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# SCALE UP OF ETHANOL PRODUCTION USING PULP MILL WASTEWATER SLUDGE BY CELLULASE AND SACCHAROMYCES CEREVISIAE

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## ABSTRACT

**SCALE UP OF ETHANOL PRODUCTION USING PULP MILL WASTEWATER SLUDGE BY CELLULASE AND SACCHAROMYCES CEREVISIAE.** This study aimed to evaluate the potential use of pulp mill wastewater sludge as substrate in ethanol production. The simultaneous saccharification and fermentation process was conducted by using *Saccharomyces cerevisiae* TISTR 5339 under optimum proportion of cellulase and pulp mill wastewater sludge. The ethanol production from cellulosic materials in simultaneous saccharification and fermentation needs cooperation between cellulase and yeast. The cellulase hydrolyzes cellulose to sugar while yeast utilizes sugar to produce ethanol. The pulp mill wastewater sludge has an average content of 73.3% hemi cellulose, 67.1% alpha cellulose, 4.7% beta cellulose and 1.4% gramma cellulose. The experimental results indicated that the volume of the ethanol tend to increase with time, providing the maximum ethanol yield of 0.69 g/g on the 7$^{th}$ day, the last day of the experiment. The ethanol production was scaled up in 5 L fermentor under optimum proportion and increased the fermentation period. It was found that the ethanol production gave the maximum ethanol yield of 1.14 g/g on the 9$^{th}$ day of the totally 13 days experimentation. These results showed that the cellulose from pulp mill wastewater sludge was as effective substrate for ethanol production and alternative energy for the future.

***Key words*** : Pulp mill wastewater sludge, Cellulase, *Saccharomyces cerevisiae*, Saccharification, Simultaneous fermentation

## ABSTRAK

***SCALE UP* PRODUKSI ETANOL MENGGUNAKAN LUMPUR AIR LIMBAH *PULP MILL* DENGAN SELULASE DAN *SACCHAROMYCES CEREVISIAE*.** Percobaan ini bertujuan untuk mengevaluasi potensi penggunaan lumpur air limbah *pulp mill* sebagai bahan produksi etanol. Proses sakarifikasi dan fermentasi simultan telah dilakukan menggunakan *Saccharomyces cerevisiae* TISTR 5339 dalam perbandingan yang optimum antara selulase dan lumpur air limbah *pulp mill*. Produksi etanol dari bahan selulase dalam sakarifikasi dan fermentasi simultan bergantung pada selulase dan ragi. Selulase menghidrolisis selulosa menjadi gula selanjutnya membentuk etanol dengan bantuan ragi. Lumpur air limbah *pulp mill* memiliki kandungan hemiselulosa 73,3 %berat, alfa selulosa 67,1 %berat, beta selulosa 4,7 %berat dan gamma selulosa 1,4 %berat. Hasil percobaan menunjukkan bahwa volume etanol cenderung meningkat seiring dengan waktu dan maksimum hasil etanol sebesar 0,69 g/g pada hari ke 7 yang merupakan hari terakhir percobaan. Produksi etanol telah di *scale up* dalam fermentor 5L, dengan perbandingan optimal dan peningkatan waktu fermentasi. Hasilnya menunjukkan bahwa produksi etanol sebesar 1,14 g/g pada hari ke 9 dari total 13 hari percobaan. Hasil ini menunjukkan bahwa selulosa dan lumpur air limbah *pulp mill* merupakan substrat yang efektif untuk memproduksi etanol dan merupakan energi alternatif pada masa mendatang.

***Kata kunci*** : Lumpur air limbah *pulp mill*, Selulase, *Saccharomyces cerevisiae*, Sakarifikasi, Fermentasi simultan

## INTRODUCTION

Due to the rising gasoline prices and constant conflicts in the oil-supply region of the world, and depletion of fossil fuels, the utilization of bioethanol as an alternative fuel has attracted a lot of attention because it results to zero net carbon dioxide output into atmosphere [1]. The raw materials used in the production of ethanol as renewable energy are mostly starch and sugar although cellulosic substrates provide cheaper raw materials for bioethanol production. Cellulosic materials are comprised of lignin, hemicellulose and

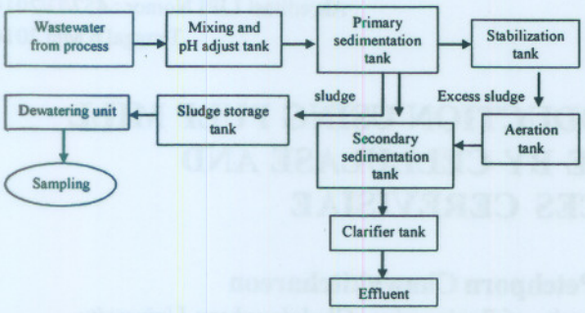


***Figure 1.*** Wastewater treatment process and sampling point

cellulose and are thus sometimes called lignocellulosic materials. Pulp mill wastewater sludge is a solid residue from paper pulp industry. It constitutes large amounts of cellulose. Its residues are usually disposed of in landfills, or subjected to incineration after dewatering which has a significant cost-increasing factor on paper production [2]. This research studied the production of ethanol by using cellulose from paper pulp factory through the joint operation of cellulase and yeast. The cellulase will decompose the cellulose to sugar, and yeast will transform the sugar to ethanol [3]. Glycolysis procedure can be presented as chemical equation to show the transformation of glucose to alcohol as shown in Equation (1).

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Ethyl Alcohol}}{2\,C_2H_5OH} + \underset{\text{Carbon dioxide}}{2\,CO_2} \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

The finding from this research can be utilized to improve the process and to yield data for future scale up.

## EXPERIMENTAL METHOD

### Materials

#### Microorganism

Saccharomyces cerevisiae TISTR 5339 from Thailand Institute of Scientific and Technological Research was used for fermentation.

#### Enzyme

Commercial cellulase enzyme solution from Trichoderma reesei ATCC 26921 (cellulase 50 mL, Sigma) was employed for saccharification.

#### Substrates

Pulp mill wastewater sludge collected from a paper pulp plant in Prachinburi province was used as raw materials in the ethanol production. The sludge was grey white in color with the sizes between 0.2-0.5 centimeters. The samples were collected after sludge pressing process as shown in Figure 1.

### Methods

The volumetric analysis of cellulose from the pulp mill wastewater sludge obtained from wastewater treatment process from paper pulp industry by using standard protocol of TAPPI (Technical Association of the Pulp and Paper Industry).

#### Preparation of Inocula

Saccharomyces cerevisiae TISTR 5339 was transferred from yeast-malt extract agar (YMA) to yeast-malt extract broth (YMB) and cultured at room temperature for 24 hr to produce yeast cells suspension. Optical density (OD) was measured on the yeast cells by using a spectrophotometer at 600 nm to have the value of 0.6 and used as inoculums for ethanol production.

The ethanol production by simultaneous saccharification and fermentation was conducted in 250 mL flask and 5 L fermentor.

In 250 mL flask : Celluloses was transformed to sugar along with yeast fermentation by sterilizing the pulp mill wastewater sludge in an autoclave at 121 °C for 15 minutes. Cellulase was then added at appropriate proportion to give the maximum amount of sugar in the 250 mL flask which contained yeast malt extract of 100 mL, and added yeast cells suspension which had been measured by spectrophotometer at 600 nm and had the value of 0.6 of 5 mL (5 % innoculum) in order to use as inoculum [4]. After that, the product was shaken at 120 rpm for 7 days. Samples were collected on the 1st, 3rd, 5th and 7th day. The ethanol volume was measured, samples were then filtered through a 0.45 µm membrane filter before inject ion to gas chromatography (Shimadzu, model GC-7AC).

***Table 1.*** Ethanol production in flask scale

| Fermentation time (day) | Ethanol (g/l) | Ethanol Yield (g/g) | Accumulation of sugar (mg/mL) |
|---|---|---|---|
| 1 | 4.73 | 0.44 | 4.15 |
| 3 | 5.52 | 0.55 | 4.90 |
| 5 | 6.94 | 0.64 | 4.15 |
| 7 | 7.26 | 0.69 | 4.52 |

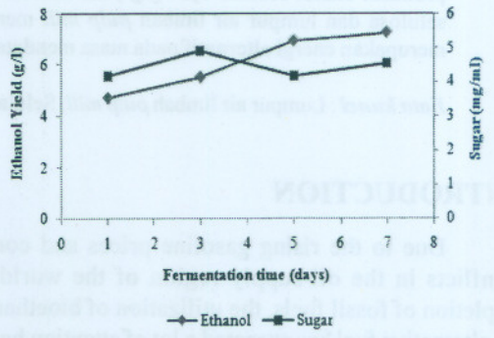


***Figure 2.*** Ethanol production and accumulation of sugar in 250 mL flask

***Table 2.*** Ethanol production in 5L fermentor

| Fermentation time (day) | Ethanol (g/l) | Ethanol Yield (g/g) | Accumulation of sugar (mg/mL) |
|---|---|---|---|
| 1 | 5.84 | 0.52 | 6.14 |
| 3 | 6.63 | 0.78 | 8.87 |
| 5 | 7.34 | 0.80 | 8.14 |
| 7 | 8.68 | 0.84 | 6.96 |
| 9 | 9.15 | 1.14 | 9.31 |
| 11 | 8.92 | 0.75 | 5.35 |
| 13 | 8.36 | 0.71 | 5.59 |

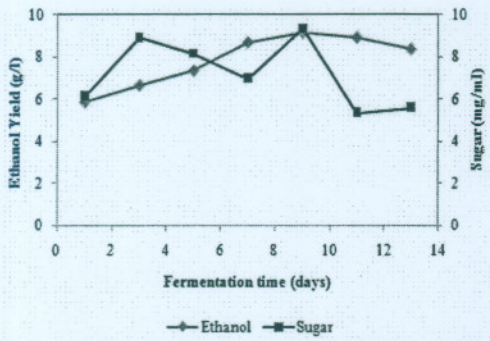


***Figure 3.*** Ethanol production and accumulation of sugar in 5 L fermentor

In 5 L fermentor : An experiment was performed at the most appropriate aforementioned condition by using a 5 L fermentor and performing 3.5 L of working volume with propeller speed of 50 rpm. The experiment was performed for 13 days, samples were collected on the 1st, 3rd, 5th, 7th, 9th, 11th and 13th day. The volume of the ethanol was measured by gas chromatography.

## RESULTS AND DISCUSSION

The sludge from wastewater of paper pulp factory contained cellulose which consisted of hemicellulose, alpha cellulose, beta cellulose and gamma cellulose of 73.3%, 67.1%, 4.7% and 1.4% respectively. The 1:15 (mL of enzyme/grams of dried sludge) was the most suitable proportion for ethanol production which provided the largest amount of sugar comparing to other proportion at the amount of 33.99 mg/mL.

### Ethanol Production in 250 mL Flask

The experimentation performed by using the proportion at 1 : 15 (mL of enzyme /grams of dried sludge) provided the highest yield of sugar for the simultaneous saccharification and fermentation process in ethanol production. The volume of the ethanol tend to increase with time, providing the maximum ethanol yield of 0.69 (g/g) on the 7th day, the last day of the experiment, as listed in Table 1. The parameters measured were sugar utilization and ethanol production as shown in Figure 2. There was a slight accumulation of sugar during the period when comparing with the initial sugar (33.99 mg/mL). Regarding the sugar utilization by yeast, as the fermentation period was increased, the ethanol volume also increased. The results obtained on the production of ethanol were in agreement with earlier study by Premjet et.al.[5] on ethanol production by using paper mulberry in the simultaneous saccharification and fermentation process along with cellulase from mixed cultures of Penicillium sp. and Candida krusei NBRC 1664. They found that the ethanol volume increased continually during fermentation, giving the maximum ethanol yield of 0.0109 (g/g).

### Ethanol Production in 5 L Fermentor

The process was scaled up by using the proportion at 1:15 (mL of enzyme/grams of dried sludge), which provided the most sugar for the simultaneous saccharification and fermentation in the 5 L fermentor. It was found that the ethanol volume increased with time. The ethanol production gave maximum ethanol yield of 1.14 (g/g), on the 9th day of the totally 13 days experimentation, as detailed in Table 2 and the parameters measured were sugar utilization and ethanol production as shown in Figure 3.

## CONCLUSION

Wastewater sludge leftover from paper pulp manufacturing industry contained 73.3% hemicellulose. Since the substances were polymeric glucose, it could be used as raw material in ethanol production process. The results of both the 250 mL flask and the 5 L fermentor of simultaneous saccharification and fermentation revealed that the volume of ethanol produced increased as time passed and it gave the maximum ethanol yield at 1.14 (g/g) in 5 L fermentor. The ethanol production in the 5 L fermentor worked much better than in the 250 mL flask.

## ACKNOWLEDGMENT

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