

Bioethanol Production from Enzymatically Saccharified Sunflower Stalks Using Steam Explosion as Pretreatment

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Abstract—Sunflower stalks were analysed for chemical compositions: pentosan 15.84%, holocellulose 70.69%, alphacellulose 45.74%, glucose 27.10% and xylose 7.69% based on dry weight of 100-g raw material. The most optimum condition for steam explosion pretreatment was as follows. Sunflower stalks were cut into small pieces and soaked in 0.02 M H₂SO₄ for overnight. After that, they were steam exploded at 207 °C and 21 kg/cm² for 3 minutes to fractionate cellulose, hemicellulose and lignin. The resulting hydrolysate, containing hemicellulose, and cellulose pulp contained xylose sugar at 2.53% and 7.00%, respectively. The pulp was further subjected to enzymatic saccharification at 50 °C, pH 4.8 (citrate buffer) with pulp/buffer 6% (w/w) and Celluclast 1.5L/pulp 2.67% (w/w) to obtain single glucose with maximum yield 11.97%. After fixed-bed fermentation under optimum condition using conventional yeast mixtures to produce bioethanol, it indicated maximum ethanol yield of 0.028 g/100 g sunflower stalk.

Keywords—Enzymatic, steam explosion, sunflower stalk, ethanol production.

I. INTRODUCTION

SUNFLOWER seed is the third largest source of vegetable oil worldwide, following soybean and palm. Sunflower oil, extracted from the commercially available sunflower varieties containing 39-49% oil in the seed, is used for cooking, as a carrier oil and for production of biodiesel. In Thailand, sunflower plantation areas mostly are located in Lopburi and Saraburi provinces. Its cultivation volumes increase by 10-20% annually as demand for vegetable oil production is raised, thus also increase huge accumulation of sunflower stalks. These wastes are generally used for medicinal and cosmetic purposes or burnt in the fields causing environmental pollution [1]. Based on previous studies by Marechal and Rigal, chemical composition of sunflower stalk and head were analysed [2]. The 16.8% of polysaccharides (mainly xylose) was detected in the head part whereas only

4.4% was found in stalk. Sharma *et al.* (2002) also showed that sunflower stalk was lignocellulosics, like other agricultural residues such as wheat straw or corn cob, which contained 33.5% hemicellulose and 38.5% cellulose. However, little attention has been focused on utilisation of sunflower wastes as substrates for bioethanol production. Sharma *et al.* (2004) experimented that the cellulose from sunflower stalks and hulls could be biotechnologically converted into bioethanol using *Saccharomyces cerevisiae* var. *ellipsoideus* under optimum condition of time 24 h, pH 5.0, 30 °C with maximum ethanol yield 12.03 g/ 100 g sunflower stalk [3].

The present study was therefore undertaken to optimise fractionation of main chemical components from sunflower stalks using steam explosion as well as to optimise enzymatically saccharification of resulting cellulose for bioethanol production.

II. MATERIALS AND METHODS

A. Materials

The sunflower stalks obtained locally in Thailand were used as raw material in this study. They were washed, chopped into small pieces and dried at 60 °C to constant weight and ground to 40-mesh size using a Wiley mill (Kinematica AG Co. Ltd., Tokyo, Japan).

The chemical composition of sunflower stalks was determined according to the Tappi standards, T204 om-88 for extractives; T222 om-98 for lignin; T203 om-88 for α -cellulose; T223 cm-84 for pentosan; T211 om-93 for ash. Holocellulose and total phenolic compounds were analysed according to the standard methods [4]. Contents of glucose and xylose was analysed by quantitative saccharification with H₂SO₄, followed by high performance liquid chromatographic analysis. After saccharification, an aliquot of sample was neutralised by solid Ba(OH)₂ and the mixture was homogenised using a magnetic stirrer. Final pH was adjusted to 7 with a solution of saturated Ba(OH)₂. The sample was centrifuged at 8000 rpm to remove barium sulfate, and an aliquot of 5 ml was filtered through a 0.45 μ m filter and analysed by a high performance liquid chromatography (HPLC) with an LC10A HPLC (Shimadzu Co. Ltd., Kyoto, Japan) using inositol as an internal standard. The HPLC

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analysis was performed with an Aminex column HPX-87C (300 x 7.8 mm., Bio-Rad, USA) in connection with a refractive index detector at 80°C using deionised water as solvent at a flow rate of 0.6 ml·min⁻¹.

B. Steam Explosion Fractionation

Air dry sunflower stalk sample (100g) was soaked in 0.02 M H₂SO₄ for overnight. After that, they were steam exploded at 207 °C and 21 kg/cm² for 3 minutes in a stainless steel batch digester of 2.5 l capacity (Nitto Koatsu Co. Ltd., Tokyo, Japan). Heating was accomplished by direct steam injection into the digester and the auto hydrolysis temperature from 203 to 223°C was reached for 2-5 min. Explosive discharge of the digester contents into a collecting tank was actuated by sudden opening a valve. The combined pulp slurry was collected and extracted with hot water (80°C) of a total volume of 2 l for 30 minutes. The pulp was filtered, dried at room temperature to determine pulp yield and to analyze chemical components with the methods described above. The pulp was stored for enzymatic hydrolysis to glucose and further fermentation to bioethanol.

C. Optimisation of Enzymatic Saccharification

After steaming process, enzymatic saccharification of the steam exploded pulp into glucose was optimised using multi-spectrum cellulose (Celluclast 1.5L, Novozyme A/S, Denmark) in final volume 250 ml. The pulp was suspended in sodium acetate buffer, pH 4.8, with different ratios of pulp:buffer at 2, 4, 5 and 6% (w/v), and incubated for 96 h at 50°C with continuous stirring. The ratio between enzyme and pulp was also varied at 0.33, 0.4, 0.5 and 1% (w/w). A portion of reaction mixture was taken off every 2 h for quantitative analysis of monomeric glucose using HPLC as mentioned above.

D. Bioethanol Fermentation

A 24 h suspension of *S. cerevisiae* at OD₆₆₀ = 0.6 was inoculated into 100 ml fermentation medium (in 250 ml Erlenmeyer flasks) containing 12 g/l glucose solution from the previous step. Glucose and sucrose were also added into samples 2 and 3, respectively, to enhance the growth of yeast. All media, except the control media (pH 6.7), were adjusted to pH 3.2 to accommodate yeast growth. The fermentation was carried out at 30 °C with 80 rpm shaking. Ethanol was estimated periodically by gas chromatography (GC) using HP5890 SeriesII apparatus equipped with Agilent 6890 Series injector.

III. RESULTS AND DISCUSSION

A. Steam Explosion Fractionation of Sunflower Stalks

The contents of ash, ethanol/benzene extractives, lignin, holocellulose, α-cellulose, pentosan, xylose and glucose of sunflower stalks were 10.65, 9.03, 21.92, 70.69, 45.74, 15.84, 7.69 and 27.10% of dry sunflower stalks, respectively. Such low content of pentosan was consistent with those shown by [5] and [2] that sunflower stalks contained 18.2 and 17%

pentosan, respectively, which actually was the property of softwood (pentosan <15%). However, this amount was rather lower when compared with those found in corn cob, bagasse and oil palm trunk of about 35-40% [6, 7] in which they belonged to the hardwood family. Pentosan content also indicates the condition used for composition separation by steam explosion. This is due to the working mechanism that high temperature steam converts acetyl groups in hemicellulose molecules into acetic acid which then hydrolyses xylan polymer into xylose and xylose oligomers dissolved in hemicellulose solution. Thus, in case of low pentosan content in sunflower stalk, steam explosion under mild acidic condition and/or stronger steaming condition was extremely necessary. Alphacellulose content indicates the content of cellulose in form of glucose polymer. The results also showed that sunflower stalks contained the same cellulose content (45.74%) as that in bagasse (45.57%) which was enough to produce for ethanol or other value-added products, but much higher than that in oil palm (37.14%) [7].

After steam explosion pretreatment, the steamed fiber was washed with hot water. The mixture of oligosaccharides and monosaccharides resulting from depolymerisation of hemicellulose were easily extracted from the exploded fiber by washing with hot water. The resulting hydrolysate and cellulose pulp contained xylose sugar at 1.68% and 3.31% based on raw material dry weight, respectively. It indicated that part of obtained xylose were released from sunflower stalks after steam explosion and dissolved in hydrolysate as monosaccharides which were ready to be used as substrate for further application. However, the combination of xylose contents as mentioned above was significantly different to the xylose content in raw material (7.69% based on raw material dry weight). This could be due to that part of xylose was in oligosaccharide form and not shown in chromatogram when detected with HPLC. This was in contrast with the contents of holocellulose and alphacellulose, which were not truly affected by the steam explosion condition. As shown by the glucose contents of 0.19% and 23.62% based on raw material dry weight in the hydrolysate and cellulose pulp, respectively. This indicated that steam explosion condition used in this experiment was not strong enough to degrade and release glucose from sunflower stalks and almost of glucose still remained in cellulose pulp (27.10% in raw material). Thus, the steam explosion technique was good and suitable for separation of hemicellulose from cellulose in sunflower stalks for further applications.

B. Enzymatic Saccharification

Effect of Sample Size on Enzymatic Hydrolysis of Cellulose

The steam exploded sunflower pulp was enzymatically hydrolysed using commercially available Celluclast 1.5L enzyme (74 FPU/ml). The ground and non-ground steam-exploded sunflower pulp as substrates for the hydrolysis was compared for its efficiency. Fig. 1 showed that size of substrate did not affect the efficiency of enzymatic hydrolysis

as observed by that the obtained reducing sugars at time intervals were not significantly different. Therefore, non-ground steam-exploded sunflower pulp was used as substrate for next experiment.

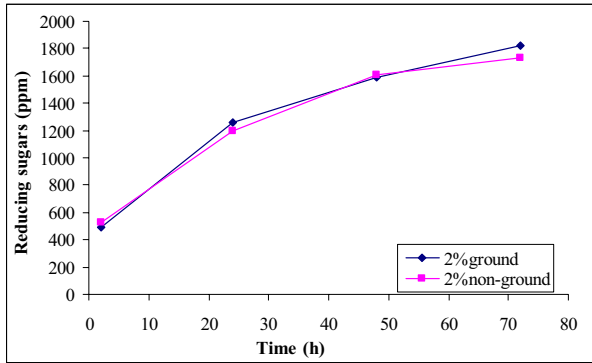


Fig. 1 The effect of sample size on enzymatic hydrolysis

Effect of Pulp Concentration on Enzymatic Hydrolysis of Cellulose

Fig. 2 showed the efficiency of pulp hydrolysis using commercial enzyme at different concentrations of sunflower pulp. It was obvious that the efficiency of enzymatic hydrolysis was relevant to the concentration of pulp. When pulp concentration increased from 2% to 6%, the efficiency of hydrolysis was also raised and would be able to constantly raise as significantly observed by the amount of reducing sugars. However, the pulp concentration used in this experiment could not be increased more than 6% since the experimented mixture would be too viscous and the reaction would not be homogeneously stirred. Thus, in next experiments, 6% pulp concentration was applied.

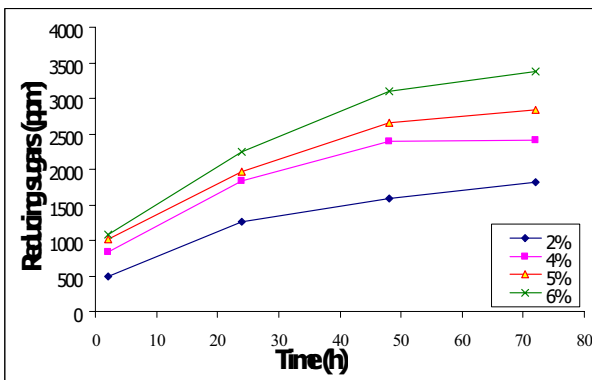


Fig. 2 The effect of pulp concentration on enzymatic hydrolysis

Effect of Celluclast1.5L Enzyme Concentration on Enzymatic Hydrolysis of Cellulose

Fig. 3 indicated the effect of Celluclast1.5L enzyme on hydrolysis of sunflower cellulose. It was clear that the efficiency of enzymatic hydrolysis of sunflower cellulose also depended on enzyme concentration as shown by the amount of released reducing sugars in the reaction. The hydrolysis efficiency significantly increased at the beginning of the

reaction and became stable after 50 h. However, the difference of reducing sugars amount between each enzyme concentration tended to decrease when 2% of enzyme concentration based on dry sunflower pulp was reached indicating the equilibrium of this hydrolysis reaction.

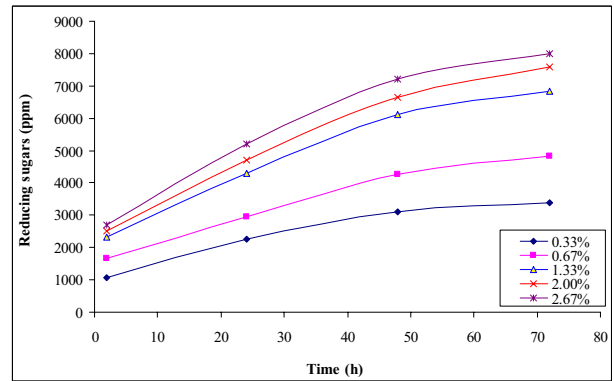


Fig. 3 The effect of enzyme concentration on enzymatic hydrolysis

C. Bioethanol Fermentation

The glucose solution from previous step was used as substrate for ethanol fermentation. Figs. 4 and 5 demonstrated the decrease in sugar concentration and increase in ethanol concentration during the fermentation reaction. It indicated a sharp decrease in sugar concentration in samples no. 2 and 3 since the beginning of fermentation. This could be due to that the addition of glucose and sucrose in samples 2 and 3, respectively, accommodated the yeast growth. The results also revealed that ethanol could be fermented either from sunflower stalks glucose (control and sample 1) or the mixture of that with pure glucose or sucrose (samples 2 and 3, respectively). However, the higher ethanol was obtained from samples 2 and 3 since more sugar was put into the reaction. Moreover, the comparison between control and sample 1 showed the effect of pH on ethanol fermentation. It indicated that the better ethanol fermentation was obtained at lower pH (pH 3.2).

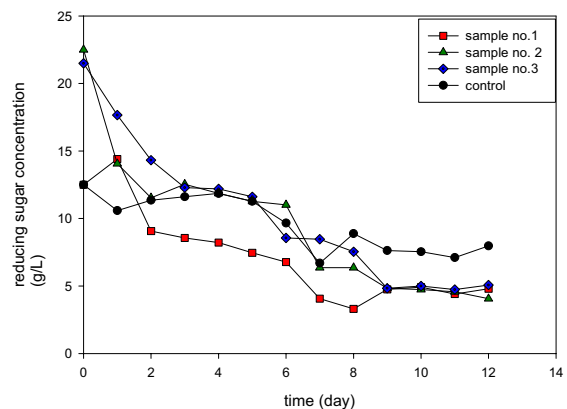


Fig. 4 The decrease in sugar concentration during fermentation reaction

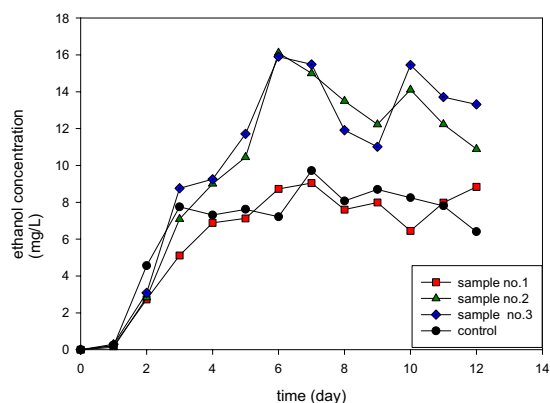


Fig. 5 The increase in ethanol concentration during fermentation reaction

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IV. CONCLUSION

Processing of sunflower stalks by steam explosion pretreatment allows the fractionation of the main polymers present in the lignocellulosic matrix. The yields of cellulose and hemicellulose are strongly dependent on the severity of the steam pretreatment. Pentosan, recovered by extraction of the exploded fiber with water, was obtained as a mixture of oligomeric and monomeric sugars. The content of monomeric sugars continuously increased with the pretreatment severity increases. The optimum condition of fractionation was 2.1 MPa, reaction time for 3 minutes at 207 °C. In addition, glucose as a raw material for ethanol production was produced by the enzymatic hydrolysis of cellulose. It indicated maximum ethanol yield of 0.028 g/100 g sunflower stalk. Even though, the yeast mixtures showed to be capable of converting glucose from sunflower stalks to ethanol, the ethanol yield was rather lower than the theoretical yield and therefore the optimal conditions still need to be further extended.

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