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Investigation of Water Hyacinth as a Feedstock for Bioethanol Production by Simultaneous Saccharification and Fermentation Process

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As an abundant biomass of surface water, water hyacinth was used as an input material to produce bioethanol in this study. Steam explosion and alkaline pre-treatment were applied on the material before simultaneous saccharification and fermentation (SSF) process was carried out. Experimental data suggested the optimal enzyme dosage be 1.0 wt% of the dry mass of the biomass, and the mass of the material be up to 8.0 wt% of the whole mixture in a one-step loading procedure to obtain the climax efficiency after 78 h. The process does not need additional nitrogen nutrition, such as corn steep liquor (CSL), as those in other common lignocellulosic bioethanol processes, because water hyacinth itself contains much protein in its chemical composition. Protein content analysis showed that it was 17.2 wt% (on dry basis), similar to what reported in some published studies. The SSF efficiency was positively 72.8 % at the optimal conditions resulted out from the experiments. Such a convenient conversion of water hyacinth to bioethanol is not only meaningful in term of biofuel production but also in term of environmental treatment.

1. Introduction

Because global energy demand is increasing while the major environmental damage is caused by the combustion of fossil fuel (Larrea et al., 2020), renewable resources have been a vital trend of world development. Among them, lignocellulosic biomass is popular, abundant, and able to be utilized from agricultural wastes (Anwar et al., 2014). More specifically, lignocellulose-based bioethanol, as known as the 2nd generation bioethanol, attracts a lot of interest from both academic and industrial fields (Tran et al., 2019).
Unlike the bioethanol production from starch and sugar-based feedstock, due to the recalcitrant structure of lignocelluloses, the large consumption of energy and chemical required for pre-treatment; and expensive enzymes needed for hydrolysis make the cost of the 2nd generation bioethanol considerably high (Wyman, 1994). Large-scale production of bioethanol from lignocelluloses in an integrated biorefinery process is expected to reduce the production cost of bioethanol (Galbe et al., 2007).
Among lignocellulosic biomass as potential materials for the second generation of bioethanol production, water hyacinth *(Eichhornia crassipes*) is a noticeable species, which is so abundant that it easily covers the groundwater-surface and makes big troubles for the movement of ships and boats. The growing rate of water hyacinths can reach 220 kg/ha/d (Bayrakci and Koçar, 2014). Removing water hyacinth from the river traffic is required in tropical countries like Vietnam. In other words, the water hyacinth is not only a waste to treat environmentally but also can be convenient biomass to utilize. Many studies have been carried out on the conversion of water hyacinth to ethanol not only in term of pretreatment techniques, but also fermentation methods (Bronzato et al., 2019). Conventionally, steam explosion can be applied on pretreatment of water hyacinth followed by acid or alkaline pretreatment to destruct the matrix structure of lignocellulosic materials, which can facilitate the penetration of hydrolysis agents (Ganguly et al., 2012).

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To ferment carbohydrate substances to ethanol, microorganisms are employed. Among the yeast strains in ethanol production, *Sacharomyces cerevisiae* is the most popular thanks to its high efficiency in metabolism of starch and sugar (Jansen et al., 2017). Fermentation can be done in-situ with enzymatic hydrolysis in simultaneous saccharification and fermentation process (SSF). During the SSF process, nutrients must be provided for the growth of yeasts.
Observantly, water hyacinths have a chemical composition with very high nitrogen protein and mineral contents (Adeyemi and Osubor, 2016). This fact leads to a question that whether one can ferment water hyacinth to ethanol without additional nutrients. However, no report was found to answer this.
In this study, SSF was applied on water hyacinth to produce bioethanol with and without the addition of a nutrient for comparison to elucidate the above problem. Some basic factors of the process, such as SSF time, enzyme dosage, pre-treatment conditions, are investigated.

2. Materials and methodology

2.1 Pretreatment of water hyacinth

Water hyacinths were collected from the Saigon River, Ho Chi Minh City, Vietnam.
After collection, the roots with mud were removed, and the plants were dried under sunlight until the moisture content reduces less than 10 wt%. The raw material was cut into 2 cm long pieces, following by being steam-exploded by an automatic continuous puffing machine. The amount of water loaded into the material during puffing was about 15-17 wt% of the dry basis to get appropriate moisture content. For alkaline pretreatment, puffed material was immersed into NaOH aqueous solution at room temperature for 12-24 h. Then the pretreated material was squeezed to remove alkaline solution and neutralized with hydrochloric acid (to obtain pH 5-6). After this step, the biomass was filtered by pressing out the solution then stored in a refrigerator.

2.2 Yeast and pre-cultivation

Before fermentation, the dry yeast (*S. cerevisiae*, Ethanol RedTM) was pre-cultivated. The pre-cultivation media (sucrose and CSL; corn steep liquor) prepared in deionized water were autoclaved at 121 ˚C for 15 min before the loading of dry yeast. The yeast was pre-cultivated in a shaking incubator (110 rpm) at 35 ˚C for 16-24 h. Optical density (O.D.) of the pre-cultivation broth was measured at 600 nm with UV-Vis instrument Hach DR 5000. The amount of yeast culture needed for SSF was calculated according to SSF experimental protocols (NREL/TP-510-42630).

2.3 Simultaneous saccharification and fermentation

Pretreated material was loaded into a 250 mL Erlenmeyer flask, with or without nutrients according to the experimental designs then autoclaved (121 °C, 20 min). After autoclaving, all flasks were cooled down to 35 °C. Pre-cultivated yeast and enzyme were added. Acremonium Cellulase (Meiji Seika Co.) with an activity of 360 FPU (filter paper unit)/g-enzyme was used as the hydrolysis enzyme. Fermentation media was incubated at 35 °C and shaken at 150 rpm during the SSF process. The flasks fitted with perforated rubber stoppers enclosing water-filled air-locks.

2.4 Analytical procedures

Dry matter was determined using an Electric Moisture Balance at 105 °C. Before analysis, samples were dried at 45 °C for one day then milled in a Braun coffee grinder to get homogeneity sizes. Fiber composition was analyzed using a two-step acid hydrolysis as described in a previous study (Tran et al., 2020). The glucose and ethanol concentration measurements were carried out by HPLC (Shimadzu) performed with an SH1011 column at 60 °C. 0.005 M H_2SO_4 solution was used as eluent at a flow rate of 1 mL/min. Samples were diluted approximately 6-fold with the eluent, carefully mixed to extract all soluble material into the liquid. The mixtures were filtered by centrifuge (4,000 rpm) and through 0.22 µm filters then analyzed by HPLC. Elemental analysis (CHNS) of materials was performed by a EuroEA3000. Amino-acid Nitrogen was measured by the Sorensen Formol titration method as described elsewhere (Vu et al., 2015).

3. Result and discussion

3.1 Chemical content analysis results

As presented in Figure 1, the raw water hyacinth had a cellulose content of 29.32 wt%. This was not a high value among popular lignocellulosic biomasses. However, the positive meaning of exploiting water hyacinths to produce bioethanol is not only for bioethanol production but also for environmental treatment.
Water hyacinth is an aqua plant, which has a typically strong fiber structure to protect the host from water penetration. This is the explanation for the low efficiency of the classical pretreatment including the steam

explosion following by alkaline pretreatment. After being pretreated by puffing and NaOH solution of 1.0 wt%, the change in the chemical content of the material was not much. Lignin content was reduced from 25.03 wt% down to only 19.91 wt% while cellulose content increased only from 29.32 wt% to 35.76 wt%. As an attempt to promote the pretreatment technique, 2.0 wt% NaOH solution was employed instead. The alkaline pretreatment efficiency just slightly increased. For further experiments, traditional 1.0 wt% NaOH solution was used to obtain compatible results for the investigation.

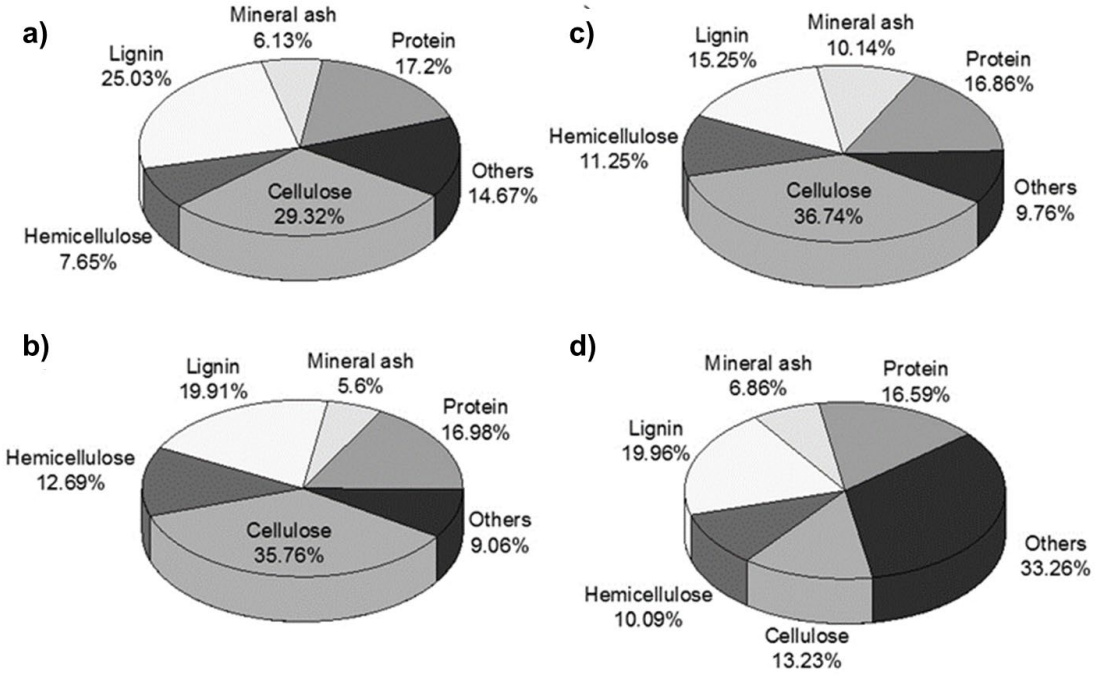

Figure 1: The chemical composition of the water hyacinth a) as the raw material; b) as pretreated with b) 1.0 wt%, and c) 2.0 wt% NaOH solution; and d) as the remaining residue after a typical SSF process.

3.2 Addition of a nutrient

During the SSF process, *Saccharomyces cerevisiae*, the yeast commonly used for ethanolic fermentation, can ferment increased amount of sugars in the medium when all required nutrients are provided in adequate amounts (Bafrncová et al., 1999). In this study, corn steep liquor (CSL) was used for SSF experiments. This supplementary nitrogen nutrient is popular in biological engineering as it is produced at industrial scales with high quality and low production cost.

Because water hyacinths contain a high content of protein, it was worth to carry out a comparative experiment of the SSF with and without the addition of CSL (0.08 wt% of the whole mixture or 1.0 wt% of biomass based on dry mass). Such dosage of CSL was calculated to be of equal amount of protein existed in the water hyacinth material as analyzed above.

As shown in Figure 2, the results were not different between the two experiments. Although CSL itself contains starch and sugar, a dosage of only 1 wt% of the water hyacinth material was eligible to make up the detected difference in ethanol fermentation.

During the initial 100 h of the processes, the ethanol concentration increasing rate (the slope of the kinetic curve) of the mixture with CSL was somewhat higher than that of the mixture without CSL. This phenomenon related to the growth of microorganisms as the yeast metabolized in-situ sugar produced from hydrolysis in the SSF flasks. During the first few hours, there was no difference between the two systems because the yeasts community had not got enough time to grow. But later on, while the protein from CSL could be soluble in the solution at the beginning, the nutrient of water hyacinth needed time to be gradually released from the solid phase when the biomass was hydrolyzed, which makes the ethanol concentration of the mixture without CSL increased more slowly than the other. After 100 h, the ethanol concentration of the two mixtures became closer and almost equal to each other at the end.

This result indicated that in the SSF process of pretreated water hyacinths, additional nutrients were not necessary. If water hyacinths are produced at industrial scales, this convenience at least can find some modest meaning of savings of production cost.

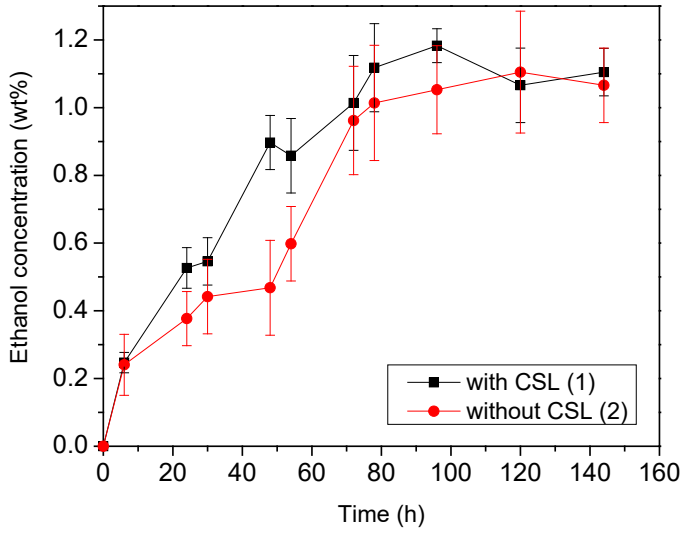

Figure 2: Produced ethanol concentration vs. SSF time when using 8.0 wt% of the mixture as the pretreated water hyacinth (dry basis), 1.0 wt% of the biomass as enzyme dosage, 2.0 wt% of the biomass as cultivated yeast solution, (1) with CSL (1.0 wt% of dry mass of the biomass) and (2) without CSL.

SSF time and enzyme dosageAs stoichiometric ratio of cellulose to ethanol is shown in Figure 3, 162 kg cellulose can be converted ideally to 92 kg ethanol. For an SSF mixture of 8 wt% material pretreated by 1.0 wt% NaOH solution (cellulose content was 35.76 % as analyzed above), if the conversion efficiency is 100 %, the bioethanol concentration of the SSF mixture will be 1.544 wt%. The low concentration of resulted ethanol from water hyacinth in this process can make the cost of energy for bioethanol refinery high.

$$\underset{\text{(cellulose)}}{H(C_6H_{10}O_5)_nOH} \xrightarrow[\text{hydrolysis}]{+\ n\ H_2O\ \text{enzyme}} \underset{\text{(glucose)}}{n\ C_6H_{12}O_6} \xrightarrow[\text{fermentation}]{\text{yeast}} \underset{\text{(ethanol)}}{2n\ C_2H_5OH} + 2n\ CO_2$$

162 kg 180 kg 92 kg 88 kg

Figure 3: Stoichiometry of the bio-conversion of cellulose to ethanol.

So it can be recommended to combine water hyacinth with other biomass to utilize its nutrient while its conversion yield is worth investigated. Based on ideal value of ethanol concentration from water hyacinth, the ethanol formation efficiencies from cellulose conversion were computed from the SSF experimental results as Eq(1):

$$\text{Conversion Efficiency } (\%) = \frac{\text{Real Ethanol Concentration (wt\%)}}{1.544\ (\text{wt\%})} \times 100\% \quad (1)$$

As presented in Figure 4, after 48-96 h of SSF, the ethanol concentration was almost not increased more. It could be explained that neither the saccharification nor fermentation got critical at that time. This will be elucidated by a discussion on the kinetics of the process later. Anyway, the SSF efficiencies were not much different when employing enzyme dosage of 1.0 wt%, 1.5 wt%, and 2.0 wt% (of biomass on dry basis). It is possible to recognize that a dosage of 1 wt% enzyme (of the dry biomass basis) is enough to hydrolyze cellulose from the biomass solid phase. More enzyme does not help increasing more the hydrolysis rate because of the limit contact between the enzyme and the solid phase surface. As a typical heterogeneous reaction, just after the cellulose on the outer surface of the biomass is hydrolyzed to glucose dissolving to the aqueous mixture, the deeper cellulose is exposed to the hydrolysis agent. More enzyme in this case does not hydrolyze more cellulose until a degree is reached. However, lower concentration of enzyme, 0.5 wt% (dry biomass basis) only yields much lower ethanol concentration. For the time being, it can be thought of the hydrophobic absorption of enzyme to lignin, which made the lower activity of low enzyme dosage (Lee et al., 2008).

In a previous study when SSF bioethanol-production process was applied on pretreated rice straw, compatible conditions of pretreatment and SSF were investigated and the similar results were observed (Vu, et al., 2015).

1.0 wt% enzyme dosage was chosen as the model factor in a single-factor approach to further investigate other factors of the process.

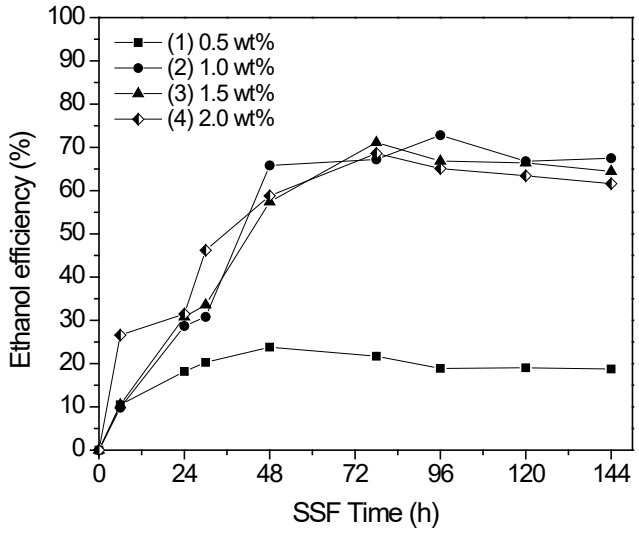

Figure 3: SSF efficiency of the mixtures consisted of 8.0 wt% as the pretreated water hyacinth (dry basis), 2.0 wt% of biomass as cultivated yeast solution, and 1) 0.5 wt%, 2) 1.0 wt%, 3) 1.5 wt%, 4) 2.0 wt% of biomass as enzyme dosage.

3.3 Kinetics of the model SSF experiment

The model SSF experiment was used to study the kinetics of the process.
As the results are shown in Figure 4, after 75 h, glucose concentration decreased to zero while ethanol concentration remained unchanged at about 1.12 wt% and the corresponding ethanol formation efficiency was 72.8 %. In other words, after 78 h, neither enzyme was not active anymore, nor the remaining lignocellulose could not be further hydrolyzed.

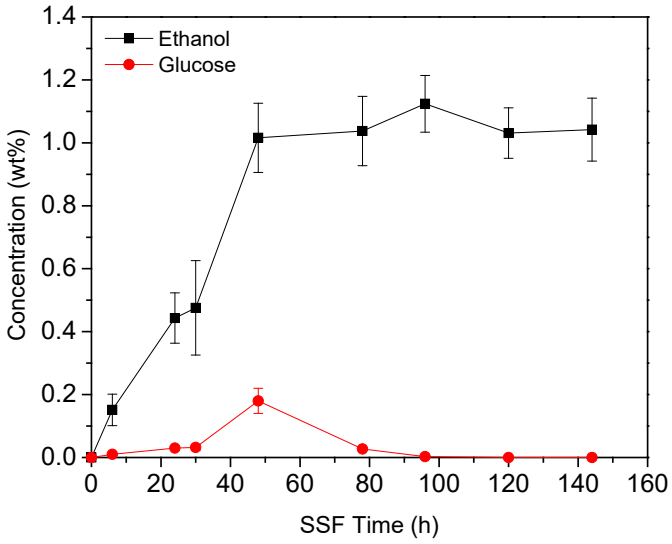

Figure 4: Ethanol and glucose concentration of the model SSF experiment.

To elucidate this point of view, another equal dosage of cellulase enzyme was supplemented to the SSF mixture after 78 h of SSF. However, there was no increase of both glucose and ethanol concentration in the broth. It is obvious that no more cellulose in the mixture was hydrolyzed by the added enzyme.

Fiber analysis was carried out for the SSF residue. As shown in Figure 1 above, the SSF residue still contains much cellulose, up to 13.23 wt% of the dry basis. This fact meant the uncompleted saccharification in the SSF process could be ascribed to the stability of crystalline cellulose and the untreated material.

4. Conclusion

In this study, water hyacinth was converted to bioethanol by a conventional SSF for lignocellulose. It was found that an additional nutrient was not needed because the protein content of the hyacinth itself was high enough for the yeast's growth. The SSF efficiency reached 72.8 % after 78 h with a mixture of 8.0 wt% of water hyacinth (dry basis) and 1.0 wt% of the biomass as enzyme dosage. This finding means that it is feasible to use water hyacinth as an input material for lignocellulosic bioethanol production without supplementary nutrients, while contributing to environmental treatment. The combination of other lignocellulosic biomass with water hyacinth without addition of nutrients in SSF processes for bioethanol production can be considered for the future work.

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