Optimal conditions to immobilize *Trichoderma reesei* on mesoporous supports

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ABSTRACT

Nowadays, the cost of bioethanol production from renewable resources is still too high. The significant barrier to improve economy and efficiency of the production process was the cost of enzyme for converting cellulosic biomass into fermenting sugar. Immobilized *Trichoderma reesei* as cellulase on mesoporous support was presented as an alternatively effective method for improving hydrolysis process in the bioethanol production. The objective of this study was to evaluate the optimal conditions to immobilize *T. reesei* on various synthesized mesoporous supports by varying immobilization temperature, immobilization time, pH, and amount of enzyme. It was found that the maximum adsorption of *T. reesei* on mesoporous SBA-15 support was 100% at pH=1–6 after 4 days at room temperature, 40°, or 60 °C without any leaching.

Keywords: *Trichoderma reesei*, mesoporous support, SBA-15, TUD-1, MCM-48.

1 INTRODUCTION

Bioethanol is a renewable bio-based resource used as transportation fuel. Bioethanol is usually used as a gasoline additive to increase octane, improve combustion process and reduce CO emission [1,2]. The bioethanol production process consists of pretreatment (first hydrolysis), saccharification (second hydrolysis), detoxification, fermentation, and separation. However, the important process for the bioethanol production is the hydrolysis process which is high cost.

The hydrolysis step for converting carbohydrate polymer in lignocellulosic materials to simple sugars before fermentation can be classified into two types: chemical and enzymatic hydrolysis [2]. The enzymatic hydrolysis process is more attractive because it operates at a much milder condition and does not cause any corrosion [3,4].

Trichoderma sp. (*from T. viride, T. reesei, T. longibrachiatum*) is commonly chosen since it provides a high yield and is a good destroyer of crystalline cellulose [5]. However, disadvantages of using enzyme are follows; high cost, difficult recovery, sensitive to pH and temperature, and not stable in organic solvent [6,7]. Immobilized enzyme is thus essential not only to overcome those drawbacks, but also for economical issue [7-10].

Major three factors affecting catalytic activity, thermal stability and additional cost of an immobilized enzyme are enzyme, matrix, and the method of attachment. The characteristics of the matrix are an important parameter in determining performance of the immobilized enzyme. Generally, supports can be either inorganic or organic component [11]. Ideal support properties include high porosity, hydrophilic character, commercial availability, biocompatibility, resistance to microbial attack, and low cost. The inorganic supports provide many advantages, especially, highly stable against physical, chemical, and microbial degradation [12].

In this work, various types of mesoporous support were studied for immobilizing *Trichoderma reesei*. Moreover, factors affecting the immobilization were investigated, as well.

2 EXPERIMENTAL

2.1 Synthesis of SBA-15, TUD-1, and MCM-48

Preparation of SBA-15 was followed Samran's method [13], using the composition of $2EO_{20}PO_{70}EO_{20}$:60HCl:4.25 silatrane:$12H_2O$. Firstly, $EO_{20}PO_{70}EO_{20}$ was dissolved in 2 M HCl, silatrane, and H_2O. The solution was mixed and stirred at room temperature for 24 h. The product was filtrated, washed with deionized water, and dried overnight.

Synthesis of TUD-1 was followed Tunglamlert's procedure [14]. A solution of 1:0.7:14 SiO_2:TEAOH:H_2O was prepared by first mixing silatrane with water and stirring continuously for 1 h. before adding TEAOH dropwise into the mixture. After aging for 2 h at room temperature, the synthesized solution was dried at 373K for 24 h. The organic residue was removed by calcination at 873K for 10 h using a heating rate of 1 °C/min.

MCM-48 was prepared by following Longloilert's synthetic method [15]. CTAB was dissolved in aqueous solution containing 2 M NaOH. The component was

continuously stirred at 50 °C until the mixture was homogeneously dissolved, followed by adding silatrane with constant stirring at 50 °C for 1 h. The adequate molar composition of the components was $1.0SiO_2$:0.3CTAB: $0.5NaOH:62H_2O$. Then, the mixture was transferred to a Teflon-lined stainless steel autoclave and treated at 140 °C for 16 h. The obtained solid product was achieved by filtration and drying. The surfactant was removed by calcination at 550 °C for 6 h to produce MCM-48.

2.2 Immobilization of enzyme on support

Trichoderma reesei (500 µl) was gradually dropped on various quantities of SBA-15, TUD-1, and MCM-48 by impregnation method. These impregnated enzymes were dried in an oven at 40 °C for 1 h.

2.3 Leaching testing

To study whether *Trichoderma reesei* absorbed on the support was leached out, the *Trichoderma reesei*-support samples are re-suspended by stirring in various pHs, temperatures, and times. The solution was tested using the Bradford assay to indicate the amount of *Trichoderma reesei* that was leached out from the support [16]. The solution was detected by UV-Vis spectrophotometer to measure the adsorption at 640 nm.

3 RESULTS AND DISCUSSION

Immobilization of *Trichoderma reesei* on three different mesoporous silica supports, viz. SBA-15, MCM-48, and TUD-1, was found that after stirring the solution for 2 h at room temperature, SBA-15 showed the best performance of adsorption, see Figure 1. It is worth noting that SBA-15 contains a bigger pore size than TUD-1 and MCM-48, thus, allowing more enzymes to be absorbed inside the pores, resulting in much higher adsorption. In addition, the higher amount of SBA-15 provided the more impressive adsorption (100%).

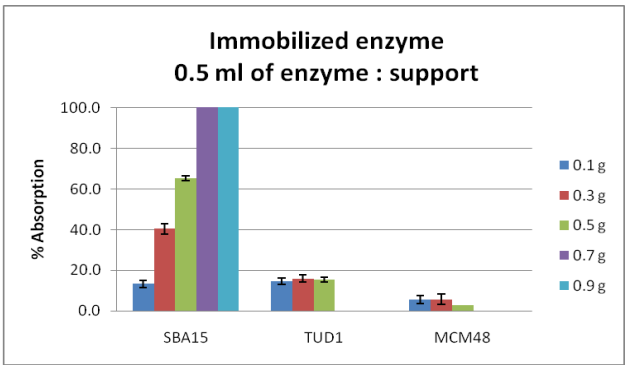

Figure 1 Influence of silica type on the enzyme adsorption

However, the higher adsorption would mean uselessness unless the enzyme did not leached out. Therefore, leaching test is necessary in this case at various studied conditions, viz. pH, temperature, and time, using SBA-15 as the support. Table 1 summarizes %adsorption of the enzyme after stirring in different pH solutions for 2 h at room temperature. As can be seen, in all acid solutions, %adsorption still maintained at 100, meaning that the enzyme was not leached out from the support. Contrarily, the enzyme was leached out as increasing the solution pH higher than 6, and the higher the pH resulted in the higher the enzyme leached out [17].

Table 1 Effect of pH on the leaching test

pH	%Adsorption	SD-%Adsorption
1.0	100.0	1.5
2.0	100.0	0.8
3.0	100.0	0.7
4.0	100.0	0.9
5.0	100.0	2.6
6.0	100.0	0.4
7.0	68.6	12.0
8.0	34.6	13

It is also known that the solution temperature has influenced on the enzyme leaching. Surprisingly, in our study at three different temperatures, room temperature, 40°, and 60 °C, it was found that after stirring the enzyme attached with SBA-15 mixture at these temperatures for 2 h, no leaching was observed, as shown in Table 2. These results are indicated that the enzyme had strong attachment with the support.

Table 2 Effect of temperature on the leaching test

Temperature	%Adsorption	SD-% Adsorption
Room temp	100.000	0.000
40 °C	100.000	0.000
60 °C	100.000	0.000

To prove this strong attachment, various much longer stirring times were studied; from 24 to 120 h. Table 3 shows that the enzyme was adsorbed and de-adsorbed with the stirring time, and became 100%adsorption after 96 h stirring.

Table 3 Effect of time on leaching test

Time (h)	%Adsorption	SD-% Adsorption
24	74.272	5.540
48	64.743	3.124
72	68.343	3.552
96	100.000	0.000
120	100.000	0.000

4 CONCLUSIONS

MCM-48, TUD-1, and SBA-15 were synthesized to be used as the support for adsorbing *Trichoderma reesei* enzyme. Among these supports, SBA-15 showed the highest performance on adsorbing the enzyme at room temperature without any leaching. This study provides an important advantage for reusability of the enzyme.

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