Enhancement of enzymatic hydrolysis and lignin removal of bagasse using photocatalytic pretreatment

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Abstract. Pretreatment for reduction of biological resistance in a lignocellulosic material, i.e. bagasse, for enzymatic hydrolysis and fermentation was investigated. Photocatalyst (TiO₂) was used as an additive composition to assist this pretreatment process. Reaction time was varied (24, 48, and 72 h) to find the optimum condition for the pretreatment, while concentration of solvent (NaOH, H₂O₂, or NH₄OH), biomass to solvent ratio, and weight ratio of catalyst to bagasse were fixed at 2 M, 1:20 g/ml (typically, solvent = 150 ml), and 1:5, respectively. Batch reaction temperature was at 25°C. After the pretreatment, the enzymatic digestibility of pretreated bagasse was carried out to find the sugar yield. Hydrolysis of pretreated bagasse with photocatalyst show higher sugar yields than the conventional reactions without photocatalyst. The maximum yields of sugars (541.03 mg glucose and 192.79 mg pentose) were obtained at the longest reaction time.

1. Introduction
Nowadays, the increase of petroleum price and the depletion of petroleum resources force many countries to find new resources to replace petroleum. There have been a number of researchers focusing on both adequacy of energy supply long-term and also the environmental implications. A lot of efforts have been carried out to develop clean sustainable renewable energy to be an alternative fuel for the current energy instead of conventional fuels. Ethanol is one of an alternative energy resources [1] since it is cleaner and does not emit any pollutant gas after use.
The utilization of lignocellulosic materials as renewable resources for ethanol production motivates a great deal of interest. The well-known lignocellulosic materials mostly use for bioethanol production are classified into: (1) sugar (e.g. sugar cane, bagasse, and molasses), (2) starch (e.g. cassava, rice, and corn), and (3) cellulose (which is mainly come from the remaining of plants). These raw materials are the most abundant carbon sources and non-food bio-feedstocks which do not compete with the food supply. They also have the benefits on disposal of problematic solid wastes, providing power opportunity to improve energy security, reducing the trade deficit, reducing greenhouse gas emissions, and improving agricultural products [2]. Several countries have been attempting to develop utilization of biomass. Although biomass utilization has been greatly improved by a variety of technologies, there are still challenges that need further investigations [3].
Since Thailand is an agricultural country with many resources of raw biomass, Thailand can highly take an advantage on these materials [1, 4]. Among various local lignocellulosic materials, a variety of agricultural biomass is considered to be an important feedstock for bioethanol production in Thailand as shown in Table 1.

Lignocellulosic biomass is a multi-structure organic compound, which consist mainly of three polymers, i.e. cellulose, hemicelluloses, and lignin, which are associated with each other (with small amounts of acids, salts, and minerals). Lignocellulosic biomass can be converted into bioenergy (biofuel) by chemical processes, i.e. thermolysis, pyrolysis, hydrolysis, fermentation, gasification, and combustion [5, 6].

Table 1. Biomass residues from rice, sugar cane, and palm oil in Thailand, 2004 [7].

| Types         | Production | Agricultural residues | Residues |
|---------------|------------|-----------------------|----------|
| Sugar cane    | 70,101     | Bagasse               | 20,399   |
|               |            | Trash                 | 21,171   |
| Rice          | 26,841     | Rice husk             | 6,173    |
|               |            | Rice straw            | 11,998   |
| Palm Oil      | 4,903      | Empty fruit bunches   | 1,226    |
|               |            | Fiber                 | 721      |
|               |            | Shells                | 240      |
|               |            | Fronds                | 12,767   |
| **Total**     | **74,695** |                      | **74,695** |

(Unit: 1,000 tons per year)

Theoretically, the main process that is used to break down hemicellulose and cellulose then converted to sugars is hydrolysis. Lignocellulosic material is highly recalcitrant to hydrolyze due to its rigid structure, particular by lignin which acts as physical barrier against hydrolytic enzymes penetration to the biomass microstructure [8]. It is noted that, due to the presence of lignin, the pretreatment of biomass is initially required for degradation and/or removal of lignin from cellulose and hemicelluloses prior to the hydrolysis process. This causes enzymes can penetrate easier to the cell wall and depolymerize celluloses and hemicelluloses leading to increase of fermentable sugars from the enzymatic hydrolysis step [8].

Currently, modifications of pretreatment of lignocellulosic biomass has been investigated by several researchers to maximize the yield of sugar with low unwanted by-product formations [1, 3]. Among the potential pretreatment technologies, alkaline-treatment is known to be an efficient pretreatment process. Nevertheless, the main drawback of this process is very long retention period in the pretreatment step. Alternatively, the use of photocatalysts (i.e. TiO₂ and ZnO) to assist pretreatment can solve this problem [9, 10].

In this work, photocatalysis was used as an alternative pretreatment process of bagasse using a commercial TiO₂ (P25) as the photocatalyst (no alkaline pretreatment). For more practical applications, the use of photocatalyst to assist in the conventional alkaline pretreatment was also investigated and compared.

2. Materials and methods

2.1. Raw material preparation

Bagasse, also called meass, fibers remain after the extraction of the sugar-bearing juices from sugarcane. Bagasse used in this work was obtained from MitrPhol Sugar Refinery in Samut Prakran. It was milled by a cutting miller and sieved through a 0.25-mm-size sieve screen. The physically processed bagasse was dried in a hot air oven at 70°C overnight and stored in a plastic bag at room
temperature before use. The composition of the bagasse was analyzed by the National Renewable
Energy Laboratory (NREL) method.

2.2. Photocatalyst-Assisted alkaline pretreatment
Bagasse was treated in aqueous solution in the presence of photocatalysts (P25). Each pretreatment
experiment was conducted in both with and without the UV irradiation (dark condition). Biomass to
solvent ratio is fixed at 1:20 (g/ml). The weight ratio of catalyst to bagasse was 1:5 (20% w/w catalyst
to biomass).
Blank experiments were prepared in screw-capped 250-ml bottles (PYREX® No.4980, STOPPER No.
6) and stirred on an Isotemp Basic-stirring hotplate, at room temperature. For the dark trial, surface of
the reactor was covered by an aluminium-foil sheet to prevent the irradiation of light.
For the pretreatment experiments, additive solution (2 M) was prepared in distilled water (150 ml).
Biomass was mixed with P25 photocatalyst in a bottle. Additive solution was then added in the dark
condition and the suspension was continuously stirred for 30 min.
A mercury lamp as a light source to irradiate the sample that located beside the reactor was turned on
to start the reaction. The photocatalytic system continuously stirred in the plastic box that was coated
with an aluminium-foil sheet inside. The reaction temperature was maintained by a cooling water
system. Then, the samples were taken from the photoreactor at specified times for analysis.
The suspension after pretreatment process was filtrated by a vacuum filter. The first liquid product was
kept in an amber bottle at room temperature for further analyzed, while the solid product was diluted
with distill water until neutral (pH = 7) and dried in an oven at 70 °C overnight and kept in a plastic
bag zipper for further product hydrolysis.

2.3. Enzymatic saccharification (Hydrolysis)
Hydrolysis test for each sample was performed to determine the improvement in enzymatic
saccharification under the different pretreatment conditions. The pretreated solid product was
hydrolyzed in a 1-ml reaction. The reaction contained 5% (w/v) pretreated biomass in 50 mM sodium
citrate buffer (pH 4.8) with 20 FPU/g and 5% sodium azide. The sample was incubated at 50°C and
shacked at 30 rpm for 72 h. The sample was then taken at specified times. Sugar profiles of the sample
were then analyzed by high performance liquid chromatography (HPLC, Shimadzu, LC-20AD pump)
and the glucose and xylose contents were calculated.

Figure 1. A configuration of photocatalytic pretreatment in the photocatalytic reactor (Mercury lamp,
90 W, \( \lambda_{\text{max}} = 253.7 \) nm).
2.4. Products analysis

Liquid product (~5 ml) was filtrated by a 0.22-μm nylon filter before analysis. The generated products were monitored by (HPLC) equipped with a refractive index detector (Shimadzu RID-10A). Separation was performed on an Aminex HPX-87H column (300 x 7.8 mm) (Bio-Rad). The mobile phase was 5-mM sulfuric acid at a flow rate of 0.5 mL/min and an injection volume of 20 μL.

3. Results and discussion

3.1. Photocatalytic treatments

Fig. 2 shows the sugar digestibility plots in cases of: (1) no any treatment (a blank, only water), (2) photolysis (with UV light only, without catalyst), (3) non-active condition (with TiO₂ in dark condition), and (4) photocatalytic pretreatment (with TiO₂ under UV irradiation). It was found that the amount of individual sugar, as well as the total sugar yields, were low in the cases of no catalyst and no UV irradiation. The highest sugar digestibility yields (80.71 mg of glucose and 53.67 mg of pentose based on grams of pretreated biomass) were achieved from the photocatalytic pretreatment. Because, under the UV irradiation, TiO₂ photocatalysts could accelerate the separation of biomass compositions during the pretreatment process. Anyhow, the sugar digestibility yields obtained after the treatment were relatively low compared with the alkaline treatment. Therefore, not only photocatalysis, but use of photocatalyst to assist the conventional alkaline process is also an interesting process.

![Sugar digestibility yields in photocatalytic pretreatment](image)

**Figure 2.** Sugar digestibility yields in photocatalytic pretreatment (solvent = water) compared with blanks (no catalyst and/or no UV irradiation).

3.2. Effects of solvents on sugar digestibility

Fig. 3 shows sugar digestibility yields in different solvent types. The solvents (NaOH, H₂O₂, and NH₄OH) were used at the same concentration of 2 M. Biomass to solvent ratio and reaction time were
fixed at 1:20 g/ml and 24 h, respectively. As illustrated in Fig. 3, it can be observed that the alkaline pretreatment (NaOH and NH₄OH) resulted in the top two ranking in enzymatic digestibility. The highest sugar yields (412.04 mg of glucose and 74.42 mg of pentose based on grams of pretreated biomass) were obtained from NaOH. Compared with the oxidative reagent (H₂O₂), the alkali treatment becomes to be the most powerful method in breaking the crystalline structure of lignocellulosic biomass.

**Figure 3.** Sugar digestibility yields with different solvent types (NaOH, H₂O₂, or NH₄OH) compared with blank (solvent = H₂O).

### 3.3. Effects of pretreatment time on sugar digestibility

Fig. 4 shows the sugar digestibility yields from the photocatalyst-assisted NaOH pretreatment of bagasse at different reaction times (24, 48, and 72 h). The photocatalyst-assisted NaOH pretreatment showed 3-times higher sugar yields compared with the photocatalytic pretreatment. It can be observed that increase of pretreatment time led to increasing solubilization period of biomass components, decreasing cellulose crystallinity, and improving enzymatic digestibility. The highest sugar yield (541.03 mg of glucose, 192.79 mg of pentose based on grams of pretreated biomass) at the longest pretreatment time (72 h).
4. Conclusion
Pretreatment is a pre-requisite step in sugar-platform biorefinery to improve the enzymatic digestibility of the intact native biomass in order to obtain feasible sugar yields for further conversion to biofuels, biochemical, and other value-added products. An experimental study on the enhancement of enzymatic hydrolysis and lignin removal of bagasse was carried out by photocatalytic pretreatment and photocatalyst-assisted alkaline pretreatment. It was found that the use of photocatalyst-assisted alkaline pretreatment could enhance higher sugar production than photocatalytic pretreatment. In addition, the increasing in pretreatment time led the increase in solubilization period, resulting in the higher sugar yields of biomass components. For the future studies, the effects of additive concentration and amount of photocatalyst loading on sugar yields will be investigated.

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