Biotransformation Methods of Paddy Straw into Bioethanol

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Abstract. Bio-fuel production can be categorized into biodiesel and bioethanol and the most common renewable fuel today is ethanol derived from sugar. Future large-scale use for ethanol will most certainly have to be based on production from lignocellulosic materials due to its abundance in food crop waste. This article gives an overview on paddy straw biotransformation into ethanol. Rice straw has lignocellulosic material for bioethanol production since it is one of the most abundant renewable resources. Paddy straw especially, has favorable characteristics such as high hemicellulose and cellulose content that can be readily hydrolyzed into fermentable sugars. However, one of the major challenges in developing technology for bioethanol production from paddy straw is selection of an appropriate pre-treatment and fermentation method.

Keywords: Fermentable sugars; Cellulose Content; Lignocellulosic; Hydrolyzed

1. Introduction
To date, biotechnology has highlighted the promise of obtaining biofuel from plant waste [1] from the industry [2]. Besides, biotransformation also aims for renewable material which will sustain the natural resources [3]. One of the most renown transformations is the production of bioethanol for petrol substitution. In addition, the conversion of lignocellulosic biomass, an abundant plant material can be used to generate biofuels thus allowing reduction of emission of greenhouse gas dramatically [4] due to cellulosic ethanol [5]. Paddy (Oryza sativa) is one of the most consumed foods in the world, as the consumption of rice per capita is 65kg annually. The production of rice is forecasted to increase in the future as the human population is growing everyday [6]. Many researchers around the world have been studying the morphology of paddy on how to increase the productivity of rice to meet the world’s demand.

The conventional C3 plants does not perform too well on this aspect as it yields lower production. Thus, to overcome this, it is crucial to generate the C4 based rice [7]. Besides being the most demanded food on earth, paddy also produce a biomass material over the residue of over-abundant dried paddy which is called paddy straw [6]. This paddy straw possesses cellulose, and hemicellulose (around 33% and 28% respectively) besides having 10% lignin and 12% silica [6]. The uses of paddy straw might vary depending on how the paddy straw being managed, for example in Japan and California, paddy straw burning have cause upheaval due to asthma hospitalization [8]. Besides being treated with burning, paddy straw is treated with soil incorporation. Wet soil incorporation, could lower the methane gas emission hence, lower the need of irrigation system as the soil is already benefited from the nutrients derived from the straw decay [9]. Bioethanol or simply ethanol is a substance derived from biological fermentation of sugar which is can be extracted from plant material. Bioethanol can be produced from various means but in particular, ethanol derived from fermentation has received a lot of
attention as a potential biofuel and substitution to fuel additives [10]. Previously, it is produced solely from high content of sugar and starch source plant and crops such as sugar cane, wheat and corn [11] but food price associated with the production of bioethanol however has gone up as the demand of bioethanol increases [12]. The production of bioethanol is not limited by sugar and starch-based crops, but also can be processed from other resources such as paddy straw and other residues from wood [13] which is relatively cheaper [14]. Besides its known use in transport fuel, bioethanol is also proven to be useful in many industrial products such as art material, perfumes, and medicine [15]. This article reviews the methods to produce sugar from rice straw in which will allow us to benefit from paddy waste, a sustainable substitute of engine fuel.

2. Paddy Straw Potential in Fuel Ethanol Production

Ethanol has become prevalent for fuel transport substitute. However, discrepancy emerges between bioethanol industry and sustenance supply when increased production of bioethanol are obtained from food crops such as complex starches; cellulose and hemicelluloses which are turned into fermentable sugars. Furthermore, the quality of the straw can be increased biotechnologically through breeding or genetic engineering or through agronomic modification in harvesting, threshing and storage methods to optimize straw production value. Consequently, paddy straw will become a better source for biofuel production. Paddy straw has a couple of characteristics perfect for feedstock that are intended for fuel. It consists of high availability of hemicellulose which helps in hydrolyzing process that produce fermentable sugars [16]. Cellulose consists of anhydrous 1→4 glucose while hemicellulose consists many types of sugars. However, both of them contain anhydrous glucose.

![Figure 1. Chemical structure of a) Lignin, b) cellulose and c) hemicellulose-xylan](image)

| Major element of paddy straw and wheat straw (Lignin, cellulose and hemicellulose) | Paddy straw (%) | Wheat straw (%) |
|----------------------------------|---------------|--------------|
| Fixed carbon                     | 15.86         | 17.7         |
| Volatile matter                 | 65.47         | 75.25        |
| Ash                             | 18.67         | 7.02         |

Table 1. Major element of paddy straw and wheat straw (Lignin, cellulose and hemicellulose)

Figure 1 depicts chemical structure of lignin, cellulose and of hemicellulose [17]. The process of breaking intermolecular hydrogen bonds in cellulose is much simpler than hemicellulose due to its structural nature. Besides that, in feedstock, there is a mixture of chemical structure contributed by rice and wheat straws. For example in table 1, high amount of particle or powder is present which is about 10–17% unlike wheat straw which only has about 3%. Table 2 on the other hand, silica is detected to be in high in the powder particle which is 75% in paddy followed by wheat at 50% [18]. In paddy, the cellulose content is 38.6%, hemicellulose-24% and lignin 15.6% [48]. Besides that, paddy straw that is commonly converted into feedstock has the advantage of having low alkalinity where Na2O and K2O are 15% in powder particle. On the other hand, wheat straws are 25% in alkalinity in the powder particle [19]. Paddy straw makes a good source of biofuel generation due to its ubiquitous source of lignocellulosic material. The overabundance supply of rice straw is one of the principles that make it one of the best choices for biofuel generation [20]. A lot of paddy straws are burned after harvest, which can cause ecological peril [21]. Thus, the implementation of paddy stalk as alternative biofuels is
plausible [16] as for every 1000 hectare harvested, 70000 kilo ton of straw is produced [2].

| Table 2. Major element of paddy straw and wheat straw |
|-----------------------------------------------|
| Elemental composition of ash (% dry fuel) | Paddy straw | Wheat straw |
| Silicon dioxide                | 74.76        | 55.32       |
| Calcium oxide                  | 3.01         | 6.14        |
| Magnesium oxide                | 1.75         | 1.06        |
| Sodium oxide                   | 0.96         | 1.71        |
| Potassium oxide                | 12.30        | 25.60       |

Source: [22]

3. Generation of Ethanol Derived from Paddy Stalk
At the initial step, hemicellulose and cellulose will turn to fermentable sugar. Combination of cellulose and hemicellulose undergoing hydrolysis process together with acid and enzyme as catalyst will produce sugars.

Pre-treatment is one of the primary practical and proficient biomass pre-treatment innovation [23]. Other than that, pre-treatment incorporates physical, chemical and also biological to produce result. Paddy straw consists of diverse sugar structure. Hemicelluloses along with cellulose were protected with some thick layer of lignin, which secure them against the process of enzymatic hydrolysis. Pre-treatment is carried out to destroy the seal of lignin to uncover hemicellulose and so with the cellulose for the enzymes to be active again. The impact of pre-treatment of lignocellulosic materials has been perceived for quite a while. The reason for pre-treatment is to remove lignin, hemicellulose, diminish cellulose crystallinity and to add porosity of the product [24].

3.1 Comparison between pre-treatment method
The goal of physical pre-treatment, for example, processing is to build surface area and reduce molecule size of lignocellulosic material [25]. Moreover, it can reduce level of polymerization and decrystallization of feedstock.

3.1.1 Electron beam irradiation (EBI)
One of the methods available besides cooking and bleaching is electron beam irradiation (EBI), a method chosen by industries to digest cellulose pulp or to assist in molecular mass alignment [26]. By irradiation, part of the cellulose found inside the lignocellulosic is dissolved where comparatively the yield of sugar from untreated paddy is 22% while after irradiation, the yield becomes 52% [27].

3.1.2 Chemical pre-treatment method
The lignocelluloses cannot be transformed into fermentable sugar without the interference of chemical pre-treatment. Alkaline pre-treatment however has been the most effective method for paddy straw to date [16]. In the basic principle of alkaline pre-treatment, biomass will be first treated with alkaline or other substance. Sodium hydroxide NaOH, Potassium hydroxide KOH, Calcium hydroxide Ca(OH)₂ and Sodium hypochloride NaOCl were some examples which being treated [28]. In this technique, rice straw was doused with 2% (w/v) of alkaline solution with various chemical and temperature setup. The treated rice is then washed to further remove impurities. Then with 1 N HCL, the sample being cleansed. Later, with 60°C temperature, the sample is left dried for a day [20]. The advantage of alkaline pre-treatment is it can operate at significantly reduced temperature and pressure, in contrast with lignin pre-treatment [29]. Unlike hardwood materials, softwood material is easier to work with [30]. In relation, to break the
bonds between polysaccharide and lignin, alkaline pre-treatment is recommended [31]. Furthermore, released lignin, acetyl group and distinctive uronic acid substitution which hinder the cellulose accessibility for enzymatic saccharification are the most essential points of interest of this pre-treatment [32]. Also, this method is known for bringing about chemical quenching of various celluloses [29], which saponification and salvation responses take place which prompt the interruption of crosslinking between hemicellulose and others thus, expanding the porosity of biomass. Table 6 shows the concentration of sugar production contrasted with untreated and alkaline treated paddy straw. The impact of various alkaline pre-treatment with NaOH, KOH, Ca(OH)₂, and NaOCl at various temperature of 30°C, 100°C and 121°C exhibited elevated sugar production than untreated. The highest yield of total sugar can be observed when the paddy straw is reacted upon 2% (w/v) 2% (w/v) KOH-treated rice straw for enzymatic saccharification in contrast with control. When the temperature is capped at 121°C, NaOH and KOH can be seen to deliver all out total sugar of 55.48 and 59.90 g/L, as oppose to the untreated paddy straw which acquired only 3.18 g/L of sugar aggregate. Other than that, the use of NaOH and KOH, yields higher sugar production than by using Ca(OH)₂ and NaOCl where this experiments witnessed solubilization and removal of the lignin component through observation with scanning electron microscope (SEM) [20]. Therefore, the alkaline pre-treatment is suggested to be an advantageous procedure to break the ester bond between lignin, cellulose and hemicellulose while expanding water holding capacity of the paddy straw. Consequently, expansion of porosity of the paddy straw may allow more prominent compound availability into the interior structure of the paddy straw which can lead to more sugar production [33].

3.1.3 Biological pre-treatment
It is possible to utilize the abundant source of cellulolytic and hemicellulolytic microorganism making biological pre-treatment an intelligent method [34]. These microorganisms show up as filamentous being growing in the very soil [35] such as white rot fungi Phanerochaete chrysosporium, Ceriporia cut, Cyathus stercoerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus, Pleurotus ostreaus and P.chrysosporium, Trametes versicolor which serve as the agents for pre-treatment procedure in most lignocellulosic based [2; 36]. It assesses the structure change prior to the treatment and reacts and incline to enzymatic hydrolysis accordingly [37]. Of these, P. ostreatus breaks the lignin of rice straw as opposed to the holocellulose segment. When rice straw was pretreated with P. ostreatus for 60 days, the aggregate observes weight reduction and the level of Klason lignin degradation were 25% and 41% respectively. After the pre-treatment, the cellulose and hemicellulose were 83% and 52% in untreated rice straw separately while with enzymatic hydrolysis of conventional preparation for 48 hours, they became solubilized under the influence of 52 % holocellulose and 44% cellulose in the pre-treated paddy straw. The aggregate sugar derived from holocellulose is 33% and cellulose is 32% respectively [37]. Meanwhile, the main disadvantage is that the rate of hydrolysis is declining, rendering it impractical for industrial application [38]. Much research must be done in isolation procedure for instance, utilizing basidiomycetes organisms for their capacity to break the plant material rapidly and productively. Thus, biological pre-treatment is not compelling enough due to its sluggishness and inefficiency [39].

3.2 Enzymatic hydrolysis
Enzymatic hydrolysis is the second step to bring ethanol from lignocellulosic materials. It is a procedure by which enzyme (biological catalyst) are utilized to separate starch or cellulose into sugar. This procedure is normally completed by cellulases and hemicellulases. Cellulose normally contains just glucans, while hemicellulose contains polymers of a few sugars, such as mannan, xylan, glucan, galactan, and arabinan. Subsequently, the main product of cellulose hydrolysis is glucose, while for hemicellulose- pentoses and hexoses. However, high lignin content limits enzyme viability, causes decreased yield of hydrolysis as lignin, celllobiose and glucose are strong inhibitors of cellulases. Different factors influence the yields of lignocellulose to monomeric sugars and the by-products, for example liquid to solid ratio, particle size, temperature, length of cellulose polymer, arrangement of the cellulose chain, association of cellulose with other protective polymeric structures inside the plant cell wall, for example, lignin, pectin, hemicellulose, proteins, and mineral components [40]. Pre-treatment
of cellulosic material may release the crystallinity of cellulose.

3.3 Fermentation process between Simultaneous Saccharification and Fermentation (SSF) and Separate Enzymatic hydrolysis and Fermentation (SHF).

The cellulose and hemicellulose part of the paddy can be converted into ethanol by simultaneous saccharification and fermentation (SSF) and separate enzymatic hydrolysis and fermentation (SHF). Comparatively, SSF is better as it is cost-effective [41] as it utilizes different temperature of hydrolyzing enzyme. The temperature around 40-50 °C with the presence of microorganism, is not a great condition for ethanol production [42] due to volatility. Other than that, SSF of alkali pre-treated paddy straw with Pachysoln tannophilus and Candida brassicae showed that P. tannophilus has the ability to produce yield more ethanol than C. brassicae. However, both C. brassicae and P. tannophilus may produce ethanol below 30% theoretically. Under optimum conditions, ethanol concentration achieved 29.1gL-1 and ethanol yield was 61.31%. In this case, production of ethanol from alkali pre-treated paddy straw to ethanol using T. reesei and S. cerevisiae is higher than alkali pre-treated paddy straw with Pachysoln tannophilus and Candida brassicae. Where give the result 61.31% and 30% respectively. Thus, the alkali pre-treated paddy straw using T. reesei and S. cerevisiae are preferable for ethanol production as it has lower enzyme loading, shorter reaction time and yields higher ethanol [43; 44].

4. Conclusion

As a conclusion, paddy straw is a good waste product for biofuel. The abundance of paddy straw contributed by paddy being a staple food has made it one of the best sources of renewable energy. As for pre-treatment method, the alkaline method is considered the best method to produce sugar. Its biological effect which the alkaline possess will act as a catalyst to produce much more fermentable sugar in the process. In contrast, the weakness of biological treatment is sluggish overall process, unlike that of the alkaline process. Besides, electron beam irradiation is too costly for many. In fermentation, ethanol utilizing cellulose T. reesei and S. cerevisiae is the best way to produce ethanol because it has lower enzyme loading, shorter reaction time and can produce higher ethanol. Nowadays, research continues to improve on the best solution to digest lignocellulose. Other than that, future prospects for the advancement of lignocellulosic biotransformation must grasp a more precise improvement of bioethanol since pre-treatment is the most costly operation and accounts for roughly 3% of the total cost [45]. Moreover, genetic improvements through fermentative and cellulolytic system by co-culture systems is attractive in increasing ethanol production under stressful conditions [46]. SSF, combined enzymatic hydrolysis [47] and CBP are likewise considered to save cost. Besides that, a general investigation of performance would give a reasonable vision of the system condition and allow implementation practical preventive intercessions aimed at upgrading biofuel production.

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