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Enzymatic Treatment of Paper Sludge

Darja Pečar*, Andreja Goršek

University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova 17, SI 2000 Maribor
darja.pecar@um.si

Paper sludge is a solid residue in paper making process that is currently disposed in land fields or burned. That consequently pollutes the environment and on the other hand this waste could be potentially valuable bioenergy convertible resource. Paper sludge, which contains cellulosic and lignocellulosic residues, is believed to be one of the most promising feedstock for the production of bio-ethanol. Bio-ethanol is the most employed liquid biofuel either as a fuel or as a gasoline enhancer. Production of ethanol for fuel is increasing because of the demand to reduce oil consumption and improving air quality, which is related to reduced emission of CO and aromatic compounds.
The main processing challenge in the ethanol production is the pretreatment of lignocellusosic biomass. During the pretreatment the degree of crystallinity of cellulose must be reduced to increase the fraction of amorphous cellulose, which is amenable to enzymatic hydrolysis. For the pretreatment one can employ physical, chemical and biological methods. The pretreatment method should improve following formation of glucose and avoid the formation of inhibitors for subsequent hydrolysis and fermentation processes.
In this study paper sludge from paper making company was firstly pretreated by milling or using phosphoric acid (H_3PO_4) and then enzymatically treated with enzyme cellulase. Enzymatic hydrolysis of cellulose using cellulase converted it to glucose. Hydrolyses were conducted at temperature, ϑ = 45 ºC, stirrer speed, f_m = 200 min^{-1}, different range of pH = (4 - 6) and amount of enzyme V = (100, 500, 1,000, 2,000 and 3,000) µL. We achieved the conversion of more than 83 % after 48 h hydrolysis at pH = 5 and amount of enzyme V = 3,000 µL.

1. Introduction

Nowadays the minimisation of emission of greenhouse gases, which mainly comprises of carbon dioxide, is becoming more and more important because of warming of the world's climate system. The atmospheric concentration of carbon dioxide increases and that is mostly a consequence of fossil fuel use (Brethauer and Wyman, 2010). Energy consumption is increasing due to the growing world's population and industrialization trend. Fossil fuels are among hydroelectric power, nuclear energy and geothermal source, the major resource of energy (Brethauer and Wyman, 2010) and that leads to the exhaustion of fossil fuels (Lakshmidevi and Muthukumar, 2010). Therefore, there is a great interest for the application of alternative energy resource (Sun and Cheng, 2002).
In contrast to fossil fuels, ethanol is a renewable energy source. It is used as a partial petrol replacement (Sun and Cheng, 2002). Researchers focus is on searching, investigating and refining of possible substances and production paths of bioethanol production (Lark et al., 1997; Chander Kuhad et al., 2010; Lu et al., 2010; Yamashita et al., 2010; López-Linares et al., 2014). Bioethanol is nowadays produced from different raw materials like starchy materials: corn, wheat, sugar cane, rise, barley; cellulosic and lignocellulose materials: straw, bagasse, corn stover, rice hulls, wood, waste paper and paper pulp (Sánchez and Cardona, 2008; Diaz et al., 2013).
Waste paper pulp is a solid residue in paper making process. Paper pulp consists of different amounts (approximately (60 – 80) %) of water depending of the efficiency of dewatering procedure and other inorganic and organic components. The composition varies with the type of paper that is produced. Most of the paper pulp is currently disposed in landfills or burned, which pollutes the environment and wastes the potentially

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valuable bioenergy resource (Peng and Chen, 2011), and creates environmental and economic problems (Prasetyo et al., 2010). The amount and type of materials in landfills are restricted by legislative trend in certain countries, because landfills are running out of storage space and cope potential ground water contamination (Prasetyo et al., 2010). This could be overcome by making waste paper pulp an attractive biomass feedstock for production of fermentable sugars and further for the production of bioethanol (Lin et al., 2012). As a consequence that could be beneficial from economic and environmental perspective (Prasetyo et al., 2010).
In this work waste paper sludge from paper making company was firstly pretreated by milling or using phosphoric acid. Enzymatic hydrolysis of pretreated paper pulp using cellulase converted it to glucose. Hydrolyses were conducted at different ranges of pH and amounts of enzyme. Afterwards the fermentation of glucose to bioethanol was conducted using immobilized yeast cells.

2. Experimental

2.1 Materials

All the chemicals, phosphoric acid (w ≥ 85 %; Kemika), acetone (p.a.; Chem-Lab), acetic acid (w ≥ 99.8 %; Fluka), sodium acetate (w ≥ 99 %; Panreac), cellulase (from Aspergillus sp.; Sigma), glucose (anhydrous; Sigma-Aldrich), glucose reagent (GOD-PAP; Roche/Hitachi), sodium alginate (Sigma-Aldrich), calcium chloride (w ≥ 97 %; Sigma-Aldrich), and yeast cells (Saccharomyces cerevisiae; Sigma-Aldrich) are commercially available, and were used as received without further purification.

2.2 Equipment

Reactor system EasyMax 102
Reactor system EAsymAx 102 consists of two 100 mL reactors that can be very easily controlled. The glass-made laboratory batch reactor was equipped with a magnetic stirrer and temperature sensor. The heating-cooling system is very accurate and can control the temperature in the range of -28 to 183 °C. The process parameters during the reaction are recorded and can be transferred to other media for further processing.

FTIR ReactiR™
For the real time in-situ monitoring of the concentration profiles of ethanol during the fermentation the FTIR based ReactIR™ iC10 analysis system coupled with a flexible Silver Halide (AgX) FiberConduit™ and 6.3 mm DiComp™ probe.

UV-VIS spectrophotometer
Glucose concentration at the end of the cellulose hydrolysis was determined using UV-VIS spectrophotometer Varian.

2.3 Pretreatment of waste paper pulp

Waste paper pulp from one of our local papermaking company was firstly dried and then approximately 20 g of milled pulp were dissolved in 70 mL of phosphoric acid at the temperature, ϑ = 50 °C. The mixture was stirred for 2 h. Undissolved inorganic compounds were separated by centrifugation for 5 min at 5,000 min^{-1}. The remaining liquid phase was poured into vigorously stirred ice-cold deionized water. The precipitated solid was separated from supernatant containing diluted phosphoric acid by centrifugation for 5 min at 5,000 min^{-1}. Solid particles were three times resuspended in deionized water and two times in acetone and each time the supernatant was removed by centrifugation. After the last centrifugation the pre-treated waste paper pulp was dried. Before it was used for enzymatic hydrolysis the content of inorganic compounds was determined by heating the solid up to 500 °C.

2.4 Enzymatic hydrolysis

The hydrolyses were performed in 100 mL reactors at an initial pretreated waste paper pulp loading of 0.15 g in 50 mL of 0.5 mol/L sodium acetate buffer with different values of pH. This slurry was heated to the temperature, ϑ = 45 °C and then different amounts of enzyme cellulose were added. The reaction mixture was stirred at f_m = 150 min^{-1} for 24 h. The samples were filtrated and heated at approximately 100 °C for 10 min to denaturize the remaining active enzyme prior to the analyse.

2.5 Immobilization

The yeast cells were immobilized so that 1.25 g of sodium alginate was dissolved in 25 mL of sodium acetate buffer with a pH = 5. To this solution was added 0.25 g yeast cells. Prepared solution was dripped into 250 mL of a solution of calcium chloride with concentration c = 0.1 mol/L. Prepared beads with entrapped enzyme were hardened in calcium chloride for 2 h. Afterwards they were thoroughly washed with deionized water and then used in fermentation.

2.6 Fermentation

The fermentation of glucose to bioethanol was carried out at the constant frequency of the stirrer, f_m = 50 min^{-1}. The reactor was charged with 50 mL of the solution. The contents of the reactor was heated to the desired temperature, ϑ = 35 ° C and then the beads with immobilized yeast cells were added. The fermentation procedure lasted for 24 h.

2.7 Analytical methods

The amount of glucose was determined by UV-VIS spectrophotometer. Analytical procedure starts by mixing 10 µL of sample to be analysed and 990 µL of GOD-PAP glucose reagent. The solution was 10 min thermostated at the temperature, ϑ = 35 °C, and then the absorbance was measured. Formed dye absorbs light at 500 nm. Concentration of glucose was determined using calibration curve prepared from glucose solutions of known concentrations.
The FTIR-based analysis system was used for the real time monitoring of ethanol concentration profiles within an automated laboratory batch reactor. For the preparation of the calibration curve ethanol was diluted in water to obtain solutions with different concentrations. The real-time concentration profiles of ethanol during the fermentation were obtained by calculating the areas to two point baseline of the corresponding peak.

3. Results and discussion

C.The purpose of our work was to enzymatically treat the obtained waste paper pulp from paper making company. The waste paper pulp consists of approximately 66 % of water, and the remaining solid represents 57 % of inorganic and 43 % of organic components mainly cellulose, which was determined by heating the solid residue up to 500 °C. It is well known that cellulose is a polysaccharide consisting of a linear chain of glucose units. The chains are bond together with hydrogen bonds and consequently cellulose has a crystalline structure. As a result of tight hydrogen bonding cellulose is extremely difficult to dissolve in water. It is therefore necessary to significantly reduce the crystallinity degree for hydrolysis purpose, especially in the case of enzymatic hydrolysis.
The choice of pretreatment method is very important, as this is the most important step for effective hydrolysis of cellulose. We investigated the difference between milling and milling in the combination with swelling by phosphoric acid.
The conversion of paper pulp pretreated by milling was much lower in comparison to paper pulp pretreated by milling and swelling in phosphoric acid as can be seen from Table 1.

Table 1: Conversion regarding the pretreatment method, at V_{enzyme} = 500 µL and a pH of sodium acetate buffer solution pH = 5

pretreatment method	X (%)
milling	3.8
milling + H_3PO_4	36.5

Chemical swelling with phosphoric acid is therefore favourable as a pretreatment method followed by enzymatic hydrolysis in comparison to milling.
We investigated the effect of pH of sodium acetate buffer solution on the conversion. The optimal pH of the solution was, pH = 5 (Table 2).

Table 2: Conversion regarding the pH of sodium acetate buffer solution, at V_{enzyme} = 500 µL

pH	X (%)
4	25.4
5	36.5
6	29.3

The amount of enzyme loading was from 0.1 to 3 mL. It turned out that the maximum conversion was achieved with the largest loading, but in fact if we compare the conversion at 1 mL of enzyme 65.1 % to 72.2 % conversion at triple the amount of enzyme, one can conclude that the conversion didn't increase significantly (Table 3).

Table 3: Conversion regarding the volume of added enzyme, at pH of sodium acetate buffer solution pH = 5

V_{enzyme} (mL)	X (%)
0.1	36.7
0.5	36.5
1	65.1
2	67.3
3	72.2

By extending the time of hydrolysis from 24 h to 48 h the conversion increased from 72.2 % to 83 %.
We proceeded with the fermentation of glucose to ethanol. The fermentations were carried out with immobilized yeast cells. Used yeast cells were immobilized, so that we could use them several times repeatedly. With immobilization the cost of yeast is reduced and the separation at the end of the reaction is easier. After some unsuccessful attempts we managed to find the appropriate immobilization technique. Yeast cells were immobilized with entrapment in calcium alginate. The formed beds were used to catalyse the reactions.
The progress of the fermentation was monitored using the FTIR-based ReactIR™ iC10 analysis system. The ReactIR iC software was used to control the spectrometer and to collect the spectra every 2 min. All the spectra collected during the experiments were measured against a background spectrum of air. As an example the FTIR spectra collected during one selected experiment is presented in Figure 1.

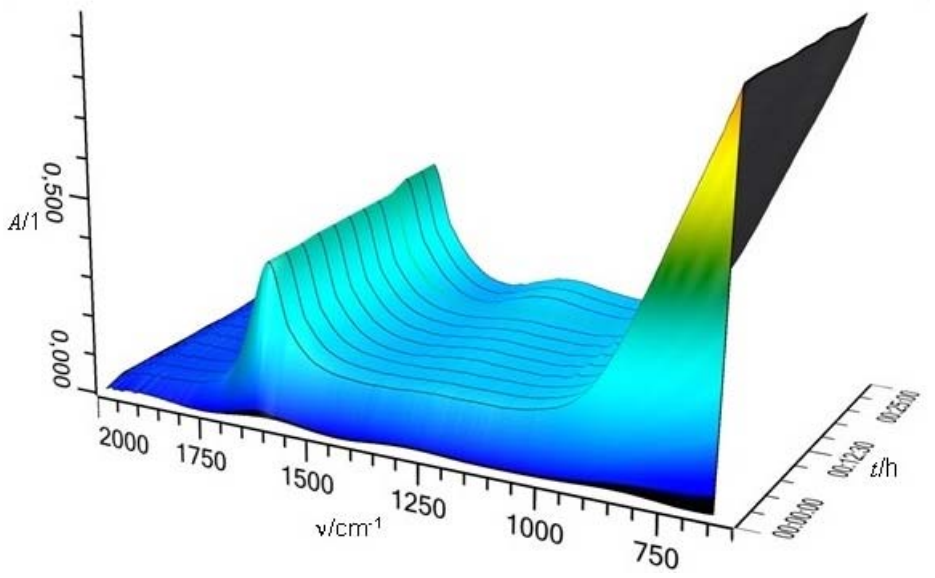

Figure 1: Waterfall plot of FTIR spectra during the fermentation at a temperature, ϑ = 35 ºC

It can be clearly seen that the intensity of the peak (absorbance, A), which is characteristic for ethanol, increases over the fermentation time. After a certain period, depending on the experimental conditions, equilibrium steady-state is formed. For reaching 98 % efficiency almost 24 h are required. Proposed simple continuous saccharification and fermentation process is schematically presented in Figure 2.

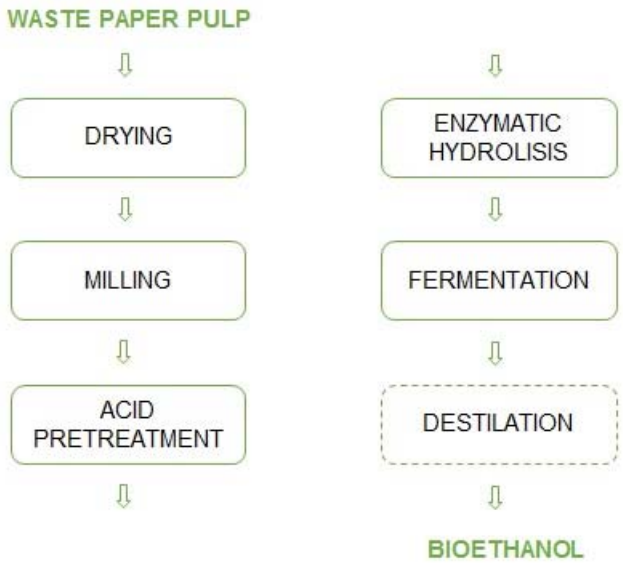

Figure 2: Process scheme for the production of bioethanol from waste paper pulp

Separate hydrolysis and fermentation approach was investigated in our study, because it enabled better matching the pH and temperature to those that are optimal for each step. Alternatively, both steps could be performed simultaneously which is economically more attractive. Distillation operation was not performed during this research.

4. Conclusion

This study presents the results of waste paper pulp processing as possible bioenergy resource and production of bioethanol. The conversion of waste paper pulp pretreated with phosphoric acid gave significantly higher values compared to those pretreated only by milling.
We achieved the conversion of more than 83 % after 48 h hydrolysis at pH = 5 and amount of enzyme V = 3000 µL. The fermentation efficiency of glucose to bioethanol was 98 % after 24 h.
We can conclude that pretreatment combining milling and swelling with phosphoric acid could be effective for further production of ethanol from waste paper pulp from existing industry.

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