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Use of membrane separation in enzymatic hydrolysis of waste paper

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Abstract−A three-stage process containing phosphoric acid pretreatment, enzymatic hydrolysis, and membrane filtration was performed on waste paper as a lignocellulosic material. In the first two stages, the effect of phosphoric acid concentration, enzyme loading, hydrolysis time, and substrate concentration on the amount of products was investigated. At the third stage using a proper membrane, the effect of substrate concentration and transmembrane pressure (TMP) on yield of the reducing sugars was studied. The novelty of the present study was to demonstrate the application of ultrafiltration membrane on the enzymatic hydrolysis process of waste paper. The reducing sugars concentration was determined by using the 3,5-dinitrosalicylic acid (DNS) reagent method. According to the results, a value of 0.5% was determined as the optimum concentration for phosphoric acid in the pretreatment stage. The reducing sugars yield was obtained as 67.4% in this concentration. Moreover, for the enzymatic hydrolysis of waste paper, the suitable amounts of cellulase enzyme loading and hydrolysis time were determined as 50 mg/g substrate and 48 h, respectively. In the filtration stage, increase of substrate concentration and decrease of TMP resulted in higher rejection of the reducing sugars. The experimental results revealed that the highest rejection was 19.2% at TMP of 3 bar and substrate concentration of 100 g/L.

Keywords: Waste Paper, Enzymatic Hydrolysis, Pretreatment, Reducing Sugars, Membrane

INTRODUCTION

Lignocellulosic materials can be classified as agricultural residues, forest products, dedicated crops, waste paper, etc [1,2]. The enzymatic method is significant because it enables regioselective depolymerization under optimum conditions [3]. Enzymatic conversion of cellulosic wastes has a considerable potential to produce fuels and chemicals [4].

Generally, hydrolysis of cellulose is more difficult than that of the other polysaccharides [5]. To enhance the enzyme accessibility to biomass and yields of fermentable sugars, pretreatment processes are necessary, which can be classified as physical, chemical, physico-chemical, and biological pretreatment [6]. Acid pretreatment is the most commonly employed chemical pretreatment for lignocellulosic materials. Acids such as sulfuric acid, hydrochloric acid, formic acid, acetic acid, peracetic acid, and phosphoric acid can be used for this purpose [6,7]. The pretreatment using a weak acid such as phosphoric acid provides an effective fractionation of lignocellulosic materials [7].

The enzymatic saccharification of cellulose is catalyzed by a complex cellulase enzyme system which typically comprises three classes of enzymes: endoglucanases, exoglucanases, and β-glucosidases. The cellulase enzyme complexes from *Trichoderma viride* and *Trichoderma reesei* are two commonly used systems [8]. Substrates, cellulase activity, and reaction conditions such as temperature and pH are the factors that affect the enzymatic hydrolysis of cellulose [9]. The slow reaction rate, exacerbated by the product inhibition of cellulases, has been recognized as the major obstacle in achieving an economically viable commercial operation of enzymatic cellulose hydrolysis [10].

Membrane filtration is one of the most selective and energy-efficient separation processes [11]. The membrane with an appropriate molecular weight cut-off can reject the enzyme and lignocellulosic substrate particles, while the low-molecular weight reaction products such as glucose pass through the membrane into the permeate side [12]. Ultrafiltration (UF) is presently regarded as a well-established unit process [13]. Some researchers have studied the use of UF for enzymatic hydrolysis of cellulose. Table 1 presents a summary of their researches.

In the present study, the enzymatic hydrolysis of the waste paper pretreated with phosphoric acid was done. Waste paper is particularly attractive as a feedstock for enzymatic hydrolysis because it is readily available and has relatively high cellulose value [17]. In the pretreatment stage, the effect of phosphoric acid concentration on the reducing sugars yield was studied. Then, in the hydrolysis stage, the effect of enzyme loading, hydrolysis time, and waste paper concentration on concentration and yield of the reducing sugars was investigated. The important aim of the study was to demonstrate the efficiency of UF membrane for enzymatic hydrolysis process. The membrane was used for the first time to separate the reducing sugars in enzymatic hydrolysis of waste paper. Thus, a polyethersulfone (PES) UF membrane was used for separation of these products. In this stage, the effect of waste paper concentration and transmembrane pressure (TMP) was studied and discussed in detail.

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Table 1. The use of UF membrane for enzymatic hydrolysis of cellulose-literature data

Substrate	Membrane	Molecular weight cut-off (kDa)	References
α-Cellulose	Polysulfone	10	Lee and Kim [14]
α-Cellulose	Polysulfone	10	Gan et al. [10]
α-Cellulose	Polyethersulfone	10	Abels et al. [15]
Microcrystalline cellulose	Polyethersulfone & ceramic	10 & 5	Lozano et al. [16]

MATERIAL AND METHODS

1. Materials

The cellulase enzyme produced by *Trichoderma reesei* (catalog no. C8546) with the molecular weight ranges of 48,000 to 52,000 Da and 3,5-dinitrosalicylic acid was supplied by Sigma-Aldrich. A flat sheet polyethersulfone (PES) membrane (PES-5) was purchased from Sepro. The other chemicals used in this research were obtained from Merck.

2. Pretreatment

Waste paper as a substrate was cut into 2 cm×2 cm pieces by scissors and was immersed into the phosphoric acid solutions (0.10-1.5%). Then, these solutions were agitated with a shaker for 1 h at 30 °C and 150 rpm. The pretreated paper was washed with deionized water until the pH became neutral. Finally, the paper was dried in an oven at 110 °C.

3. Enzymatic Hydrolysis

After pretreatment, the waste papers were cut into 0.2 cm×0.2 cm pieces. Batch hydrolysis experiment was conducted by adding a specified amount of the pretreated waste paper into the Erlenmeyer flasks containing 250 mL of 0.05 M acetate buffer (pH 4.8). The cellulase enzyme was added into the mentioned hydrolysis system. The range of cellulase enzyme concentration was from 20 to 80 mg/g substrate. The content of the flasks was preheated to 50 °C before the addition of the enzyme. The flasks were incubated in a water bath and placed on a magnetic stirrer at 140 rpm and 50 °C for 72 h. Afterward, the effect of substrate concentration on enzymatic hydrolysis was investigated at fixed enzyme to substrate ratio and also fixed hydrolysis time. The substrate concentration was varied from 20 to 100 g/L. For reducing sugars analysis, the samples were heated to 100 °C for 5 min to inactivate the enzyme and then were cooled to ambient temperature. The cooled samples were centrifuged at 5,000 rpm for 10 min and their reducing sugars content was determined using the 3,5-dinitrosalicylic acid (DNS) reagent method [18,19]. Sugar yield of waste paper was calculated using the following equation [17]:

$$\text{Sugar yield (\%)} = \frac{(\text{sugar concentration})(\text{volume of reaction mixture})}{\text{mass of waste paper}} \times 100 \quad (1)$$

4. Membrane Filtration

After the enzymatic hydrolysis, the reaction mixture was cooled to room temperature and then filtered through the UF membrane. The membrane filtration was performed according to the levels of TMP and substrate concentration presented in Table 2. The reaction mixture was continuously pumped to the membrane module and recycled back to the reaction mixture tank. The total reducing sugars concentration in the permeate and feed was determined by using the DNS method. The reducing sugars rejection (R) was calculated using the following equation [20]:

$$R\ (\%) = (1 - C_P/C_F) \times 100 \quad (2)$$

where C_P and C_F are the reducing sugars concentration in the permeate and feed, respectively.

Table 2. Parameters and their levels for the experiments of membrane separation stage

Experiment number	Substrate concentration (g/L)	TMP (bar)
1	20	3
2	20	5
3	20	7
4	40	3
5	40	5
6	40	7
7	60	3
8	60	5
9	60	7
10	80	3
11	80	5
12	80	7
13	100	3
14	100	5
15	100	7

RESULTS AND DISCUSSION

1. Effect of Phosphoric Acid Pretreatment

The effect of phosphoric acid concentration in the pretreatment stage on the reducing sugars yield is depicted in Fig. 1. As observed, the reducing sugars yield was increased with an initial increase in concentration of phosphoric acid. This result implies that the pretreatment is effective for this purpose. However, the reducing sugars yield was almost constant with the variation of phosphoric acid concentration from 0.5% to 1.5%. At phosphoric acid concentrations of 0.5%, 1%, and 1.5%, the reducing sugars yield was obtained 67.4%, 69.3%, and 68.7%, respectively. Therefore, we selected 0.5% as the optimum phosphoric acid concentration for the pretreatment.

2. Effect of Enzyme Loading, Hydrolysis Time, and Substrate Concentration

In enzymatic hydrolysis processes, the correct choice of enzymes and the optimization of the operating conditions are crucial for the success of the process. Enzymes are costly and are used in concentrations as low as possible [21]. The optimum ratio between enzyme and substrate is very important for the efficient use of cel-

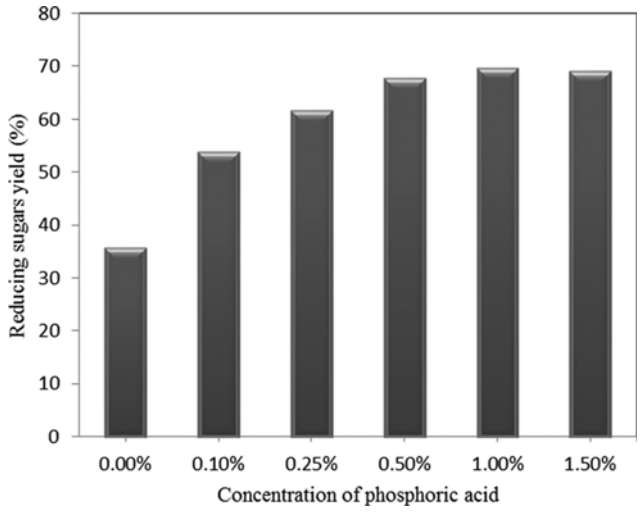

Fig. 1. Effect of phosphoric acid concentration on the reducing sugars yield.

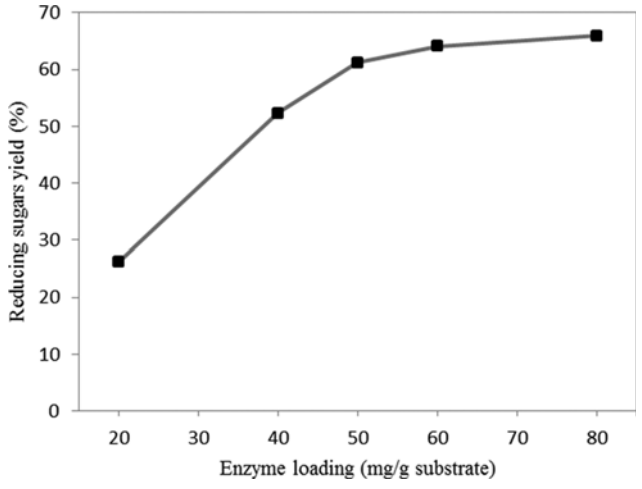

Fig. 2. Concentration of the reducing sugars in terms of enzyme loading at 48 h.

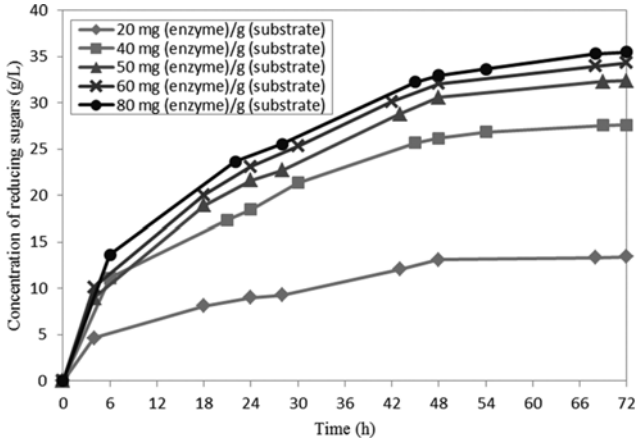

Fig. 3. Effect of time and enzyme loading on the reducing sugars concentration.

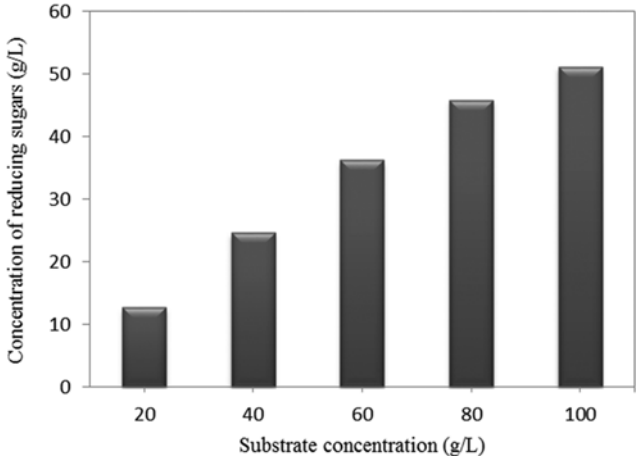

Fig. 4. Concentration of the reducing sugars in terms of substrate concentration.

lulase enzyme complex [14]. Thus, the effect of this ratio on the reducing sugars yield was studied. The results are presented in Fig. 2. Various loadings of cellulase enzyme (20-80 mg/g substrate) were used for hydrolysis of 50 g/L pretreated waste paper at pH 4.8 and temperature of 50 °C. As observed in Fig. 2, higher enzyme loading resulted in increase of the reducing sugars yield. However, the mentioned increase is negligible at the enzyme loadings above 50 mg/g substrate. At the enzyme loadings of 50 to 80 mg/g substrate, the reducing sugars yield increased from 61.2% to 65.9%. The high cost of enzymes makes high dosage impractical for this purpose [22]. For this reason, an enzyme loading of 50 mg/g substrate was selected for the next experiments.

Fig. 3 shows the effect of time on the reducing sugars concentration at different enzyme loadings in enzymatic hydrolysis of waste paper. As observed, the reducing sugars concentration during three days was examined. As expected, for a given enzyme loading, the reducing sugars concentration increased with the increase of hydrolysis time. Furthermore, for a given hydrolysis time, higher reducing sugars concentration was obtained for higher enzyme loading level. The hydrolysis rate was noticeable at the initial hours, and gradually became smaller with passing of time. The slowdown of the hydrolytic rate is mainly caused by enzyme deactivation, products inhibition, and decrease of utilizable substrate [23]. The majority of the reducing sugars were released during initial 48 h, and then the reducing sugars concentration increased slowly during the last day of enzymatic conversion. Thus, 48 h was selected as the optimal time to produce the reducing sugars.

The effect of the substrate concentration on enzymatic hydrolysis of waste paper was investigated at fixed enzyme to substrate ratio of 50 mg/g substrate and hydrolysis time of 48 h. The effect of the substrate concentration on concentration and yield of the reducing sugars is shown in Figs. 4 and 5, respectively.

As observed in Fig. 4, the increase of substrate concentration resulted in higher production of the reducing sugars. The final concentration of the reducing sugars after enzymatic hydrolysis for 48 h was 12.8, 24.7, 36.3, 45.9 and 51.2 g/L for the substrate concentration of 20, 40, 60, 80 and 100 g/L, respectively. When the ratio of

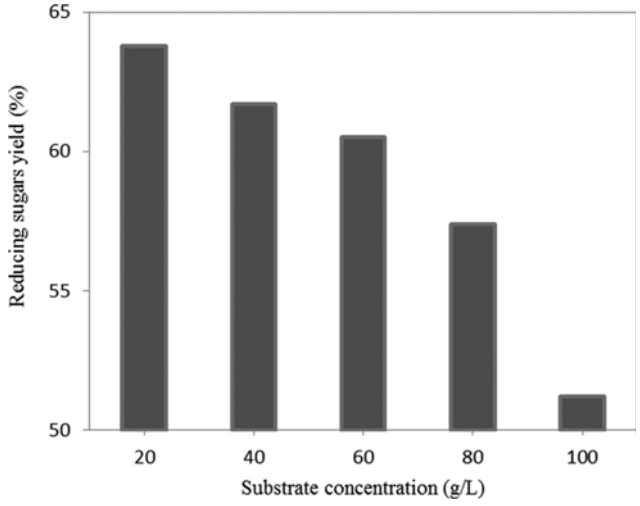

Fig. 5. The reducing sugars yield in terms of substrate concentration.

enzyme to substrate is constant, the increase of substrate concentration, along with the increase of enzyme concentration, facilitates the production of the reducing sugars.

With respect to Fig. 5, the reducing sugars yield decreased slightly in the range of the substrate concentration of 20 to 60 g/L. However, further increase of substrate concentration from 60 to 100 g/L resulted in a lower reducing sugars yield. For example, at the substrate concentration of 20 and 60 g/L, the reducing sugars yield was 63.8% and 60.5%, respectively, whereas at the substrate concentration of 100 g/L, the yield was 51.2%. Gregg and Saddler also reported an inverse relationship between the substrate concentration and the yield [24].

3. Filtration of Reaction Mixture

The important aim of this research was the separation of the products of the enzymatic reaction using the UF membrane. In this stage, the effects of substrate concentration and TMP were investigated. The rejection should be included in defining the optimum conditions for the UF process [25]. The rejection of the reducing sugars in terms of substrate concentration and TMP is presented in Fig. 6.

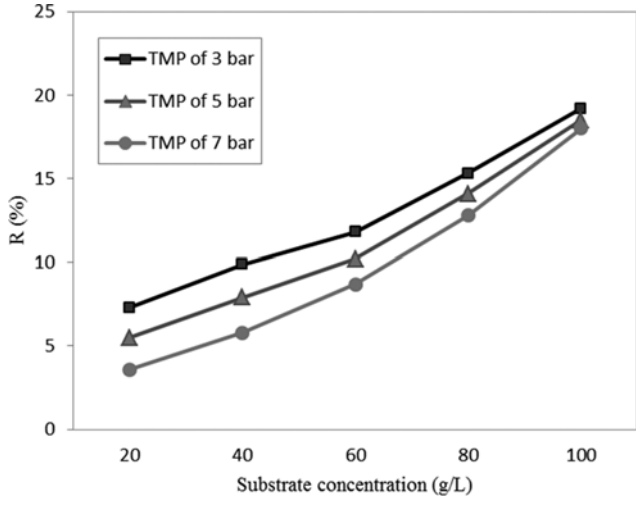

Fig. 6. Rejection percent of the reducing sugars in terms of substrate concentration and TMP.

As observed, the rejection of the reducing sugars was gradually increased with the increase of substrate concentration. At the TMP of 3 bars, the rejection increased from 7.3% to 19.2% as the substrate concentration increased from 20 to 100 g/L. One possible explanation for this result may be to form a kind of second or dynamic membrane by higher molecular weight solutes, retained by the membrane, which causes higher rejection of lower molecular weight solutes.

Fig. 6 shows that rejection of the reducing sugars decreases at higher TMP. For example, at the substrate concentration of 20 g/L, the rejection at 3 and 7 bar was 7.3% and 3.6%, respectively, and at the substrate concentration of 100 g/L and the mentioned pressures, the rejection was 19.2% and 18%, respectively. As observed, the pressure had little effect on rejection of the reducing sugars.

CONCLUSION

The enzymatic hydrolysis of waste paper using a commercial cellulase enzyme was performed and then the reaction mixture was filtered by a suitable membrane. First, waste paper was pretreated with phosphoric acid.

The results obtained from the pretreatment stage showed that the reducing sugars yield was increased with an initial increase in concentration of phosphoric acid. Furthermore, the optimum amount of the acid concentration was obtained as 0.5%. It was proved that the pretreatment using phosphoric acid is highly effective. For enzymatic hydrolysis of waste paper, the reducing sugars yield was increased with increase of enzyme loading. Finally, in the ultrafiltration stage, the results showed that TMP had little effect in comparison with substrate concentration on rejection of the reducing sugars.

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