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Production of Bioethanol from Office Waste via Simultaneous Saccharification Fermentation

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Abstract

Bioethanol is an attractive and sustainable fuel that can be used in the transportation sector. It is recognized as being a good alternative to conventional fossil based fuel as it is biodegradable and emits zero net carbon output into the atmosphere. In this study, bioethanol was produced from office waste by using a commercial cellulase preparation and *Saccharomyces cerevisiae* via Simultaneous Saccharification Fermentation (SSF). The office waste feedstocks were loaded at 5.0%, 7.5% and 10.0% (w/v) against two cellulase dosages at 25 and 50 FPU/g paper. The fermentation products and residual sugars were then analyzed via High Performance Liquid Chromatography (HPLC). From the results obtained, the combination of 5.0% feedstock loading with 50 FPU/g paper cellulase produced the highest concentration of ethanol at 96 hr, which was at 67.64% of theoretical ethanol yield (TEY). While the combination of 10.0% feedstock loading with 50 FPU/g paper cellulase produced the lowest concentration of ethanol at 32.69% of TEY. This study has shown that office waste can be converted to bioethanol via SSF, and may assist in reutilization and the waste management of office waste.

INTRODUCTION

Liquid biofuel such as bioethanol is a promising alternative to substitute and supplement petroleum based fuel [1]. Bioethanol is an oxygen-containing organic chemical with unique combination of properties as a solvent, antifreeze and especially of its versatility as a chemical intermediate for other chemicals [2]. This biodegradable and low toxic liquid fuel also functions as an octane enhancer in conventional petrol [3]. The application of bioethanol as transportation fuel is currently attracting worldwide interest because of its zero net carbon output into the atmosphere [1, 4]. Furthermore, bioethanol is biodegradable, and unlike fossil fuel, it only emits carbon dioxide without other poisonous emissions [2].

Bioethanol or ethanol is traditionally produced from microbial fermentation of sugar, although it can also be manufactured by the chemical process of reacting ethylene with steam [2]. Mature technologies for bioethanol production are crop-based, utilizing substrates such as sugar cane and cornstarch [6]. However, the cost of these raw materials can be as high as 40% of the bioethanol cost [7]. Therefore, recent efforts are concentrating on utilizing lignocelluloses as these cheap and abundant polymer is found as agricultural waste, forestry residue, municipal solid waste as well as office waste [1, 2, 6].

According to the Global Environment Centre (n.d.) [8], over 23,000 tonnes of waste is produced each day in Malaysia. This amount is expected to rise to 30,000 tonnes by the year 2020. A middle-income country such as Indonesia, Malaysia and Thailand generates 0.52 and 1.0 kg of municipal solid waste (MSW) per capita per day, and it is projected to increase to 511 kg/capita in 2025 [9]. The major components of MSW are office waste (papers), plastics, metal and glass. Paper products are considered to be the largest group in MSW which is about 55% of the total volume [10]. These cellulosic materials can be broken down into glucose and converted into ethanol by processes such as saccharification and fermentation, and because of its abundance and low costs, they are very ideal feedstock for ethanol production [11].

In this study, we performed ethanol production from office waste (paper) by using a commercial cellulase preparation and *S. cerevisiae*, focusing on the effects of the different enzyme concentration and effect of the feedstock loading on the simultaneous saccharification and fermentation (SSF) rates of the office waste.

MATERIALS AND METHODOLOGY

Experimental Setup

A flow-chart of the overall experimental setup is shown in **Figure 1**. All experiments were conducted in duplicate (n=2) and analyses were done in triplicate (n = 3).

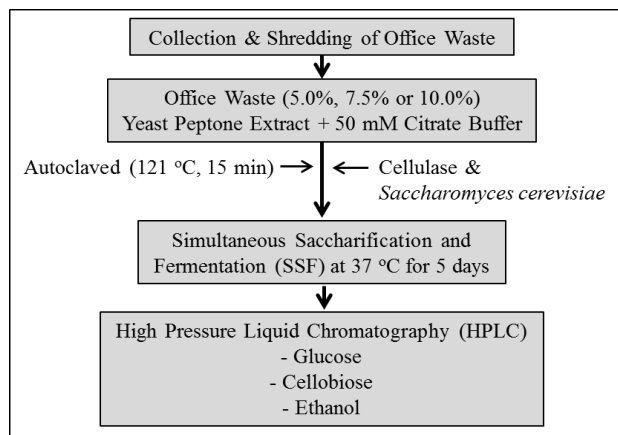


Figure 1. Flow-chart of process outlining the steps for simultaneous saccharification and fermentation (SSF) of office waste using cellulase and *S. cerevisiae*.

Cellulase Activities Determination-Filter Paper Unit (FPU)

The cellulase activity assay was performed according to the International Union of Pure Applied Chemistry (IUPAC) [12].

Saccharomyces cerevisiae culture preparation

Saccharomyces cerevisiae (ATCC 24859) used in this study was obtained from the American Type Culture Collection (Rockville, MD, USA). The *S. cerevisiae* culture inoculum was prepared in sterile 100 ml Yeast Malt (YM) Broth overnight at 32 °C with constant agitation (150 rpm), and harvested via centrifugation in 50 ml conical centrifuge tubes. The cell concentration density was adjusted by using sterile YM Broth to 10^7 – 10^8 cells/ml [1].

Simultaneous Saccharification and Fermentation (SSF)

Simultaneous saccharification and fermentation (SSF) experiments were carried out in 250 ml flasks with batch cultures of 150 ml final volume, consisting of 1.5 g yeast extract and 3.0 g peptone dissolved in 0.05 M citrate buffer. Office waste (paper) which was the substrate for SSF was added at 5.0%, 7.5% and 10.0% (w/v) before autoclaving. After autoclaving, the enzyme cellulase (Accellerase® 1000, Genencor, CA, USA) was added at 25 FPU/g paper and 50 FPU/g paper and the bottles were aseptically inoculated with the harvested *S. cerevisiae* culture. These batch cultures were incubated at 37 °C and agitated at 150 rpm for 5 days. The SSF experiments were performed in duplicate (n=2).

Sample Collection

Sample aliquots of 1.5 ml were taken at 0 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr and 120 hr. The samples were centrifuged and filtered through a 0.45 µm nylon syringe filter.

High-Performance Liquid Chromatography (HPLC)

Ethanol, glucose and cellobiose were analyzed using a High Performance Liquid Chromatography (Shimadzu/LC-20A, Tokyo, Japan) system that was equipped with a refractive index detector (RID-10A, Tokyo, Japan), Column Oven (CTO-20A, Tokyo, Japan), Communication Bus Module (CBM-20A, Tokyo, Japan) and computer controller. The separation and analysis of ethanol and sugar residues were done on a Bio-Rad Aminex HPX-87H column (150 x 7.8 mm; Bio-Rad Chemical Division, CA, USA) using 0.005 M H₂SO₄ as the mobile phase at a rate flow of 0.8 ml/min, a 20 µl injection volume and a column temperature of 65 °C [6, 11].

RESULTS AND DISCUSSION

In this study, we performed simultaneous saccharification and fermentation (SSF) on office waste using a commercial cellulase preparation and *S. cerevisiae*. SSF is a process that utilizes the enzymatic saccharification of cellulose by cellulolytic enzymes and fermentation of resulting sugars to ethanol by a fermentation microorganism that occurs at the same time, in the same vessel [6]. This process has been studied for several decades and has great potential for the economic production of bioethanol [13]. According to Vincent (2010) [2], SSF is the chosen method to produce ethanol from lignocellulosic biomass as this process alleviates end-product inhibition of the enzymes, and it is also less capital intensive than other procedure such as separate hydrolysis and fermentation (SHF). In addition, the presence of ethanol in the culture medium reduces the potential for microbial contamination [14]. Vincent et al. (2010) [2] further noted that SSF is superior to SHF in terms of overall ethanol yield. Although the current market price of cellulases makes the process less favourable compared to technologies using acid catalysts, working with enzymes are significantly milder, making it possible to combine the cellulose hydrolysis with the ethanol fermentation [2, 15]. To perform fermentation of the sugars released during saccharification, *S. cerevisiae* was chosen as the fermenting organism because this yeast effectively converts sugar to ethanol due to its rapid rate of glycolysis [11].

Office waste was used as the substrate in our study to produce ethanol because of its abundance and low cost. The most common office waste is paper, consisting mainly of cellulose. These cellulosic materials therefore can be broken down into glucose and finally converted into ethanol via anaerobic fermentation [11]. Saccharification of the office waste was performed using Accellerase® 1000, a commercial cellulase enzyme, to hydrolyze the office waste into fermentable monomeric glucose units. The progression of this hydrolysis can be seen in **Figure 2**. On day 0, at 0 hr up to 12 hr (**Figure 2A** and **Figure 2B**), the shredded paper can be seen to be still intact. As SSF enters the 48 hr period, the shredded paper appeared less distinguishable (**Figure 2C**) and at 120 hr (**Figure 2D**), all the paper substrate was hydrolyzed into thick slurries.

Initial evaluation on the efficiency of the cellulolytic enzyme in hydrolyzing the office waste can be gauged and validated via the presence of saccharification products such as oligosaccharides, disaccharides and monosaccharides using HPLC. **Figure 3** shows the time course of glucose at 25 and 50 FPU/g paper cellulase dosings. Based on graph, for the samples with 25 FPU/g paper cellulase dose, the glucose concentration was higher at 10% of

substrate loading compared to 5.0% and 7.5%. The glucose levels were detectable from 6 hr and increased steadily until 12 hr, decreasing thereafter for all substrate loads. The same profiles were observed for the fermentation broth dosed with 50 FPU/g paper cellulase. Glucose was produced immediately after 0 hr and extensively increased until 12 hr and decreasing after 12 hr for 5.0%, 7.5% and 10.0% of paper.

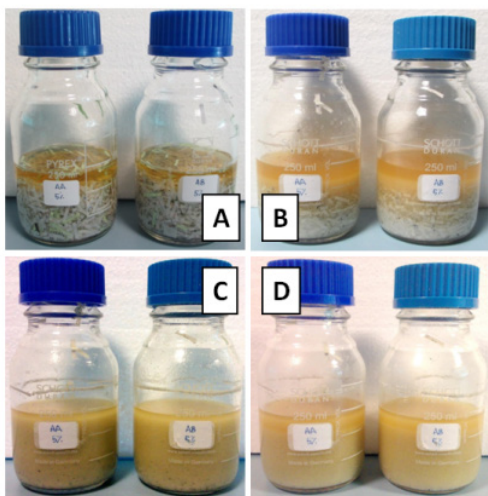


Figure 2. Simultaneous saccharification and fermentation (SSF) of office waste at (A) 0 hr, (B) 12 hr, (C) 48 hr and (D) 120 hr.

However, the glucose concentration slowly increased from 24 to 48 hr and declining until 120 hr. In general glucose concentration remained low for all samples (< 4 g/l) after 24 hr.

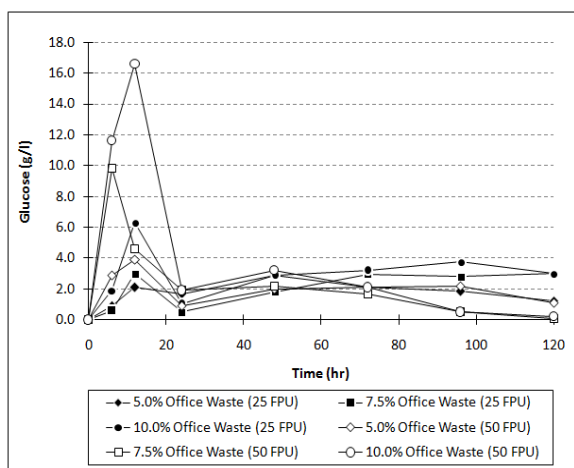


Figure 3. Time course of glucose production.

Cellobiose is another product of SSF from cellulosic materials [2]. The time course of cellobiose production is shown in **Figure 4**. For the office waste dosed with 25 FPU/g paper cellulase, the amount of cellobiose peaked at approximately 6 g/l for all substrate loadings. After 12 hr, the cellobiose level decreased at different rates until 120 hr. For the office waste dosed with 50 FPU/g paper cellulase, the cellobiose production profiles were also similar. One interesting observation was that all the

broth containing 10.0% office waste had significantly higher cellobiose concentration at the end of the SSF period, suggesting incomplete hydrolysis of cellobiose into glucose. One possible explanation is that due to high substrate content, conversion efficiencies were compromised due to the increasing viscosity which required higher energy for mixing, and shear in activation of enzymes as well as poor heat transfer [16].

Figure 5 shows the time course of ethanol production for the office waste at three different feedstock loadings against 25 FPU/g paper cellulase and **Figure 6** shows the time course of ethanol production for then office waste of three different feedstock loadings against 50 FPU/g paper cellulase. Both graphs show sigmoidal curves, typical of lignocellulosic ethanol production profiles [1, 6, 11]. Except for the 5.0% office waste dosed with 25 FPU/g paper cellulase, ethanol was only produced after 12 hr for all samples. After 12 hr, all the fermentation broths with the three different substrate loading continued to produce ethanol until 120 hr. However, for the 5.0% office waste dosed with 25 FPU/g paper cellulase, ethanol production started to decrease at 96 hr, after peaking at 52.95% TEY (Theoretical Ethanol Yield). For the fermentation broths dosed with 50 FPU/g paper cellulase, production of ethanol started at 12 hr, and increasing steadily until 72 hr (10.0% substrate) and 96 hr (5.0% and 7.5% substrate). However, the ethanol yield slowly decreases from 67.64% to 58.41% TEY after 96 hr until for the 5.0% substrate load until the end of the SSF period. At 7.5% of substrate load, ethanol production also plateaued after the 96 hr.

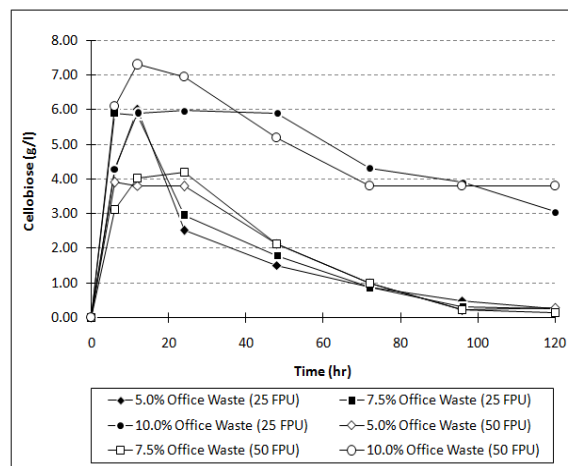


Figure 4. Time course of cellobiose production.

Based on **Table 1**, results indicate that 5.0% of paper loading for both 25 FPU/g paper and 50 FPU/g paper enzyme doses produced the highest ethanol yield compared to 7.5% and 10.0% of paper loading. Adding more enzyme at 50 FPU/g paper also significantly produce more ethanol which was at 67.64% TEY, compared to 52.95% TEY of ethanol for 25 FPU/g paper cellulase dose. According to Baiet *al.*, (2007) [17], sugar concentrations also affect the production of ethanol. Therefore, the complete hydrolysis of office waste into glucose by the cellulase can affect the rate production of ethanol. Therefore, in general, less ethanol was produced in the fermentation broth with 10.0% substrate as significant amount of cellobiose was still detected until 120 hr, as shown in **Figure 4**, suggesting partial hydrolysis.

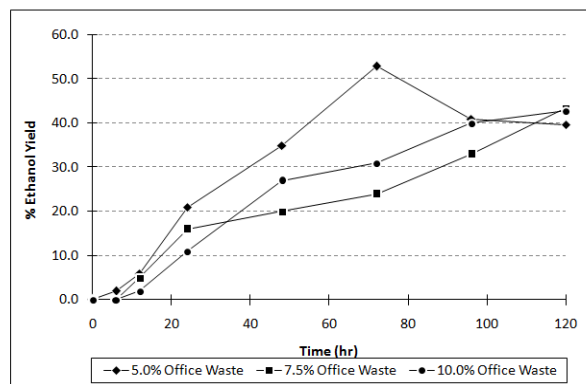


Figure 5. Time course of ethanol production at 25 FPU/g paper cellulase.

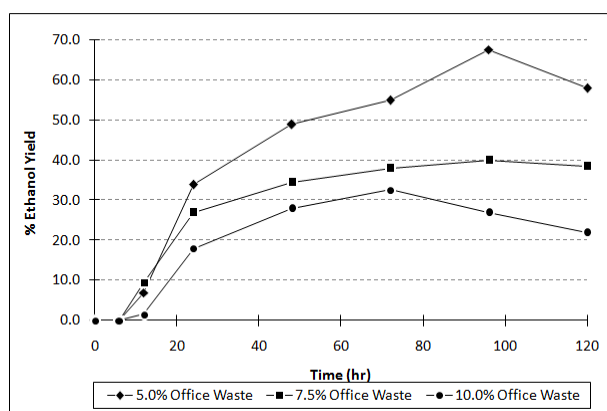


Figure 6. Time course of ethanol production at 50 FPU/g paper cellulase.

Table 1. Highest theoretical ethanol yield (TEY) data.

Enzyme Dosage	Paper Loading (%)	Highest TEY (%)
25 FPU/g paper	5	52.95
	7.5	43.50
	10	42.79
50 FPU/g paper	5	67.64
	7.5	40.03
	10	32.69

CONCLUSION

This study has shown that office waste can be converted to bioethanol using *S. cerevisiae* via SSF. From the results obtained, office waste loading at 5.0% w/v coupled with 50 FPU/g paper cellulase was found to produce the highest theoretical ethanol yield at 67.64%. One recommendation is to perform a similar study by conducting fed-batch protocol to attempt higher theoretical ethanol yield.

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