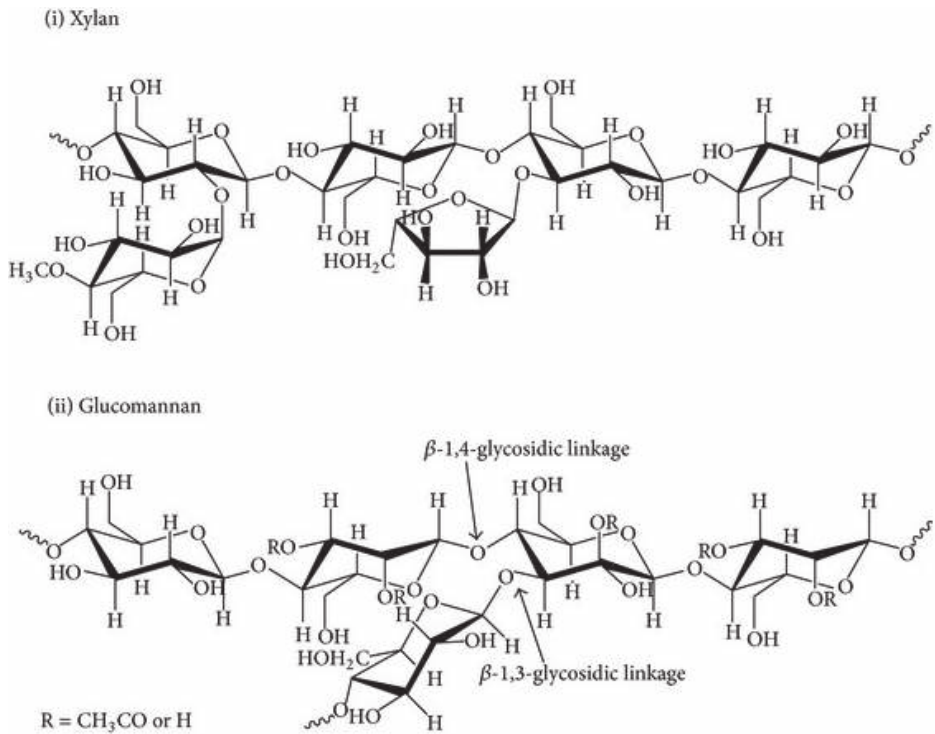


Figure 6. Chemical structure of hemicellulose. Source: available from https://www.researchgate.net/figure/Chemical-structure-of-hemicellulose-compounds-xylan-and-glucomannan-are-the-most_fig4_266026683 (accessed on February 26, 2018).

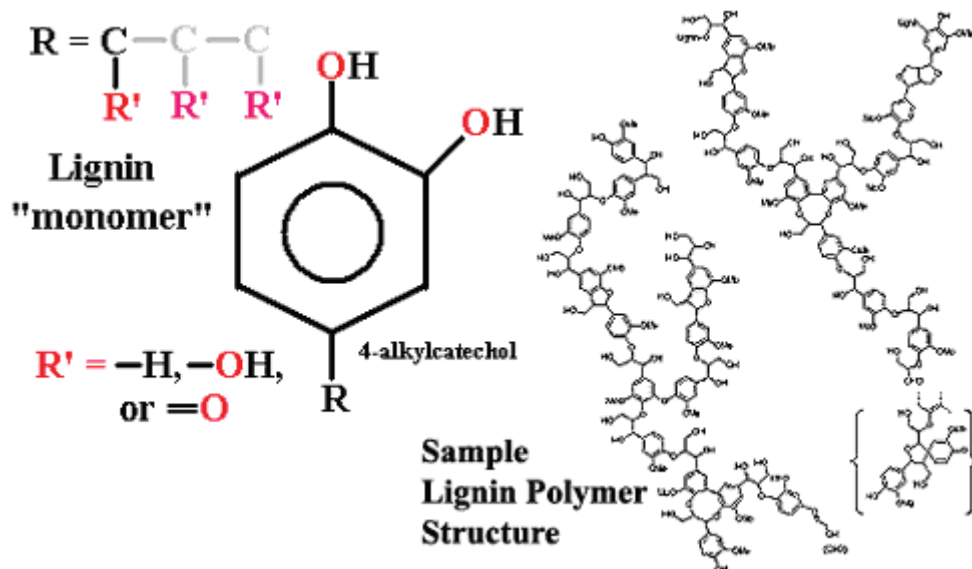


Figure 7. Chemical structure of lignin. Source: available from <http://palaeos.com/plants/glossary/images/Lignin.gif> (accessed on February 26, 2018).

3. Pretreatments

Pretreatment is actions given to biomass materials such as EFBOPT for enhancing the cellulose reactivity with cellulase enzymes and for increasing the yield of fermentable sugars. There are eight requirements for effective and economical pretreatment; they are (a) producing higher reactive cellulose fibers for enzymatic attachment, (b) producing less residues, (c) avoiding formation of compound inhibitors for hydrolytic enzymes and fermenting microorganisms, (d) avoiding destruction of celluloses and hemicelluloses, (e) reducing of material cost for setting upper-treatment reactors, (f) minimizing the energy demand, (g) reducing the cost of size reduction for feedstock, (h) consuming little or no chemical, and (i) using a cheap or no chemical [40]. The goal of pretreatment is to disrupt the crystallinity of cellulose, to open lignin and hemicellulose protection, to increase EFBOPT surface accessibility, and to decrease the degree of hemicellulose acetylation [41]. Pretreatment can increase significantly bioethanol or biogas yield and productivity. Effects of pretreatment on the degrading enzyme accessibility, bioethanol or biogas yield, and productivity from lignocellulosic materials were diagrammatically (**Figure 8**) shown by Taherzadeh and Karimi [40].

Based on actions given to biomass materials, pretreatment methods are classified into three groups, namely physical, chemical and physicochemical, and biological pretreatment methods [40]. The physical methods include milling (ball milling, colloid milling, hammer milling, two-rollmilling, and vibrant energy milling), irradiation (electron-beam irradiation, gamma-ray irradiation, and microwave irradiation), and expansion, extrusion, high pressure, hydrothermal,

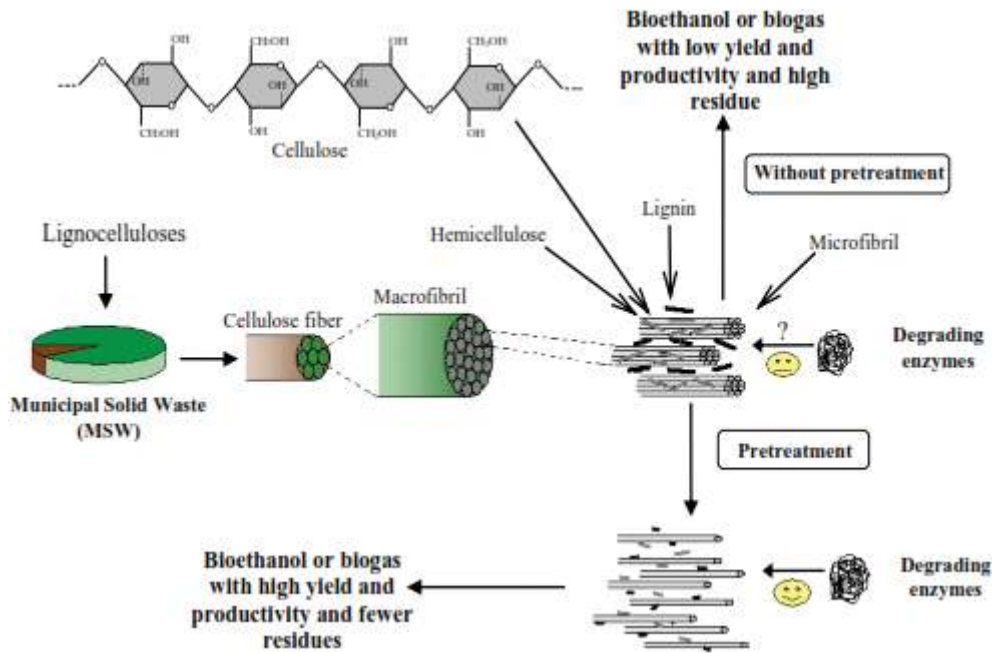


Figure 8. Effects of pretreatment on degrading-enzyme accessibility, and bioethanol or biogas yield and productivity. Source: available from Taherzadeh and Karimi [40].

and pyrolysis. The chemical and physicochemical pretreatments consist of acid (hydrochloric acid, phosphoric acid, and sulfuric acid), alkali (ammonia and sodium hydroxide), explosion (ammonia fiber explosion/AFEX, CO₂ explosion, SO₂ explosion, and steam explosion), gas (chlorine dioxide, nitrogen dioxide, and sulfur dioxide), oxidizing agents (hydrogen peroxide, ozone, and wet oxidation), and solvent extraction of lignin (benzene-water extraction, butanol-water extraction, ethanol-water extraction, ethylene glycol extraction, and swelling agents), and biological. Biological pretreatments commonly utilize fungi and actinomycetes as microbial producing enzymes which can degrade lignin compounds. Advantages and disadvantages of some different pretreatments are well tabulated (**Table 1**) by Brodeur et al. and Maurya et al. [42, 43].

3.1. Acid pretreatments

The main objective of the acid pretreatment is to solubilize lignin and hemicelluloses chemically so that the cellulose is more accessible to enzymes. Either diluted or concentrated acid can be utilized to perform this acid pretreatment. The diluted acid pretreatment method is more attractive because of less inhibitor compound formation, such as furfural, 5-hydroxymethylfurfural, phenolic acids, and aldehydes. In addition, diluted acids are less toxic, corrosive, hazardous, and corrosive, as well as more feasible for industrial scale. The diluted acid pretreatment methods have been developed in different types of reactors including percolation,

Pretreatment method	Advantages	Disadvantages
Acid	<ul style="list-style-type: none"> • Yield of glucose is high • Hemicellulose is solubilized 	<ul style="list-style-type: none"> • Costs of acids are high • Acid recovery is needed • Costs of corrosive resistant equipment are high • Fermentation inhibitors are formed
AFEX	<ul style="list-style-type: none"> • Effectiveness for herbaceous material is high • It is suitable for low lignin content biomass • Cellulose becomes more accessible • Formation of inhibitors is low 	<ul style="list-style-type: none"> • Recycling of ammonia is needed • Process effectiveness decreases with increasing biomass lignin content • Lignin structure changes • Costs of ammonia are high
Alkali	<ul style="list-style-type: none"> • Lignin is efficiently removed • Formation of inhibitors is low 	<ul style="list-style-type: none"> • Costs of alkaline catalyst are high • Alteration of lignin structure
Ammonia recycle percolation	<ul style="list-style-type: none"> • Majority of lignin is removed • Cellulose content after pretreatment is high • Herbaceous materials are most affected 	<ul style="list-style-type: none"> • Energy costs and liquid loading are high
Biological	<ul style="list-style-type: none"> • Energy requirements are low • Lignin is removed • Degree of cellulose polymerization is reduced • Hemicelluloses are partial hydrolyzed • Chemicals are not required • Environmental conditions are mild 	<ul style="list-style-type: none"> • Process rate is slow • Treatment rate is very low • It is not very effective for commercial application
CO ₂ explosion	<ul style="list-style-type: none"> • Accessible surface area increase • Inhibitory compounds are not formed • It is non-flammability and relatively cheap • Recovery after extraction is easy • It is environmentally acceptable 	<ul style="list-style-type: none"> • Pressure requirements are very high
Liquid hot water	<ul style="list-style-type: none"> • Hemicellulose is separated from rest of feedstock • It is no need for catalyst • Hemicellulose is hydrolyzed 	<ul style="list-style-type: none"> • Energy/water input is high • Solid mass left over will need to be dealt with (cellulose/lignin)

Pretreatment method	Advantages	Disadvantages
Milling	<ul style="list-style-type: none"> Cellulose crystallinity and degree of polymerization are decreased Particle size decreases Specific surface area and pore size increase 	<ul style="list-style-type: none"> Power and energy consumptions are high
Steam explosions	<ul style="list-style-type: none"> It is low cost Lignin transforms Hemicellulose is solubilized Yield of glucose is high 	<ul style="list-style-type: none"> Partial hemicellulose degrades Acid catalyst is needed to make process efficient with high lignin content material Toxic compound is formed
Wet oxidation	<ul style="list-style-type: none"> Majority of hemicellulose and lignin are solubilized Inhibitor compounds are avoided 	<ul style="list-style-type: none"> Costs of oxygen and alkaline catalyst are high

Table 1. Advantages and disadvantages of different pretreatment methods of lignocellulosic biomass [42, 43].

plug flow, shrinking-bed, batch, flow-through reactor and countercurrent reactors [40]. There are two approach processes of dilute acid pretreatment methods, namely (a) high temperature (e.g., 180°C) during a short period of time and (b) lower temperature (e.g., 120°C) for longer retention time (30–90 min). The most widely used acid is dilute H₂SO₄ which provides high hydrolysis yields [37, 44]. Other acids used for cellulosic material pretreatments are acetic acid, C₂H₄O₃, formic acid, hydrochloric acid (HCl), maleic acid, oxalic acid, phosphoric acid, and nitric acid [45–49].

3.2. AFEX pretreatment

AFEX is one of the physicochemical pretreatments which treated lignocellulosic biomass with liquid ammonia at relatively moderate temperature (90–100°C) for about 30–60 min, then followed by a rapid pressure release [50]. A rapid expansion of the liquid ammonia causes swelling and physical disruption of lignocellulosic fibers and partial reduction of cellulose crystallinity. AFEX process is able to either modify or effectively decrystallization of cellulose and lignin fractions [51]. AFEX removes the least acetyl groups of biomass by deacetylation process, so that the digestibility of lignocellulosic biomass increases [52, 53]. The main advantage of the AFEX is that it does not produce inhibitors for the downstream biological processes, so water wash is not needed. AFEX is more effective for agricultural residues [54]. The AFEX process conditions (ammonia loading, temperature, blowdown pressure, moisture content of biomass, and residence time) have been optimized [55]. At the optimal conditions, AFEX can convert over 90% cellulose and hemicellulose to fermentable sugars for a broad variety of biomass materials including EFB OPT. Due to high volatility, ammonia is easy to be recovered and recycled [56], and leaving the dried biomass ready for enzymatic hydrolysis [57]. After pre-pretreatment, ammonia must be recycled in order to reduce the cost and protect the environment [56].

3.3. Alkali pretreatment

Alkali pretreatment is commonly utilized ammonium, calcium, sodium, and potassium hydroxides at certain temperature and pressure. The main advantage of this pretreatment is that lignin is efficiently removed from the biomass (**Table 1**). This process eliminates acetyl and uronic acid groups at hemicelluloses; as a result, the accessibility of enzyme that degrades hemicellulose increases [58]. Xylan ester linkages on hemicellulose residues are also hydrolyzed [56]. The advantages of alkali pretreatments are able to largely improve the cellulose digestibility, solubilize lignin more effectively, exhibit less cellulose, and hemicellulose solubilization compared to the acid pretreatments [59]. Alkali pretreatments can also be performed at lower temperature, pressure, and time ranging from hours to days. NaOH solution is more effective than other alkalis [60, 61]. Alkali pretreatments were shown to be more effective on decreasing the degree of polymerization and crystallinity, increasing the internal surface area of cellulose, and disrupting the lignin structure [41].

Besides NaOH, Ca(OH)₂ (lime) is another alkali widely used. It also eliminates lignin-carbohydrate ester and acetyl groups, and enhances cellulose digestibility [37]. Lime pretreatment has been proven successful for several biomass pretreatments, such as wheat straw, poplar wood, switchgrass, and corn stover [61, 62]. This pretreatment has lower reagent cost and less safety requirements compared to NaOH or KOH pretreatments. In addition, lime can be easier recovered from hydrolysate by reaction with CO₂ [37]. The air/oxygen addition to the alkaline pretreatments (NaOH or lime) can increase lignin removal [59].

3.4. Ammonia recycle percolation

Ammonia recycled percolation (ARP) is another type of ammonia-based pretreatment in which aqueous ammonia (5–15 wt %) passes through a packed bed reactor along with biomass materials at high temperature (140–210°C) for 90 min and the rate of percolation is maintained at 5 mL/min [56, 64]. ARP can remove hemicellulose and lignin from the biomass as the liquid phase [31] although requires high liquid loading or process temperature. To reduce energy cost, soaking in aqueous ammonia (SAA) at lower temperatures (40–90°C) for longer reaction times has been used. This approach can preserve most of the glucan and xylan in the biomass samples which are then fermented using the simultaneous saccharification and cofermentation (SSCF) method [63].

3.5. Biological pretreatment

Biological pretreatments are treatments to biomass materials with microbes such as white rot fungi. Like conventional physicochemical methods, the objectives of biological pretreatments are to degrade lignin. The biological pretreatment is considered as a cheap, ecofriendly, and efficient method [64]. This method is carried out using cellulolytic and hemicellulolytic microbes, such as filamentous fungi which are ubiquitous and can be isolated from soil, living plants or lignocellulosic waste material [65, 66]. The most effective microorganisms for the pretreatment of most of the biomass materials are white-rot fungi [52], such as *Ceriporia lacerata*, *Ceriporiopsis subvermispora*, *Cyathus stercoreus*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus*, and *P. chrysosporium*. These fungi produce

lignin peroxidases—lignin-degrading enzymes and manganese-dependent peroxidases—which show high delignification efficiency on various biomass materials [52, 67]. Some advantages of biological pretreatments include low-capital cost, low energy requirement, no chemical requirement, and mild environmental conditions (**Table 1**). However, the main drawback of the biological methods is that hydrolysis rate is very low [56]. To solve this drawback, some researches have to perform to find out isolates which have ability to delignify the biomass materials more quickly and efficiently.

3.6. CO₂ explosion pretreatment

CO₂ explosion pretreatment utilizes CO₂ as a supercritical fluid. The fluid displays gas like mass transfer properties and a liquid-like solvating power. This method can remove lignin effectively so that enzymes can digest biomass materials effectively [68]. Supercritical CO₂ has been mostly used as an extraction solvent because of its several advantages including easy recovery after extraction, environmental acceptability, non-toxicity, non-flammability, and relatively low cost (**Table 1**) [69]. In aqueous solution, CO₂ reacts with H₂O to form carbonic acid and increases hydrolysis rate. Because of their small size, CO₂ molecules are able to penetrate small pores accessible to ammonia and water molecules. In this pretreatment, cellulose and hemicellulose structures disrupt so that the surface area of the substrate increases and can easily attack by the digestive enzymes. For several substrates, the CO₂ explosion pretreatment is more cost-effective than ammonia expansion and produces lower inhibitors than steam explosion [70].

3.7. Liquid hot water pretreatment

Liquid hot water (LHW) is one of the hydrothermal pretreatment without rapid decompression and any catalyst or chemical additions and performs under high pressure in order to maintain the water in the liquid state at high temperatures. It is usually carried out at temperature range between 170 and 230°C and pressure (5 MPa) [71]. LHW eliminates hemicellulose from biomass materials so that the cellulose is more accessible to enzymatic attack (**Table 1**). After pretreatment, the obtained slurry is able to be filtered to yield two fractions, namely a solid cellulose-enriched fraction and a liquid fraction containing high hemicellulose derived sugars. Better pH (4–7) of this pretreatment can be controlled in order to minimize the non-specific degradation of polysaccharides and also to avoid the formation of inhibitors [37]. To promote more effective contact between the biomass materials and the liquid water, three methods have been developed, namely co-current, countercurrent, and flow-through methods. In co-current method, water and biomass slurry are heated to the desired temperature and held at the pretreatment conditions for a certain residence time before being cooled. Countercurrent method is designed to move water opposite to biomass through the pretreatment system. Hot water flows through passage system over a stationary bed of biomass which hydrolyzes and dissolves biomass components and brings them out of the system [72, 73]. LHW pretreatments are generally preferred because it is required lower costs due to no need chemicals and corrosion-resistant materials for hydrolysis reactors. In addition, the LHW pretreatments produce lower concentration of the solubilized hemicellulose and lignin products due to high water input (**Table 1**). Lower formation of inhibitory components and higher

pentose recovery can be achieved in this LHW pretreatment compared to the steam explosion. However, this method has not yet developed at a commercial scale due to higher water demand and high energy input.

3.8. Milling pretreatment

Milling pretreatment is commonly used for reducing size biomass and altering the inherent ultrastructure of biomass and degree of crystallinity so that the biomass can be easier accessed by cellulase enzymes [74]. This pretreatment is performed prior to enzymatic hydrolysis or even other chemical pretreatment processes [74, 75, 76, 77]. There are several kinds of milling, such as ball, tow roll, hammer, colloid, and vibro energy millings [40]. Milling is able to improve susceptibility to enzymatic hydrolysis because of reducing the biomass size [78], decreasing the biomass crystallinity, [79] and increasing the biomass area.

3.9. Steam explosion pretreatment

Steam explosion pretreatment is a treatment with high pressure saturated steam for few seconds (30 s) to several minutes (20 min), and then pressure is suddenly reduced. These pretreatments are the most commonly used for treating biomass materials [80, 81]. Steam explosion—typically a combination of mechanical forces and chemical—is able to hydrolyze (autohydrolyze) acetyl groups of hemicellulose. At high temperatures (160–260°C), autohydrolysis occurs and produces acetic acid from acetyl groups of the biomass materials [82, 83]. In addition, water is also able to act as an acid at high temperatures. Reducing pressure suddenly produces explosive decompression so that biomass fibers separate each other. This process is able to degrade hemicellulose and lignin because of explosive decompression, and thus increase the potential of cellulose hydrolysis [82].

Steam explosion processes have several advantages compared to other pretreatment methods (Table 1). The advantages include high sugar recovery, less hazardous process chemicals and conditions, lower environmental impact, lower capital investment, possibility of using larger chip size, no acid catalyst additions except for softwoods, more efficient in energy usage, and significant improvement in enzymatic hydrolysis as well as its feasibility at industrial scale [84]. The main drawbacks of steam explosion pretreatment are the partial degradation of hemicelluloses producing inhibitor compounds that can affect the enzymatic hydrolysis and fermentation process [85, 86]. Thus, an inhibitor compound separation becomes necessary (e.g., addition of activated charcoal, over liming, and ion exchange) and will increase the overall process cost [31, 87].

3.10. Wet oxidation pretreatment

Wet oxidation is treated biomass materials with water and air/oxygen at temperatures higher than 120°C for 30 min [88]. This wet oxidation pretreatment is suitable for pretreatment of biomass materials containing high lignin content. The most effective parameters in the wet oxidation process are temperature, reaction time, and oxygen pressure [87]. This pretreatment yields acid compounds from the hydrolytic processes and oxidative reactions of biomass materials.

Wet oxidation pretreatments affect all three main components of lignocellulosic materials, namely, cellulose, hemicellulose, and lignin. The hemicelluloses are extensively hydrolyzed to low molecular weight sugars that become soluble in water. Lignin is cleaved and oxidized, and cellulose is partly degraded and becomes highly susceptible to enzymatic hydrolysis. Addition of some alkaline compounds, such as sodium carbonate make easier to hydrolyze hemicellulose components and also minimizes the formation inhibitor compounds, such as furan-based degradation products [89].

The main advantage of wet oxidation is efficient lignin removal and less inhibitor formations (**Table 1**). The main disadvantage of this method is requirements for high temperature and pressure maintenances and the presence of strong oxidizing agents such as H_2O_2 . These requirements cause to high costs of maintenance and also require large-scale reactors. Therefore, application of this process is limited in large-scale pretreatment of biomass materials.

4. Conclusions and future perspectives

Empty fruit bunch of oil palm trees have been described in terms of their physical and chemical characteristics. As solid waste products of palm oil factories, physical EFBOPT characteristics are brown, not uniform, low bulk density, varying in length from 17 to 30 cm, and varying in width from 25 to 35 cm depending on the size of fresh fruit bunch. EFBOPT fiber is mainly constructed from a complex matrix of three main polymers, namely, cellulose, hemicellulose, and lignin. The main chemical component of EFBOPT is cellulose (44.2–50.0%) and the others are hemicellulose (22.0–33.5%) and lignin (20.4–21.0%) which should be removed through pretreatments before the cellulose is hydrolyzed enzymatically.

There are three kinds of pretreatment methods, namely, physical, chemical, and physico-chemical and biological pretreatment methods. The various pretreatment methods for biomass materials have been described to improve enzymatic saccharifications and ethanol production. Each method has its advantages and disadvantages. There is no one treatment method yielding 100% conversion of biomass into fermentable sugars. There is always a loss of biomass materials, which affects the final yield and increases the cost of finished product, i.e., bioethanol. Although a combination of two or more pretreatment methods has indicated promising results, we still feel that there is a need for extensive researches in this area so that either a new efficient treatment method is discovered or an existing method is upgraded to yield better results. Predictive models will enable the selection, design, optimization, and process control of pretreatment methods that are suitable for biomass materials.

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References

- [1] EIA. Annual energy outlook, 1999, with projections to 2020. In: DOE/EIA-383. Washington, DC, USA: Energy Information Administration, Department of Energy; 1998
- [2] Jergensen H, Kristensen JB, Felby C. Enzymatic conversion of lignocellulose into fermentable sugars: Challenge and opportunities. *Biopr*. 2007;**1**:119-134
- [3] Brown MA, Levine MD, Romm JPP, Koomey JH. Engineering-economic studies of energy technologies to reduce green house gas emissions: Opportunities and challenges. *Annual Review of Energy and the Environment*. 1998;**23**:31-39
- [4] Gomez LD, Steel-King CG, McQueen-Mason J. Sustainable liquid biofuels from biomass: The writing's on the wall. *New Phytologist*. 2008;**178**:473-485
- [5] Espadafor FJ, García MT, Villanueva JB, Gutiérrez JM. The viability of pure vegetable oil as an alternative fuel for large ships. *Transportation Research Part D: Transport and Environment*. 2009;**14**:461-469. CrossRefGoogle Scholar
- [6] Isariyakul T, Dalai AK. Biodiesel from vegetable oils. *Renewable and Sustainable Energy Reviews*. 2014;**31**:46-471. CrossRefGoogle Scholar
- [7] Argun H, Kargi F. Effects of sludge pre-treatment method on bio-hydrogen production by dark fermentation of waste ground wheat. *International Journal of Hydrogen Energy*. 2009;**34**:8543-8548
- [8] Chakraborty D, Karthikeyan OP, Selvam A, Wong JWC. Co-digestion of food waste with chemically enhanced primary treated sludge in a continuous stirred tank reactor. *Biomass and Bioenergy*. 2017. DOI: 10.1016/j.biombioe.2017.06.002
- [9] Hoekman SK. Biofuels in the US-challenges and opportunities. *Renewable Energy*. 2009;**34**:14-22
- [10] Zabed H, Faruq G, Sahu JN. Bioethanol production from fermentable sugar juice. *Scientific World Journal*. 2014;**2014**:1-11
- [11] Nuwamanya E, Chiwona L, Kawuki R, Baguma Y. Bio-ethanol production from non-food parts of cassava (*Manihot esculenta* Crantz). *Ambio*. 2012;**41**:262-270

- [12] Sukowati A. Bioethanol Production from Banana Peel Hydrolyzed with H_2SO_4 [Produksi Bioetanol Dari Kulit Pisang Melalui Hidrolisis Asam Sulfat]. Thesis of Graduate Program of Agroindustrial Technology Magister, Faculty of Agriculture, Lampung University, [Tesis. Program Pasca Sarjana Magister Teknologi Agroindustri, Fakultas Pertanian. Universitas Lampung]. Bandar Lampung; 2014
- [13] Sucihati. Pretreatment and Enzymatic Hidrolisis Optimations Of Cacao Pod For Bioethanol Production [Optimasi Perlakuan Awal dan Hidrolisis Enzimatik Kulid Cacao untuk Produksi Bioetanol]. Thesis of Graduate Program of Agroindustrial Technology Magister, Faculty of Agriculture, Lampung Univerwsity, [Tesis. Program Pasca Sarjana Magister Teknologi Agroindustri, Fakultas Pertanian. Universitas Lampung]. Bandar Lampung, 2014
- [14] Riswandi B. Effects of Rice Strow Size, H_2SO_4 Concentration, and Hydrolysis Time on reducing sugar of Rice Straw as raw materials of bioethanol production. [Pengaruh Ukuran Jerami, Konsentrasi H_2SO_4 , dan Lama Hidrolisis Terhadap Kadar Gula Reduksi Jerami Padi Sebagai Bahan Baku Prouksi Bioetanol], Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung]. Bandar Lampung, 2011
- [15] Yulianingsih H. Rice Straw Hydrolysis with Sulfuric Acid to Glucose as Raw Materials of Bioethanol Production, petrolium replacement [Hidrolisis jerami padi dengan asam sulfat menjadi glukosa sebagai bahan baku bioethanol pengganti BBM], Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung]. Bandar Lampung; 2010
- [16] S Hidayati, S Nawansih, O Nurainy, F Rizal, S Marniza, Dan Arion R. Bagasse Lignin Degradation resulting of Alkali Pre-Treatment at Various Condition [Tingkat degradasi Lignin Bagas Tebu Akibat Perlakuan Basa Pada Berbagai Kondisi]. Presented at National Seminar of Applied Technology in Politeknik Negeri Lampung in April 2010 [Disampaikan Dalam Seminar Nasional Teknologi Tepat guna di Politeknik Negeri Lampung Pada Bulan April]. Bandar Lampung: Universitas Lampung; 2010. <http://blog.unila.ac.id/sutiknounila/category/research-activities>
- [17] Septiyani R. Effects of Cellulase Concentration and Hydrolysis Time on reducing sugar of bagasse. [Pengaruh Konsentrasi dan Lama Hidrolisis enzim Selulase Terhadap Kadar Gula Reduksi Ampas Tebu], Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung]. Bandar Lampung; 2011
- [18] Ningtys KR. Effects of Reaction Time and HCl Concentration on Bagasse Lignocellulose Degradation. [Pengaruh lama reaksi dan konsentrasi asam klorida (HCl) terhadap degradasi lignoselulosa bagas tebu], Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University

- [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung]. Bandar Lampung; 2010
- [19] Kismurtono M, Sutikno S. Effect of alkali pretreatment and enzymatic saccharification on bagasse reducing sugar for bioethanol production. Proceeding of International Conference on Sustainable Energy Engineering and Application; ISBN 978-602-17952-1-7. Grand Hotel Preanger, Bandung, Indonesia. 14-15 October 2014; 2014
- [20] Carere CR, Sparling R, Cicek N, Levin DB. Third generation biofuel via direct cellulose fermentation. *International Journal of Molecular Sciences*. 2008;9:1342-1360
- [21] Putri FY. Effects of NaOH concentration and immersion time on hemicellulose, cellulose, and lignin contents of empty fruit bunch of oil palm tree for bioethanol production [Pengaruh Konsentrasi NaOH dan Lama Perendaman TKKS (*Elaeis guineensis* JACQ) Terhadap Kadar Hemiselulosa, Selulosa dan Lignin untuk Produksi Biobutanol], Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung]. Bandar Lampung. 96 hlm, 2010
- [22] Widyasari R. Effects of Cellulase Concentration and Hydrolysis Time on reducing sugar of cellulose and hemicellulose of empty fruit bunch of oil palm tree as raw materials of bioethanol production [Pengaruh Konsentrasi dan Lama Inkubasi Enzim Selulase untuk Menghidrolisis Selulosa dan Hemiselulosa TKKS menjadi Gula Reduksi sebagai Bahan Baku Bioetanol]. Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University, 61 pages [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung, 61 halaman]. Bandar Lampung; 2011
- [23] Feriandi. Study of chemical and enzymatic pretreatment on empty fruit bunch of oil palm tree into reducing sugar as bioethanol raw material [Kajian Perlakuan Awal secara Kimiawi dan Enzimatik Limbah Tandan Kosong Kelapa Sawit (TKKS) menjadi Gula Reduksi sebagai Bahan Baku Bioetanol]. Thesis of Graduate Program of Agroindustrial Technology Magister, Faculty of Agriculture, Lampung Univerwsity, [Tesis. Program Pasca Sarjana Magister Teknologi Agroindustri, Fakultas Pertanian. Universitas Lampung]. Bandar Lampung; 2011
- [24] Fitri M Y. Effects of sulfuric acid concentration and hydrolysis time on reducing sugar of empty fruit bunch of oil palm tree as raw materials of bioethanol production [Pengaruh Konsentrasi Asam Sulfat (H_2SO_4) dan Waktu Hidrolisis Tandan Kosong Kelapa Sawit (TKKS) Terhadap Kadar Gula Reduksi Sebagai Bahan Baku Bioetanol]. Mini Thesis for Bachelor Degree, The Department of Agricultural Product Technology, Faculty of Agriculture, Lampung University [Skripsi. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian, Universitas Lampung]. Bandar Lampung. 48 hlm; 2013
- [25] Sheil D, Casson A, Meijaard E, Gaskel J, Sunderland-Groves J, Wertz K, Kanninen M. The impact and opportunities of oil palm in South East Asia. In: *What Do we Know and What Do we Need to Know?* Bogor: Indonesia. Cifor; 2009

- [26] Misson M, Haron R, Fadhzir M, Kamaoddin A, Amin NAS. Pretreatment of empty palm fruit bunch for lignin degradation. *Jurnal Teknologi*. 2009;**50**:89-98
- [27] Carere CR, Sparling R, Cicek N, Levin DB. Third generation biofuel via direct cellulose fermentation. *International Journal of Molecular Sciences*. 2008;**9**:1342-1360
- [28] Gomez LD, Steel-King CG, McQueen-Mason J. Sustainable liquid biofuels from biomass: The writing's on the wall. *New Phytologist*. 2008;**178**:473-485
- [29] Jagger A. Brazil invests in second-generation biofuel. *Biofuels, bioprod. BioReference*. 2009;**3**:8-10
- [30] Knauf M, Moniruzzaman M. Lignocellulosic biomass processing: A perspective. *International Sugar Journal*. 2004;**106**(1263):147-150
- [31] Yang B, Wyman CE. Pretreatment: The key to unlocking low cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*. 2008;**2**:26-40
- [32] Katamaneee A. Appropriate technology evaluation for oil palm product utilization in Krabi province. 2006. Mahidol University, 1 February 2011. Available from: www.li.mahidol.ac.th/thesis/2549/cd388/4637145.pdf
- [33] Quintero-Ramirez R. Hydrolysis of Lignocellulosic Biomass. In: Luis Augusto Barbosa Cortez (Coord.). *Sugarcane bioethanol—R&D for Productivity and Sustainability*, São Paulo: Editora Edgard Blücher, 2014. p. 717-732. http://dx.doi.org/10.5151/BlucherOA-Sugarcane-SUGARCANEETHANOL_60
- [34] Astimar AA, Husin M, Anis M. Preparation of cellulose from oil palm empty fruit bunches via ethanol digestion: Effect of acid and alkali catalyst. *Journal of Oil Palm Research*. 2002;**14**:9-14
- [35] Bari MN, Alam MZ, Suleyman AM, Jamal P, Mamun AA. Improvement of production of citric acid from oil palm empty fruit bunches: Optimization of media by statistical experimental designs. *Bioresource Technology*. 2009;**100**:3113-3120
- [36] Palonen H. Role of lignin in the enzymatic hydrolysis of lignocellulose. VTT Publications. 2004;**520**:1-80
- [37] Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*. 2005;**96**:673-686. DOI: 10.1016/j.biortech.2004.06.025. [PubMed] [Cross Ref]
- [38] Chang VS, Holtzapple MT. Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology*. 2000;**84**:5-37. DOI: 10.1385/ABAB:84-86:1-9:5. [PubMed] [Cross Ref]
- [39] Jeffries TW. Biodegradation of lignin-carbohydrate complexes. *Biodegradation*. 1990;**1**:808-812
- [40] Taherzadeh MJ, Karimi K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *International Journal of Molecular Sciences*. 2008;**9**:1621-1651. DOI: 10.3390/ijms9091621. [PMC free article][PubMed] [Cross Ref]

- [41] Wyman CE. Handbook on Bioethanol: Production and Utilization. Washington DC, USA: Taylor & Francis; 1996
- [42] Brodeur G, Yau E, BAdal K, Collier J, Ramachandran KB, Ramakrishnan S. Review article: Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Enzyme Research*. 2011;**2011**:787532. 17 pages. DOI: 10.4061/2011/787532
- [43] Maurya PA, Singla A, Negi S. An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech*. 2015;**5**:597-609. DOI: 10.1007/s13205-015-0279-4
- [44] Sindhu R, Kuttiraja M, Binod P, Janu KU, Sukumaran RK, Pandey A. Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. *Bioresource Technology*. 2011;**102**:10915-10921
- [45] Rodriguez-Chong A, Ramirez JA, Garrote G, Vazquez M. Hydrolysis of sugarcane bagasse using nitric acid: A kinetic assessment. *Journal of Food Engineering*. 2004;**61**:143-152
- [46] Gámez S, González-Cabriales JJ, Ramírez JA, Garrote G, Vázquez M. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. *Journal of Food Engineering*. 2006;**74**:78-88
- [47] Hernández-Salas JM, Villa-Ramírez MS, Veloz-Rendón JS, RiveraHernández KN, González-César RA, Plascencia-Espinosa MA, Trejo-Estrada SR. Comparative hydrolysis and fermentation of sugarcane and agave bagasse. *Bioresource Technology*. 2009;**100**:1238-1245
- [48] Lee J, Jeffries TW. Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors. *Bioresource Technology*. 2011;**102**:5884-5890
- [49] Lee J, Houtman CJ, Kim H, Choi I, Jeffries TW. Scale-up study of oxalic acid pretreatment of agricultural lignocellulosic biomass for the production of bioethanol. *Bioresource Technology*. 2011;**102**:7451-7456
- [50] Kim JW, Kim KS, Lee JS, Park SM, Cho HY, Park JC, Kim JS. Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid. *Bioresource Technology*. 2011;**102**:8992-8999
- [51] Laureano-Pérez L, Teymouri F, Alizadeh H, Dale BE. Understanding factors that limit enzymatic hydrolysis of biomass. *Applied Biochemistry and Biotechnology*. 2005;**121**:1081-1099
- [52] Kumar R, Wyman CE. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnology Progress*. 2009;**25**:302-314
- [53] Kumar R, Wyman CE. Does change in accessibility with conversion depend on both the substrate and pretreatment technology. *Bioresource Technology*. 2009;**100**:4193-4202
- [54] Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY. Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology*. 2005;**96**:1959-1966

- [55] Teymouri F, Laureano-Pérez L, Alizadeh H, Dale BE. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn Stover. *Bioresource Technology*. 2005;**96**:2014-2018
- [56] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*. 2002;**83**:1-11
- [57] Sendich E, Laser M, Kim S, Alizadeh H, Laureano-Perez L, Dale B, Lynd L. Recent process improvements for the ammonia fiber expansion (AFEX) process and resulting reductions in minimum ethanol selling price. *Bioresource Technology*. 2008;**99**:8429-8435
- [58] Chang VS, Holtzaple MT. Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology*. 2000;**84**:5-37
- [59] Carvalho F, Duarte LC, Gírio FM. Hemicellulose biorefineries: A review on biomass pretreatments. *Journal of Scientific and Industrial Research*. 2008;**67**:849-864
- [60] Sun RC, Lawther JM, Banks WB. Influence of alkaline pretreatments on the cell-wall components of wheat-straw. *Industrial Crops and Products*. 1995;**4**:127-145
- [61] Chang VS, Nagwani M, Kim CH, Holtzaple MT. Oxidative lime pretreatment of high-lignin biomass: Poplar wood and newspaper. *Applied Biochemistry and Biotechnology*. 2001;**94**:1-28
- [62] Kim S, Holtzaple MT. Delignification kinetics of corn stover in lime pretreatment. *Bioresource Technology*. 2006;**97**:778-785
- [63] Kim TH, Taylor F, Hicks KB. Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. *Bioresource Technology*. 2008;**99**:5694-5702
- [64] Wan C, Li Y. Fungal pretreatment of lignocellulosic biomass. *Biotechnology Advances*. 2012;**30**:1447-1457
- [65] Vats S, Maurya DP, Shaimoon M, Agarwal A, Negi S. Development of a microbial consortium for the production of blend enzymes for the hydrolysis of agricultural waste into sugars. *Journal of Scientific and Industrial Research*. 2013;**72**:585-590
- [66] ISROI. Characteristic of oil palm empty fruit bunch pretreated with *Pleurotus florida* *menara*. *Menara Perkebunan*. 2017;**85**(2):67-76. p-ISSN: 0215-9318/e-ISSN: 1858-3768. DOI: <http://dx.doi.org>. DOI: 10.22302/iribb.jur.mp.8v5i2.234
- [67] Shi J, Chinn MS, Sharma-Shivappa RR. Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. *Bioresource Technology*. 2008;**99**:6556-6564
- [68] Kim KH, Hong J. Supercritical CO₂ pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. *Bioresource Technology*. 2001;**77**:139-144
- [69] Schacht C, Zetzl C, Brunner G. From plant materials to ethanol by means of supercritical fluid technology. *Journal of Supercritical Fluids*. 2008;**46**:299-321
- [70] Zheng Y, Lin HM, Tsao GT. Pretreatment for cellulose hydrolysis by carbon dioxide explosion. *Biotechnology Progress*. 1998;**14**:890-896

- [71] Sánchez OJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology*. 2008;**99**:5270-5295
- [72] Liu C, Wyman CE. The effect of flow rate of compressed hot water on Xylan, lignin, and total mass removal from corn stover. *Industrial and Engineering Chemistry Research*. 2003;**42**:5409-5416
- [73] Yang B, Wyman CE. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. *Biotechnology and Bioengineering*. 2004;**86**:88-95
- [74] Mais U, Esteghlalian AR, Saddler JN, Mansfield SD. Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling. *Applied Biochemistry and Biotechnology*. 2002;**98**:815-832. [PubMed]
- [75] Tassinari T, Macy C. Differential speed two roll mill pretreatment of cellulosic materials for enzymatic hydrolysis. *Biotechnology and Bioengineering*. 1977;**19**:1321-1330. [PubMed]
- [76] Zhang RH, Zhang ZQ. Biogasification of rice straw with an anaerobic-phased solids digester system. *Bioresource Technology*. 1999;**68**:235-245
- [77] Muller CD, Abu-Orf M, Novak JT. Application of mechanical shear in an internal-recycle for the enhancement of mesophilic anaerobic digestion. *Water Environment Research*. 2007;**79**:297-304. [PubMed]
- [78] Zeng M, Mosier NS, Huang CP, Sherman DM, Ladisch MR. Microscopic examination of changes of plant cell structure in corn stover due to hot water pretreatment and enzymatic hydrolysis. *Biotechnology and Bioengineering*. 2007;**97**:265-278. [PubMed]
- [79] Fan LT, Lee Y, Beardmore DH. Mechanism of the enzymatic hydrolysis of cellulose: Effects of major structural features of cellulose on enzymatic hydrolysis. *Biotechnology and Bioengineering*. 1980;**22**
- [80] Chandra RP, Bura R, Mabee WE, Berlin A, Pan X, Saddler JN. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics. *Advances in Biochemical Engineering/Biotechnology*. 2007;**108**:67-93
- [81] Singh J, Suhag M, Dhaka A. Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: A review. *Carbohydrate Polymers*. 2015;**117**:624-631
- [82] Pan X, Xie D, Gilkes N, Gregg DJ, Saddler JN. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. *Applied Biochemistry and Biotechnology: Part A, Enzyme Engineering and Biotechnology*. 2005;**124**:1069-1079
- [83] Quievy N, Jacquet N, Sclavons M, Deroanne C, Paquot M, Devaux J. Influence of homogenization and drying on the thermal stability of microfibril-lated cellulose. *Polymer Degradation and Stability*. 2009;**95**:306-314

- [84] Avellar BK, Glasser WG. Steam-assisted biomass fractionation. I. Process considerations and economic evaluation. *Biomass and Bioenergy*. 1998;**14**:205-218
- [85] Oliva JM, Sáez F, Ballesteros I, González A, Negro MJ, Manzanares P, Ballesteros M. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*. *Applied Microbiology and Biotechnology*. 2003;**105**:141-154
- [86] Hamelinck CN, van Hooijdonk G, Faaij APC. Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle- and long-term. *Biomass and Bioenergy*. 2005;**28**:384-410
- [87] Schmidt A, Thomsen A. Optimization of wet oxidation pretreatment of wheat straw. *Bioresource Technology*. 1998;**64**:139-151
- [88] Varga E, Klinke HB, Reczey K, Thomsen AB. High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol. *Biotechnology and Bioengineering*. 2004;**88**:567-574
- [89] Ahring BK, Jensen K, Nielsen P, Bjerre AB, Schmidt AS. Pretreatment of wheat straw and conversion of xylose and Xylan to ethanol by thermophilic anaerobic bacteria. *Bioresource Technology*. 1996;**58**:107-113

