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Irradiation Pretreatment of Tropical Biomass and Biofiber for Biofuel Production

Mohd Asyraf Kassim, H.P.S Abdul Khalil, Noor Aziah Serri, Mohamad Haafiz Mohamad Kassim, Muhammad Izzuddin Syakir, N.A. Sri Aprila and Rudi Dungani

Abstract

Interest on biofuel production from biomass and biofiber has gain great attention globally because these materials are abundant, inexpensive, renewable, and sustainable. Generally, the conversion of biomass and biofiber to biofuel involves several processes including biomass production, pretreatment, hydrolysis, and fermentation. Selecting the most efficient pretreatment is crucial to ensure the success of biofuel production since pretreatment has been reported to contribute substantial portion on the production cost. The main goal of the pretreatment is to enhance digestibility of the biomass and biofiber, and to increase sugar production prior to fermentation process. To date, several pretreatment methods have been introduced to pretreat biomass and biofiber including irradiation. This book chapter reviews and discusses different leading irradiation pretreatment technologies along with their mechanism involved during pretreatment of various tropical biomass and biofiber. This chapter also reviews the effect of irradiation pretreatment on the biomass and biofiber component, which could assist the enzymatic saccharification process.

Keywords: irradiation, pretreatment, biomass, biofiber, biofuel

1. Introduction

Rapid development and increase growth of population has led to global environmental problems. Furthermore, increasing demand on energy source has contributed to a reduction of
petroleum reverse. To overcome this problem, production of biofuel from renewable resources such as biomass and biofiber has gained great attention to partially replace fossil fuels in the future. Production of biofuel from these materials is environmentally friendly and sustainable. Mass production of biomass and biofiber as waste residue from agriculture and forestry industries has created a great concern for environmental sustainability globally. Generally, biomass and biofiber can be generated from various origins, either direct cultivation, or as residue from agro-waste and forestry industries. The biomass and biofiber residue generated from these industries including sugarcane, bagasse, rice straw, empty fruit bunch (EFB), oil palm trunk (OPT), oil palm frond (OPF), and sago bark. Meanwhile, the examples for the cultivated biofiber are kenaf and hemp.

In order to produce biofuel from these materials, it has to undergo several processes such as biomass production, pretreatment, saccharification, and fermentation. Pretreatment of biomass has been reported to contribute a substantial portion of the liquid biofuel production cost. There are four established pretreatment methods such as thermal, chemical, biological, and physical pretreatment that have been applied to pretreat biomass and biofiber. However, among the pretreatments mentioned, physical pretreatment method especially irradiation method is considered as one of the promising approaches applied to reduce the recalcitrance of biomass and biomaterials. Generally, this method utilizes both thermal and non-thermal effects generated by intermolecular collision during the realignment of biomass molecule. Irradiation pretreatment offers great advantages such as having a very selective process, and it is energy efficient. Since there are wide ranges of tropical biomass types renewably available in tropical countries, thus, exploring the potential of this pretreatment is really much needed.

This chapter discusses comprehensively on irradiation pretreatment of tropical biomass prior to the subsequent enzymatic saccharification and fermentation processes. The emphasis is given on the type of irradiation pretreatments and mechanism that could be beneficial for scientists and researchers to understand the process, which can be applied as an alternative pretreatment approach for biofuel production.

2. Biomass and biofiber

Renewable biomass and biofiber are a carbon-based biological material derived from living organism. It is composed of a mixture of organic molecules containing hydrogen, oxygen, nitrogen, and small quantities of other atoms such as alkali, alkaline earth, and heavy metals. These materials are abundant, eco-friendly, low cost, and sustainable biomaterials. The biomass and biofiber produced from various industries and manufacturing can be used in composite, textile, food, and chemical industries. On the other hand, these materials have also gained a great attention as a liquid biofuel feedstock due to low cost feedstock materials and environment friendly conversion process.

The renewable biomass and biofiber materials can be categorized into five major categories based on their origin as presented in Figure 1. Five distinct biomass and biofiber categories include: (1) Wood and non-wood (softwood, hardwood, and residue), (2) animal fiber (wool,
silk, hair) (3) aquatic plant (algae and hyacinth) (4) plant fiber (cultivated, residue), and (5) other renewable resource (animal residue, municipal solid waste [MSW], industrial residue, sewage).

Figure 1. Schematic classification of renewable resources for biofuel production.

These biomass and biofiber are natural biomaterials and can be described as lignocellulosic materials comprised of cellulose, hemicellulose, and lignin. These materials can be further categorized into five categories based on which part it comes from. The five categories are (1) leaf, (2) seed-hair, (3) coir, (4) bark or stem, and (5) other than part mentioned above [1].

Most of the biomass and biofiber generally have a very low economic value. However, due to a broad range of characteristics especially in chemical composition, distribution has provided a variety of applications (Table 1).

The biomass and biofiber produced from agro-industry can be used in the plywood, hybrid composite, and animal feed. In any case, the biomass generated from the industry also can be converted into bioenergy and other chemicals, for instance acid and solvent, and liquid biofuel. Due to environmental concern and reduction of fossil fuel reserve, production of liquid biofuel from biomass has gained a great attention because the process is environment friendly. In order to produce biofuel from biomass, it has to go through several processes such as biomass production, pretreatment, followed by enzymatic saccharification, and fermentation (Figure 2).
<table>
<thead>
<tr>
<th>Biomass</th>
<th>Extractive (%)</th>
<th>Hemicellulose (%)</th>
<th>Cellulose (%)</th>
<th>Lignin (%)</th>
<th>Ash (%)</th>
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<td>40</td>
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<td>26.0</td>
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<td>4.9</td>
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<td>63.9</td>
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<td>9.8</td>
<td>69.8</td>
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<td>1.1</td>
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<td>32.3</td>
<td>45.3</td>
<td>19.0</td>
<td>1.4</td>
<td>[8]</td>
</tr>
</tbody>
</table>

*nd, Not determined.*

Table 1. Chemical composition of various types of renewable biomass and biofiber.

Production of liquid fuel and value-added chemicals from biomass is believed to be one of the approaches to increase the value of biomass and biofiber generated. However, one of the main huddles to ensure the success of this process is the pretreatment process. Pretreatment process has been reported to contribute substantial portion in biofuel production cost. Thus, selecting the most efficient and low cost production could reduce biofuel production cost.

![Figure 2. Process flow diagram for biofuel production from biomass and biofiber through biochemical conversion.](image-url)
3. Pretreatment of biomass and biofiber for liquid biofuel

Pretreatment is one of the most important processes involved in liquid biofuel production through a biochemical conversion pathway. The main goal of the pretreatment is to increase the enzyme accessibility and improve the digestibility of polysaccharides or carbohydrate available in the biomass [9]. The highly organized structure makes plant biomass recalcitrant to physical, chemical, and microbial attack [10]. Thus, the challenge of using lignocellulosic biomass is to have a fast and economical process by integrating variety of pretreatment during the conversion of biofuel. Appropriate selection of pretreatment method must be taken into consideration accordingly to the type of biomass [11]. The pretreatment step involves reduction in biomass size, depolymerization, fractionation, and solubilization of the major components in the biomass, such as hemicellulose, cellulose, lignin, and extractives, making the remaining solid biomass more accessible for further subsequent process. Cellulose is a linear polymer composed of D-anhydroglucopyranose unit which is linked together by β-(1–4) glucosidic bond. This cellulose chains are packed into microfibrils that are attached to each other by hemicelluloses and amorphous polymer. These structures are attached together and covered by lignin. Lignin is an amorphous polymer that provides rigidity to the plant cell wall and protect against microbial attack.

Figure 3. Pretreatment of biomass and biofiber for sugar production.
In order to provide better access for enzymatic saccharification, the lignin, and hemicellulose needs to be separated from the cellulose through pretreatment process (Figure 3). Generally, pretreatment of biomass is totally dependent on the chemical composition of the biomass. The key factors for the effectiveness of the pretreatment of biomass and biofiber are: highly digestible, less sugar degradation, and produce less inhibitors that could reduce fermentation performance [12]. Biomass and biofiber that possesses a high recalcitrant component requires a harsh pretreatment condition to disrupt the cell structure.

The pretreatment of biomass and biofiber can be categorized into four different methods, namely thermal, physical, chemical, and biological (Figure 4). Thermal pretreatment is a treatment used to solubilize the biomass by applying heat in the pretreatment system. This method is one of the most common method used for the pretreatment of biomass and biofiber. Generally, the thermal pretreatment is sub-divided into three categories: (1) thermal treatment (temperature = < 100°C under atmospheric pressure); (2) hydrothermal treatment (temperature = > 100°C with gradual pressure release after treatment); and (3) thermal treatment with steam explosion (temperature > 100°C with sudden pressure drop after pretreatment). Temperature and reaction time are the most important factor that plays a major role in this pretreatment process [13]. This method proved to display a significant effect on the disruption of biomass and biofiber such as pelletized corn stover, rice hulls, kenaf, Tahoe mix, and switch grass [14–16]. Although this method was reported to display a positive effect on enzymatic saccharification process, this method is not selective and less effective for the biomass with less lignin content. Thermal pretreatment at high temperature would partially degrade hemicellulose and produce more inhibitors that could influence fermentation process [12, 17]. The major fermentation inhibitors such as hydroxymethylfurfural (HMF) and furfural are one of the major products made from the thermal pretreatment process [13].

![Figure 4. Pretreatment methods of biomass and biofiber for biofuel production.](image-url)
Chemical pretreatment is one of the most promising methods used to pretreat biomass and biofiber. Generally, this process has been proven successful, particularly when combined with heat [18, 19]. The chemical most commonly applied in this process is either an acid or alkali reagent. The main goal of chemical pretreatment is to solubilize polymers, favoring the availability of carbohydrate in the biomass for enzymatic saccharification. The most common acids used for biomass pretreatment are hydrochloric acid (HCl) and sulfuric acid (H₂SO₄). In this process, the acid will catalyze the linkage bond and solubilizes hemicellulose. Unlike acid pretreatment, the alkaline pretreatment method is considered very mild and environment friendly as this method uses low concentration of alkali [20]. Pretreatments with alkali such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)₂), hydrazine, and anhydrous ammonia cause swelling of biomass, disrupts the lignin structure, and breaks the linkage between lignin and the other carbohydrate fractions. Chemical pretreatment using acid and alkaline has been reported to be a promising approach to pretreat biomass and biofiber prior to enzymatic saccharification due to its capability to remove lignin and hemicellulose from the biomass. However, this pretreatment has few disadvantages such as, generation of inhibitors during the pretreatment and chemical used, which could affect subsequent fermentation process and is not environment friendly [21]. Thus, it has encouraged more exploration on other alternative pretreatment process that is sustainable and could be beneficial for the whole production line.

Another pretreatment that is commonly used to pretreat biomass and biofiber is biological pretreatment. This pretreatment involves microbes and enzymes to degrade the chemical compound and release fermentable sugar from the biomass and biofiber. In this method, microorganisms such as brown-, white-, and soft-rot fungi are used to degrade the biomass and biofiber cell wall. White rot fungi such as *Phanerochaete chrysosporium*, *Cleriponopsis subremospera*, *Phlebia subserialisis*, and *Pleurotus ostiiosis* are commonly used in biological pretreatment [22]. While, brown rot fungi for instance *Gleophyllum sepiarum*, *Fomitopsis pinicola*, and *Laetiporus sulphureus* are among the common brown rot fungi used to pretreat biomass and biofiber via this process [23]. During the biological pretreatment, hydrolytic enzyme such as lignin peroxidase (LiP) is produced by the bacteria or fungi and it will attack biomass and biofiber cell wall to a small compound with a low molecular weight, which subsequently, can be used in anaerobic fermentation for biofuel production. Currently, research on the direct enzymatic saccharification of biomass is still scarce.

This method appears to have a few advantages, for example, it requires low energy input and this process is mildly environment friendly. However, the large diversity of chemical composition among different types of biomass, enzyme production, and low hydrolysis rate are among the drawbacks that needs to be considered before the method is applied in a large-scale biofuel production. In spite of the many pretreatment methods tested, currently available pretreatment techniques can hardly meet the requirements of commercial application due to long processing times, chemical recycle problems, or high operational costs [9, 24]. Therefore, more works are required to understand and generate more information on the pretreatment of biomass and biofiber.
Physical pretreatment is a process that acts directly at breaking the cells through physical force. This method is widely used as a preliminary step for biomass pretreatment process. Physical pretreatment will reduce biomass size and increase the accessible surface area and pore size. Besides, it could also decrease the cellulose crystallinity and polymerization degrees. Various types of physical pretreatment have been introduced to pretreat biomass including comminution, milling (ball milling, colloid milling, and vibro energy milling), extrusion, and irradiation. The biomass pretreatment using irradiation has been reported to require less energy compared to other approaches mentioned. Furthermore, this approach is selective and easy to control, thus it is more efficient for production of the desired product [25]. The details on the irradiation pretreatment is described in the next section.

4. Irradiation pretreatment and its mechanisms

Among various physical methods, irradiation is considered an attractive method for biomass and biofiber pretreatment. In biomass irradiation process, biomass and biofiber is exposed to high-energy radiations such as ultrasonic waves, microwaves, γ-rays, and electron beam. The irradiation effect on the biomass and process mechanisms varies according to the method applied. Generally, radiation processing technology is defined as: a radiolysis reaction, which uses γ-rays from radioisotopes such as cobalt-60 or cesium-137, or an electron beam produced by an electron accelerator to induce degradation of cellulose. In this process, the high energy radiation generated could change the characteristic of cellulosic biomass including: enhance specific surface area, reduce the degree of polymerization and crystallinity of cellulose, hydrolysis of hemicellulose, and partial depolymerization of lignin [26–29]. Typically, the irradiation pretreatment mechanism mode significantly depends on the technology applied during the pretreatment process. The effect of irradiation pretreatment is assessed based on the reducing sugar production during enzymatic saccharification and the solid residues left after pretreatment. The effectiveness of the treatment depends on several factors such as frequency of radiations, time of exposure, composition of the biomass, and resistance to the radiations by medium between radiations and biomass [30, 31]. Besides, the pretreatment combination used of the irradiation pretreatment and chemical treatment also gives a significant effect on the reducing sugar production during the enzymatic saccharification process [32, 33]. The detailed explanation on the effect of irradiation pretreatment on the biomass and biofiber structure and functional group is described in the next section.

4.1. Type of irradiation pretreatments

There are four different irradiation pretreatment methods that is commonly being used to pretreat biomass prior to enzymatic saccharification process. The irradiation methods are gamma-ray irradiation, electron-beam irradiation, microwave, and ultrasonication. Aforementioned in the previous section, the pretreatment mechanism of each process is different according to the method applied.
4.1.1. Gamma-ray irradiation

Gamma ray is a high-energy ionizing radiation in electromagnetic spectrum that easily penetrates most materials. This irradiation is extremely large high frequency waves and largely depends on the radiation source. This technology is commonly applied in radiotherapy as a tracer in food and medical apparatus sterilization. Recently, the utilization of this technology has gain great attention especially in a biomass and biofiber pretreatment for liquid biofuel production. Radioactive nuclides such as cobalt-60 and cesium-137 are the common radioactive used in this pretreatment [28]. The main goal of this irradiation is to decrease intra and intermolecular order in cellulose due to the breakdown of the intermolecular hydrogen bonds. In this process, the radiation will travel from the seal source and penetrates (bombard) the biomass and biofiber. The energy carried by gamma radiation is transferred to the biomass component by collision of radiation, resulting to the loss of electron by the atom and lead to the ionization. Under exposure to radiation, the biomass component mainly cellulose molecules undergo scission, and various short and long-lived radicals are formed [34]. Also, the content of fragments with a low degree of polymerization generated from the process gradually increases, leading to the alteration of biomass structure, thus, providing ease of access for subsequent process such as enzymatic saccharification process.

The potential of gamma irradiation technology in biomass and biofiber pretreatment has been studied on various types of biomass for instance, jute fiber, poplar sawdust, wheat straw, and cotton-cellulose [33, 35]. There were only scanty studies on gamma irradiation pretreatment on tropical biomass and biofiber that has also been reported. A study on gamma irradiation of empty fruit bunches (EFB) indicated that the pretreatment has reduced the lignin and increased the cellulose content in the EFB [36]. Scanning electron microscopy – EDX (SEM-EDX) analysis showed that there is a significant change on the carbon and oxygen content in the EFB biomass. Typically, untreated EFB contains high carbon and low oxygen content, while the study found a decrease of carbon (9% increment) and decrease of oxygen content (16% decrease), indicating the reduction of lignin content in the EFB.

A comparison on gamma ray irradiation pretreatment on soft and hardwood has also been carried out using different level of dosage ranges between 10–100 kGy [37]. The study found that the most suitable condition for softwood was at 40 kGy, while higher dosage is required to pretreat hardwood (90 kGy). The study also concluded that gamma ray pretreatment process is species-dependent, wherein higher dosage is needed to disrupt hardwood cell structure compared to softwood.

4.1.2. Electron-beam irradiation

Electron-beam is one of the irradiation pretreatment used to pretreat biomass prior to enzymatic saccharification. This technology has been widely used in various applications such as welding, drilling, and surface treatment [38]. For commercial use, the most important characteristics of an accelerator are its electron energy and average beam power. Therefore, industrial electron accelerators are usually classified according to their energy ranges, which are divided into low (80–300 keV), medium (300 keV–5 MeV), and high-energy ranges (above 5 MeV). In the electron beam pretreatment, the biomass and biofiber is exposed to a highly charged stream
electron. The electron is emitted from an electron beam gun and accelerated by accelerator (Figure 5). In this pretreatment process, the electron energy can be controlled and modulated by varying the irradiation dose. The high-energy electrons emitted travel into biomass and biofiber component and transfer the energy within the materials. The heating process initiates chemical and thermal reaction in the biomass including cellulose depolymerization, and production of carbonyl group, resulting from the oxidation of the biomass. Crosslinking of biomass component has also been reported to occur when the biomass is exposed to irradiation beam [39]. Also, reduction of the biomass mechanical strength has been observed from the biomass exposed to electron beam. This could be due to the disruption of hydrogen bond between cellulose chains making it less crystalline and more amorphous [40].

![Figure 5. Experimental set-up for electron beam irradiation.](image)

Recently various research groups have studied the potential of electron beam radiation on various type of biomass including tropical biomass and biofiber such as bamboo, rice straw, oil palm, fruit bunch, and kenaf [30, 41, 42]. Overall, most of the EBI pretreatment indicated that a significant cellulose degradation was observed after the process [39]. Moreover, the study also showed that this pretreatment has enhanced enzymatic saccharification and reduce sugar production from biomass [31, 41]. A study on EBI pretreatment of hybrid grass biomass indicated that the pretreatment could enhance 59% of glucose yield from the biomass compared to untreated sample. This is similar to a study by Bak et al. [43] who reported that EBI pretreatment on rice straw could increase enzyme digestibility and energy during the pretreatment process.

Similar to other pretreatment process, EBI pretreatment process could be influenced by several factors. EBI dosage is one of the factors that play a major role in the EBI pretreatment of biomass process [39, 42]. A study on the EBI pretreatment of bamboo chips at various EBI dosage range 0.5–50 kGy, indicated that significant cellulose degradation was attained from the pretreatment dosage between 0–50 kGy. Furthermore, the study showed no significant changes on the hemicellulose content. This indicates that EBI pretreatment process is a selective process and the degradation level can be controlled by the EBI dosage [39].
4.1.3. Microwave irradiation

Microwave is electromagnetic waves between the frequency range of 0.3–300 GHz, and most of the microwave systems used for industrial and domestic purposes range between 0.9 GHz to 2.45 GHz [44]. Microwave radiation is a radiating wave movement and takes a straight-line path type of energy. This radiation do not require any medium to travel through and could penetrate non-metal materials such as plastic and glass. Microwaves can affect the material thermally and non-thermally. Thermally, microwaves heat the material by the interaction of the molecules of material with electromagnetic field produced by microwave energy (Figure 6). Non-thermally, microwaves affect and interact with the polar molecules and ions in the materials causing physical, chemical, and biological reactions [45].

![Figure 6. Conventional and microwave heating mechanisms of biomolecule.](image)

Many studies on the potential of microwave pretreatment towards various types of biomass and biofiber digestibilities such as switchgrass, sweet sorghum bagasse and mischantus have been reported [46–48]. Generally, the microwave pretreatment can be carried out through three different approaches:

a. Combination of mechanical and microwave pretreatment

b. Combination of microwave and chemical pretreatment

c. Combination of microwave and steam explosion pretreatment

Mechanical pretreatment is used to reduce biomass particle size and provide more surface area for further microwave pretreatment. Pretreatment through combination of microwave and chemical approaches will generally involve either acid or alkaline as catalyst. In this process, alkaline is used to swell the biomass structure and remove lignin component from the biomass [49]. On the other hand, acid catalyst used in this process will convert hemicellulose and cellulose component into a small monomer sugar such as glucose and xylose, which is the main platform for biofuel production [49]. In contrast to the combination of microwave and steam explosion approach, the high pressure and temperature radically disrupts the lignocellulosic
biomass structure and provide better access for hydrolytic enzyme to degrade cellulose and hemicellulose.

Study on microwaves pretreatment on biomass such as palm biomass has been widely reported \[50–52\]. Akhbar et al. \[53\] compared the microwave assisted chemical pretreatment of empty fruit bunches (EFB) with conventional method and found higher lignin removal of up to 72% using microwave assisted chemical treatment. The presence of chemical such as alkaline or acid in microwave pretreatment could assist fractionation of the biomass and biofiber.

The microwave pretreatment has also been applied on other types of palm biomass. Lai and Idris \[54\] in their study on the microwave pretreatment of oil palm trunk (OPT) and frond (OPF), found that this pretreatment was able to disrupt the OPT and OPF. In this study, the biomass was pretreated at 700 W at 80°C for 60 min, and approximately 41.6% and 64.42% of cellulose was released from the OPT and OPF respectively. They also suggested that pretreatment at this condition is more effective in extracting hemicellulose and cellulose component compared to lignin in both OPT and OPF. In their other study on the determination of optimum condition for lignin extraction from OPT indicated that the highest lignin reduction (22.38%) was attained when the pretreatment was performed at 100°C for 80 min at 900 W. This study is in agreement to the conclusion that microwave pretreatment is significantly influenced by the temperature, reaction time, and microwave power \[52\].

Apart from oil palm biomass, several studies on the microwave pretreatment on other biomass such as kenaf, sago pith, sago bark waste, banana trunk, and miscanthus have also been reported elsewhere \[55, 56\]. Study by Ooi et al. \[57\] on the microwave alkali-assisted pretreatment of kenaf pulp showed that the pretreatment at 50°C is the suitable temperature to convert crystalline cellulose to amorphous form, and produce higher sugar yield compared to untreated sample. In another study on microwave pretreatment of sago pith, a starch-based crop that contain substantial amount of starch and fiber, indicated that direct heating of sago pith in water by microwave treatment can swell and gelatinize the starch, resulting to a more amorphous and more susceptible fiber for subsequent enzyme reaction \[55\]. In a study on microwave chemical assisted pretreatment of miscanthus under different temperature range of 130–200°C, found that the suitable condition for miscanthus pretreatment is at 180°C for 20 min \[58\]. This study concluded that temperature plays an important role in microwave pretreatment process. Pretreatment at high temperature increases biomass solubility, shorten the pretreatment reaction period, and reduce recalcitrant characteristic of the biomass. However, the pretreatment process at high temperature also produced a substantial amount of inhibitor that is harmful to the subsequent enzymatic saccharification and fermentation.

4.1.4. Ultrasonication

Another irradiation pretreatment that is widely used to pretreat biomass and biofiber for biofuel production is ultrasonication. This process can be performed either using probe-type ultrasonication or an ultrasonic bath. In this process, ultrasonic waves can be generated via piezoelectric or magnetostrictive transducers in the frequency range of 20–1000 kHz, in which the waves induced provide pressure difference in the medium. The pressure wave that travels through the liquid medium has high pressure (compression) and low pressure (rarefaction)
regions. The rarefaction of the cycle can stretch the liquid molecules apart and create cavities also known as bubbles. As the wave cycles through the liquid, the bubbles expand and contract with the rarefaction and compression of the wave, respectively, drawing more liquid molecules into the bubbles as they grow. The bubbles that either continue to expand and then float to the surface, are subjected to coalescence due to the forces or collapse during compression of the wave (Figure 7). This collapse is almost adiabatic and can result in localized temperatures of around 5000 K and pressures of 1000 atm [59]. The collapse results in the formation of radicals through dissociation of the molecules within and around the bubbles, luminescence due to excited molecules formed losing energy, and microjets shooting out of the bubbles of speeds in the realms of hundreds of km per hour.

Figure 7. Ultrasonication pretreatment of biomass and biofiber mechanisms.

Ultrasonic pretreatment has been performed on a great variety of lignocellulosic biomass and biofiber including kenaf powder, kenaf bast fiber, corn meal, and corn stover [60–63]. This approach has also been performed on tropical biomass such as EFB and kenaf fiber. Most of the study concluded that ultrasonication pretreatment is capable to enhance conversion of biomass to biofuel. A study on ultrasonic pretreatment of EFB at low temperature indicated that this pretreatment could assist the acid hydrolysis performed at low temperature and pressure [64]. The study showed that xylose production from the pretreated EFB was two times higher than that of un-pretreated sample when the pretreatment was performed at 100°C for 40 min. Similar to the study on the ultrasonic pretreatment of kenaf powder in ionic liquid indicated that higher reducing sugar was attained from pretreated sample [60]. In this study, a significant change on the hemicellulose content was observed in the pretreated biomass.

5. Irradiation effect on biomass and biofiber

The main goal of the pretreatment process in liquid biofuel production is to modify the surface morphology structure and properties aiming to improve digestibility in the subsequent enzymatic saccharification. The pretreatment has also been reported to affect the chemical
composition of the biomass (Table 2). A significant reduction of lignin and hemicellulose were observed from the EFB after it went through the EBI pretreatment process.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total lignin (%)</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
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<td>OPEFB-CI 500 kGy</td>
<td>7.86</td>
<td>63.81</td>
<td>13.45</td>
</tr>
</tbody>
</table>

Table 2. Chemical composition content of oil palm empty fruit bunches (OPEFB) untreated and gamma irradiated OPEFB. (Adapted from Kristiani et al. [36]).

5.1 Surface morphology and chemical structure

The changes in chemical composition and surface morphology structure of biomass and biofiber are the main obvious effect observed from the irradiation pretreatment process. Typically, biomass or fiber with high crystallinity may consist a significant amount of crystallinity cellulose, and appear to be relatively smooth. The biomass with high degree of crystallinity indicates that it has high tensile strength properties [65].

Various investigations on the effect of irradiation pretreatment on tropical biomass including EFB, kenaf, rubberwood, and bamboo have been reported [29, 42, 64]. The study agreed that the pretreatment applied on these biomass have a significant effect on the biomass structure and chemical properties. For instance, a study on irradiation pretreatment of EFB at 300 kGy indicated a significant change in the surface morphology before and after pretreatment process [36]. The study found that the EFB, which is solid, intact, rough, and rigid structure becomes brittle and flaky after irradiated with gamma ray. Similar observation has been reported on the irradiation pretreatment of rubberwood. Darji et al. [66] compared the rubberwood structure before and after pretreatment and found that most of the fibrous in the rubberwood lost and disappeared after the pretreatment process.

The surface morphology structure change after pretreatment could be attributed to the irradiation process that is able to break the intermolecular hydrogen bond, resulting to the decrease of intra and intermolecular order in cellulose. Furthermore, under high energy and pressure, the cellulose macromolecule will undergo scission and increase the fragment with low degree of polymerization [67]. On the other hand, irradiation has also been reported to influence the biomass pore size. Brunauer–Emmett–Teller (BET) analysis on the kenaf core and cellulose, indicated that a significant increase of pore size was observed for both materials after irradiation pretreatment process [42, 68]. High pore size is a very important characteristic that could provide easy access for subsequent process prior to biofuel production.
Most of the studies reported that the change of the biomass surface morphology is correlated to the chemical structure in the biomass. A change on the degree of crystallinity was found to change surface morphology [69]. Typically, X-ray diffraction (XRD) analysis is applied to evaluate the effect of irradiation on biomass crystallinity. Chen et al. [70] reported that the major diffraction peak for cellulose crystallography can be identified for 2θ ranging between 22° and 23° as a primary peak, whereas a secondary peak is in the range of 16° to 18°. As reported by Liu et al. [28], the I₀₀₂ peak intensity (the maximum intensity of the 002 lattice diffraction) represents the primary peak and is classified as the diffraction intensity of crystalline regions, whereas the secondary peak represents the diffraction intensity of the amorphous zone. XRD analysis of irradiated cellulose at different irradiation dosage between 10–100 kGy indicated that increase of dosage could reduce crystallinity index and crystallite size [68]. In another study on irradiation of OPTT and OPF, it was found that an obvious peak reduction on the primary and second peak, indicates the transformation of cellulose molecular hydrogen bond due to rapid heating during the irradiation pretreatment process [54].

Apart from XRD analysis, the effect of irradiation pretreatment can also be evaluated by Fourier transform infrared spectroscopy (FT-IR). This method is widely used to determine the chemical structure changes after pretreatment of various types of biomass [71, 72]. FT-IR analysis on the irradiated oil palm trunk and oil palm frond indicated that radiation has affected the intensity of all bands in the IR spectra [51, 54]. Obvious changes were observed at absorbance between 3500–3200 cm⁻¹, 2840–2690 cm⁻¹, 1740–1720 cm⁻¹, 1500–1450 cm⁻¹, 1300–1000 cm⁻¹, 1315–1318 cm⁻¹, and 900–898 cm⁻¹. These bands represent a specific chemical structure in biomass as summarized in Table 3.

<table>
<thead>
<tr>
<th>Infrared band (cm⁻¹)</th>
<th>Functional groups</th>
<th>Infrared band (cm⁻¹)</th>
<th>Biomass component assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500–3200</td>
<td>O-H (H-bonded)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2840–2690</td>
<td>C-H (aldehyde C-H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1740–1720</td>
<td>C=O (saturated aldehyde)</td>
<td>1735</td>
<td>C=O in xylan (Hemicellulose)</td>
</tr>
<tr>
<td>1695–1630</td>
<td>C=O, C=C</td>
<td>1647</td>
<td>Absorb OH and conjugate C=O</td>
</tr>
<tr>
<td>1500–1450</td>
<td>C=C (in ring)</td>
<td>1505</td>
<td>Aromatic skeletal vibration in lignin</td>
</tr>
<tr>
<td>1421</td>
<td>CH deformation in lignin and carbohydrate</td>
<td>1435</td>
<td>Syringyl ring and CO stretch in lignin and xylan</td>
</tr>
<tr>
<td>1300–1000</td>
<td>C-O</td>
<td>1371</td>
<td>CH deformation in cellulose and hemicellulose</td>
</tr>
<tr>
<td>1319</td>
<td>CH vibration in cellulose, CO vibration in syringyl derivative</td>
<td>1319</td>
<td>CH deformation in cellulose and hemicellulose</td>
</tr>
<tr>
<td>1155</td>
<td>C-O-C vibration in cellulose and hemicellulose</td>
<td>1155</td>
<td>CH deformation in cellulose and hemicellulose</td>
</tr>
<tr>
<td>1030</td>
<td>C-O vibration in cellulose and hemicellulose</td>
<td>1030</td>
<td></td>
</tr>
<tr>
<td>900</td>
<td>C-H</td>
<td>897</td>
<td>CH deformation in cellulose</td>
</tr>
</tbody>
</table>

Table 3. FT-IR band assignment in biomass [54].
Normally, the structure of lignin consists of guaiacyl propane units (G) and syringyl propane units (S) containing one and two methoxy groups. It is known that the presence of guaiacyl propane could restrict the swelling of biomass [73, 74]. These chemical structures can be identified by FT-IR spectra with frequencies in the region of 1509, 1464, and 1422 cm\(^{-1}\). Reduction of spectra in this region indicated that most of the lignin in OPT and OPF have been removed from the biomass during the pretreatment process. Removal of lignin in the biomass after irradiation gives a better access for enzyme to attack cellulose and hemicellulose.

The FT-IR analysis of the irradiation pretreatment on biomass also indicated that significant changes on absorbance was observed at 1732 cm\(^{-1}\) and 3300 cm\(^{-1}\), attributed to the vibration of hydrogen bonded OH-group. Liu et al. [75] reported a shifting and reduction of band 2899 cm\(^{-1}\), indicating to the disruption of biomass resulting from the C-H shifting vibration. The study also found that high-energy irradiation pretreatment could interrupt and destroy the intra-molecular and inter molecular hydrogen bond in the cellulose. The degradation of cellulose generated carbonyl group could be determined at band at 1603 cm\(^{-1}\). Apart of this region, the shifting of band region between 1164 cm\(^{-1}\), 1112 cm\(^{-1}\), and 1058 cm\(^{-1}\) attributed to the vibration of C-O-C of cellulose.

5.2 Enzymatic saccharification

The pretreatment method aims at facilitating maximum saccharification of cellulose and hemicellulose by enzymatic hydrolysis. In this process, the cellulose and hemicellulose present in the biomass will be hydrolysed by cellulase and hemicellulase enzyme produced from fungi into simple monomer sugar such as glucose and xylose. This monomer sugar is the main chemical platform for biofuel and other chemicals (Figure 8). Previous research obtained the cellulose from untreated biomass and biofiber upon enzymatic hydrolysis can yield not more than 15–25% glucose due to the recalcitrance [76]. Most pretreatment methods have some disadvantages in terms of cost, recovery, secondary pollution, and formation of intermediate compounds that will inhibit enzymatic hydrolysis, but implementation of laser, microwave, and electron beam irradiation have become more attractive because of its fast and effective result during experimentation [77].

Biomass pretreated with electron beam irradiation (EBI) enhance enzymatic saccharification by decreasing the crystallinity and molecular weight and simultaneously increase the surface area [36, 68]. Irradiation induces a chain-cleavage mechanism by depolymerizing the polymeric material [78]. Higher cellulose content was found in chemical-irradiated pre-treated oil palm empty fruit bunch (OPEFB) than untreated OPEFB. Higher cellulose content can produce higher glucose, hemicelluloses content can be converted to xylosa, while lignin can produce derivatives compound of phenol [36]. The effectiveness of EBI treatment also depends on the nature of biomass with respect to energy delivered, and sources and concentration of enzymes used [77]. The earliest study by Kumakura and Kaetsu [79] found the pre-irradiation (dosage 107 rad) with presence of chlorine yields six times higher reducing sugar than its absence with subsequent enzymatic hydrolysis on rice straw. Then Ardica et al. [80] used gamma-ray irradiation (doses range from 1 kGy to 1000 kGy) on wood chips, kapok, papers, hays, and grain straw to enhance the enzymatic hydrolysis. Combined pretreatment of gamma-ray and
diluted acid on poplar bark biomass observed a drastic increased in reducing sugar yield from 56.1 to 83.1% compared to gamma-ray pretreatment alone [81]. **Table 4** shows previous study using electron beam irradiation with various types of biomass.

Zhu et al. [32] reported the rice straw pretreated with microwave-alkali method obtained higher hydrolysis rate than alkaline pretreated alone. The amount of glucose obtained from the enzymatic hydrolysis using *Trichoderma reesei* cellulase was higher (24.8 g l\(^{-1}\)), and lower for xylose (2.6 g l\(^{-1}\)) concentration for microwave/alkali pretreated rice straw which is more suitable for subsequent fermentation process to produce biofuel. The microwave-alkali assist irradiation has been proven to remove more hemicellulose and lignin, and simultaneously increasing enzyme accessibility [32]. In 2006, they have presented comparison between three techniques for the enzymatic hydrolysis of rice straw by pre-treating them with microwave/alkali, microwave/acid/alkali, and microwave/acid/alkali/H\(_2\)O\(_2\) treatment. The result shows that the rice straw pretreated with microwave/acid/alkali/H\(_2\)O\(_2\) treatment had the highest hydrolysis rate and glucose content in the hydrolysate. Furthermore, recovery of xylose content could be recovered compared to microwave/alkali treatment [83]. [84] using microwave-assisted alkali treatment. They have presented at optimal condition of 190°C, 50g/l solid content, and 30 min treatment time. The sugar yield from the combined treatment and hydrolysis was 58.7g/100g biomass which is equivalent to 99% of potential maximum sugars. This study was further investigated by using scanning electron microscope, and showed the advantage of microwave over conventional heating was due to the disruption of recalcitrant structures of lignocellulose. In conclusion, microwave/chemical pretreatment is more effective than conventional heating.

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**Figure 8.** Enzymatic saccharification of cellulose and hemicellulose to monomer sugars by cellulase and hemicellulase enzyme.
### Table 4. Summarization of previous research using EBI pretreatment on various types of biomass and biofiber.

<table>
<thead>
<tr>
<th>Biomass type</th>
<th>EBI dose</th>
<th>Glucose yield (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Pretreated</td>
<td></td>
</tr>
<tr>
<td>Rice straw (RS)</td>
<td>80 kGy</td>
<td>35</td>
<td>52</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>500 kGy</td>
<td>8.60</td>
<td>10.72</td>
</tr>
<tr>
<td>Poplar bark</td>
<td>0–1000 kGy</td>
<td>44.2</td>
<td>66.7a</td>
</tr>
<tr>
<td>Napier Grass</td>
<td>250 kGy</td>
<td>20</td>
<td>79</td>
</tr>
<tr>
<td>Rice straw (RS)</td>
<td>80 kGy</td>
<td>37</td>
<td>70.4b</td>
</tr>
<tr>
<td>OPEFB</td>
<td>100–500 kGy</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

a, pretreatment added with diluted sulfuric acid.  
b, pretreatment added with water soaking-based EBI.  
na, not available.

6. Biofuel from biomass and biofiber

In general, sugar substrates from tropical biomass and biofiber are potential sources for biofuel production such as ethanol and butanol because they are abundant, cheap, and renewable [85]. Biomass and biofiber utilization will reduce the dependency on fossil fuel and at the same time it could help in reducing toxic gases emission with an abundant feedstock that can support for a very long period of time. This second generation biofuel does not compete with human food resources which are non-edible in nature [86]. In lignocellulosic biomass conversion for biofuels such as ethanol and butanol, pretreatment plays a major role in separating the major components (lignin, cellulose, and hemicellulose) of the biomass. The conventional chemical and enzymatic pretreatment methods have disadvantages such as producing byproducts and low conversion of biomass components [86]. Numerous numbers of publications reported the potential of bioenergy from biomass wastes through irradiation pretreatment [43, 45, 62, 87, 88]. Most of the studies agreed that irradiation pretreatment could assist the reduction of particle size that provide better access for subsequent process. This pretreatment clearly proved able to enhance enzymatic saccharification and fermentation performance.

Presently, Malaysia is dependent on fossil fuels such as coils, oil, and natural gas as well as renewable energy sources such as hydro, biomass, and solar energy. The demands for energy is increasing by years with some challenges such as the decreasing source of fossil fuels, food versus fuel crisis, and greenhouse gas (GHG) emission that needs to be taken into consideration [11]. In Malaysia, the development of renewable energy is still rather slow. Although, in 10th Malaysia plan, renewable energy usage has to increase >1% in 2009 to 5.5% of total electricity generation in 2010, although several fiscal incentives have been launched by the Malaysian government [89]. On the other hand, Malaysia is geographically located in the tropical and humid climate region which provides easy access to variety of biomass resources. Biomass resources are mainly from palm oil, wood, and agro-industries [90]. Malaysia devotes 11% of
the total land area with 62% of the economy agricultural land for planting palm oil. If 20% of palm oil productions are turned into biofuels, it can replace 64% of diesel consumption, and at the same time cutting off 41% of imported crude oil [91]. From these facts, we can estimate the amount of biomass and biofiber waste produced yearly. Thus, there is a potential need to convert the residue into a valuable product by converting them into biomass energy feedstock.

Economically, biomass waste from palm oil plantation such as empty fruit bunch (EFB) can be used as resources for conversion of bioethanol, since the production is 6.1 million tons dry EFB and is forecasted to increase to 7.6 million tons in 2025 as shown in Table 5 [92].

<table>
<thead>
<tr>
<th>Year</th>
<th>Projected EFB production (Million tons dry matter/year)</th>
<th>Potential bioethanol production (Million/year)</th>
<th>Energy content in ethanol: 22MJ/litre (GJoule/year)</th>
<th>Potential Bioethanol (ktoe/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>6.14</td>
<td>2382</td>
<td>54,793,360</td>
<td>1863</td>
</tr>
<tr>
<td>2015</td>
<td>7.59</td>
<td>2945</td>
<td>67,733,160</td>
<td>2303</td>
</tr>
<tr>
<td>2025</td>
<td>7.66</td>
<td>2972</td>
<td>68,357,840</td>
<td>2324</td>
</tr>
</tbody>
</table>

Table 5. Potential ethanol and forecasted EFB production by MPOB based on 22% EFB to FFB and moisture at 65% (93).

Currently, biorefineries are increasingly focused on integrated process design for maximum valorization of fractionated biomass components for fuels and a spectrum of co-products. This multi-product “integrated biorefineries concept” is a platform for development of modern biorefineries with economic competitiveness to the current petroleum industry [10]. According to the IEA (International Energy Agency) report from the assessment of available residue in 2030, it was predicted that 10% of global residues could yield around 155 billion lge (5.2 EJ) lignocellulosic ethanol or almost around 4.1% of the projected transport fuel demand in 2030, and 25% of global residues converted to either ethanol, diesel, or syngas that could contribute to 385–554 billion lge (13–23.3 EJ) globally [94].

In conclusion, the viability of biomass and biofiber materials should concentrate more on developing a complete understanding of these materials to form a foundation for significant advancement in sustainable energy. Development in characterization and overcoming the difficulty for enzymatic saccharification of different raw materials is crucial for the development of economically competitive processes based on enzymatic treatment.

7. Important and challenges

Pretreatment process is an important process prior to enzymatic saccharification. Applying the most efficient pretreatment process could reduce production cost, hence reduce the final product price. As per date, the irradiation pretreatment process shows a promising approach and has some advantages compared to other pretreatment processes.

Irradiation pretreatment is an environment friendly process due to less chemical used during the pretreatment process. Also, this process requires less time (<10 minutes) compared to other
process, especially biological pretreatment, which requires more than 7 days to remove lignin material from the biomass [95].

The most important advantage of the irradiation process is that this process is very selective to the degradation of the biomass component, unlike chemical pretreatment that could degrade some part of cellulose and hemicellulose during the process [45]. On the other hand, this process also produced less inhibitor that could affect enzymatic saccharification and fermentation process. According to Bak et al. [43], there was no inhibitors produced from the irradiated biomass when the pretreatment was carried out using water as soaking buffer. Hence, could increase the enzymatic saccharification and fermentation process performance for liquid biofuel production.

Even though this irradiation pretreatment procedure is quite simple, it is undeniable that the high-energy consumption associated with it makes the process not preferable for implementation on a commercial scale [40, 96]. Besides, this process requires a special reactor that could affect during large-scale process. For the large-scale pretreatment of biomass, a large microwave irradiator or reactor is required, which is costly, energy consuming, and limits its use in large-scale operations. This drawback hence also could increase the operational cost.

8. Conclusions

There is a wide range of chemical composition distribution in tropical biomass and biofiber making these resources a great potential to be used for biofuel and other value added products. To convert these materials, it has to go through series of processes, and the most environment friendly and efficient method is important to ensure the feasibility of the product produced. Irradiation pretreatment has been reported to have more advantages on the biomass pretreatment because this process is environment friendly, it requires less chemical, and the process can be performed in a short period of time. Irradiation pretreatment such as gamma ray, electron beam, microwave, and ultrasonication proved able to disrupt cell wall structure and provide better access for enzymatic saccharification. Hence, this could increase biofuel production and other chemicals from the tropical biomass and biofiber generated from agro-industry. However, this process still requires high energy and it could give negative impact especially at the large-scale production. Thus, further research to attempt maximum performance using low energy is very crucial to ensure the feasibility of the biofuel production from tropical biomass and biofiber using irradiation pretreatment.

Acknowledgements

The author, would like to thank for the financial support provided by University Sains Malaysia (USM) Short Term Research Grant 304/PTEKIND/6313194
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