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Preliminary Study of the Potential of *Phanerochaete chrysosporium* Immobilized in Agar for Degradation of Sugarcane *Bagasse*

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ABSTRACT: Previous research has shown that the culture of *Phanerochaete chrysosporium* in stationary phase was able to degrade sugarcane bagasse to glucose in a single reaction step. The aims of this study were to study the ability of *P. chrysosporium* immobilized in agar with or without rejuvenation to degrade bagasse and to determine the optimum process conditions comprising method, amount of bagasse, temperature and incubation time. The immobilized *P. chrysosporium* without rejuvenation was incubated with bagasse directly, while the immobilized *P. chrysosporium* within rejuvenation first incubated in N-limited growth media for 48 hours and washed before being incubated with bagasse. Glucose as a parameter to determine the ability of degradation is determined by the Somogy-Nelson method. The yield of bagasse degradation by the immobilized *P. chrysosporium* without or with the rejuvenation was 1.78% and 1.07% respectively, with 2g bagasse, for 7 days incubated, at 37°C, and shaking rate of 50 rpm. The optimum condition of bagasse degradation was conducted by the immobilized stationary phase *P. chrysosporium* (0.065 g dry weight) in 3.3% agar without rejuvenation for as much as 4 g of bagasse, the incubation temperature of 37 ° C, for 7 days, giving degradation rate of 1.045%.

Keywords: *Phanerochaete chrysosporium*, immobilized, degradation, sugarcane bagasse

INTRODUCTION

Sugarcane bagasse is one of the high potential biomass as bioethanol raw material. The content of cellulose in the bagasse is quite high at 37.65%,[1] but unfortunately this cellulose in the form of lignocellulose is relatively difficult to be degraded enzymatically.[2] Degradation of bagasse is to change the cellulose into glucose, which can be fermented into bioethanol.[3,4] So far, degradation of bagasse was conducted through two stages i.e. pretreatment and hydrolysis using cellulase enzymes. The pretreatment is to disrupt lignocellulose so that the cellulose in the structure of bagasse will be exposed, which in turn will facilitate cellulase enzymes to bind and break glycosidic bonds between monomers on cellulose.[3–6] Various studies show that the optimum pretreatment of bagasse involves steaming at high temperature followed by immersion in certain chemicals, such as sulfuric acid, organic solvents or sodium hydroxide solution.[4,5] This condition causes the operational costs of the degradation quite high.

P. chrysosporium is one of white rot fungi basidiomycetes that is able to degrade and metabolize lignin polymer,[7] because of its ability to produce multiple proteins are glycosylated heme manganese peroxide (MNP), lignin peroxide (LIP) and laccase.[8,9] All three enzymes produced when the *P. chrysosporium* enters secondary metabolism triggered by a limited number of carbon, nitrogen and sulfur.[10] On the other hand, we known that *P. chrysosporium* has cellulolitic machinery similar to *Trichoderma reesei*. Thus, *P. chrysosporium* has the potential to degrade cellulose in the bagasse directly without pretreatment step. Previous research showed that the yield of degradation of bagasse with stationary phase of *P. chrysosporium* was 2.91%. The aim of this study is to learn the ability of the immobilized *P. chrysosporium* in agar without or within the rejuvenation to degrade bagasse and optimum process conditions include the method, amount of bagasse, temperature and incubation time. The resulting informations are usefull for the development of research on exploration of lignoselulotic degrading enzymes from *P. chrysosporium* and its application in the bioethanol process production from lignocellulose.

METHODS AND EXPERIMENTAL DETAILS

Preparation of Immobilized *P. chrysosporium*

A total of 300 mL culture of *P. chrysosporium* in stationary phase was sentrifuged at 2500 rpm for 15 min to obtained cell suspension that equivalent to ± 0.65 g dry weight. Preparation the culture of *P. chrysosporium* in stationary phase as desribed in previous research[10]. Homogenized cell suspension in the warm 3.3% steril agar solution, then poured into three petri dishes. The mixture was allowed to solidify and incubated 37°C for 24 hours. The results obtained in each petri dish is ±0.13 g dry weight of *P. chrysosporium* immobilized in agar.

Degradation of Bagasse by Immobilized *P. chrysosporium* without Rejuvenation

As many as half of the petri dish contain immobilized *P. chrysosporium* was cut aseptically with size 1×1 cm. The immobilized culture was then placed into 75 mL of treatment media (N-limited media + 2 g bagasse) in a 500 mL Erlenmeyer. This mixture was incubated in a 37°C with shaking speed of 50 rpm. On 7^{th} and 14^{th} days of incubation, as much as ±3 mL mixture aseptically taken. Next, it was centrifuged at 3000 rpm for 20 minutes. The filtrate was tested for glucose levels by Somogy-Nelson method.

Optimization of Bagasse Degradation by Immobilized *P. chrysosporium* without Rejuvenation

Optimization of bagasse amount. Five 500 mL erlenmeyer containing 75 mL of treatment media and bagasse as much as 2, 4, 6, 10, and 15 g were prepared. As a control for each treatment, solid media without immobilized *P. chrysosporium* were used. Controls were given the same treatment and the conditions treated by the media.

Optimization of incubation time. During day 0, 5, 7, 14, and 21, as much as ± 3 mL mixture aseptically taken, and it was sentrifuged at 3000 rpm for 20 minutes. The filtrate obtained was tested of glucose levels by Somogy-Nelson method.

Optimization of temperature. The variation of temperature was 37°C and room temperature.

Determination of glucose level by Somogy-Nelson Methods. Preparation of glucose standard curve were done by adding 1 mL of Nelson reagent into several glucose solution containing 0 (blank), 20, 40, 60, 80, 100 ppm glucose, respectively, stirred and heated in a boiling water bath for 20 minutes. It was then cooled at room temperature and added with 1 mL of reagent arsenomolybdate and 7 mL of distilled water to reduce the thickness. The absorbance of the solution was measured at wavelength of 540 nm using Spectronic-20. To determine the levels of glucose in the sample, similar procedures were applied with only 1 mL samples. The absorbance was also measured at wavelength of 540 nm.

Data analysis. The ability of bagasse degradation by immobilization of *P. chrysosporium* was expressed as percentage of glucose mass-produced per mass of bagasse.

RESULTS AND DISCUSSION

Comparison of Bagasse Degradation Using Immobilized *P. chrysosporium* without and with Rejuvenation

Immobilized *P. chrysosporium* were used to improve efficiency of bagasse degradation as alternative method to reduce the operational cost, using immobilized system due to multiple cycles. Bagasse degradation by immobilized *P. chrysosporium* can be done in two ways, which are: (1) **without rejuvenation,** bagasse is contacted with immobilized *P. chrysosporium* directly and (2) **with rejuvenation,** immobilized *P. chrysosporium* inoculated in the growth medium at 37°C for 48 hrs first and then contacted with bagasse.

Figure 1 showed that degradation percentage obtained using immobilized *P. chrysosporium* on day 7 without rejuvenation gave higher rate (1.78%) than those with rejuvenation (1.07%). This is presumably because the rejuvenated immobilized cells were on primary metabolism in which the glucose carbon source present in the growth medium. This condition is believe resulting production of ligninase enzymes and expressed by the rejuvenated immobilized cells which become less than immobilized cells without rejuvenation. The less amount of ligninase enzyme make less lignocellulosic structures damage and cause cellulase enzymes more difficult to bind and consequently harder to break glycosidic bonds in cellulose. Thus the yield of degradation was very low. This argument is still to be proven through further study of the expression lignoselulotic system of *P. chrysosporium* on that media.

Moreover, these result presumably because the decrease of cells number of *P. chrysosporium* that immobilized during rejuvanation process. This is shown from the turbidity of the growth medium that was used for the rejuvenation process. Figure 1 also shows the decrease in percent degradation on day 14. That is due to high glucose levels on day 7 is used as a carbon source by *P. chrysosporium* for growing up to day 14. Based on the experimental results, it is concluded that bagasse degradation by immobilized *P. chrysosporium* without rejuvenation is more effective than those in immobilized *P. chrysosporium* by rejuvenating for 7 days incubation.

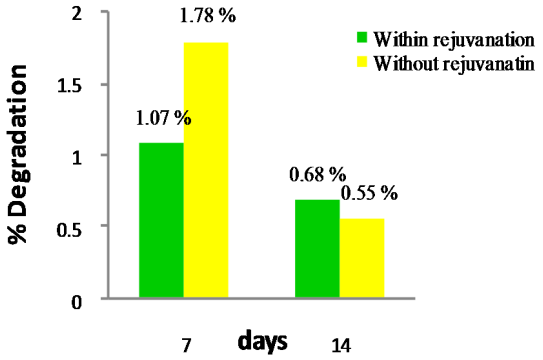

Figure 1. Degradation percentage of bagasse using immobilized *P. chrysosporium* without and with rejuvenation (dry weight *P. chrysosporium* ± 0.065 g; weight of bagasse = 2 g, pH = 5; temperature 37 °C; erlenmeyer 500 mL).

Optimization of Bagasse Degradation Condition by Immobilized *P. chrysosporium* Without Rejuvenation

The results of the condition optimization on bagasse degradation by immobilized *P. chrysosporium* without rejuvenation for the amount of bagasse, the incubation time, and temperature are presented in Figure 2, 3 and 4, respectively.

Degradation percentage for day 7 in Figure 3 (1.08%), when it is compared to the optimum one in Figure 2 (1.53%) was different but actually it was not. It is because both data, in which showed the actual degradation, have not been reduced by the degradation of control yet. The net data was actually 1.03 % in Figure 2, while in Figure 3 was 1.045 %. The different between these values were not significant.

Figures 2 and 3 are inline one another, the culture of *P. chrysosporium* at stationary phase that is equivalent to 0.065g dry weight and was immobilized on 3.3% agar optimise the degradation by as much as 4g of bagasse with a time of 7 days incubation.

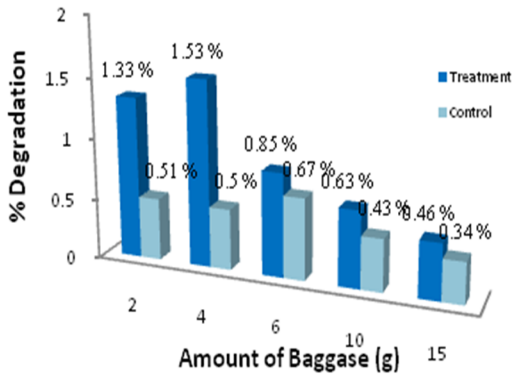

Figure 2. Degradation percentage of bagasse by immobilized *P. chrysosporium* on variation of bagasse amount (dry weight of *P. chrysosporium* ± 0.065 g, 37°C, pH = 5, time = 7 days; erlenmeyer 500 mL).

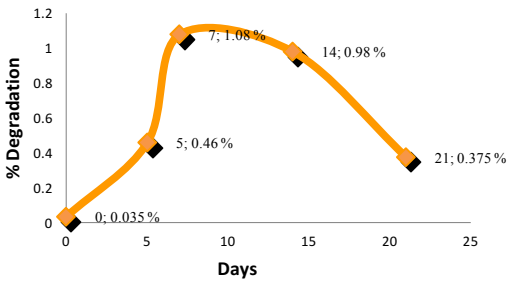

Figure 3. Degradation percentage of bagasse by immobilized *P. chrysosporium* on variation of incubation time (weight of bagasse= 4g, dry weight of *P. chrysosporium* = ± 0.065 g, 37°C, pH = 5,; erlenmeyer 500 mL).

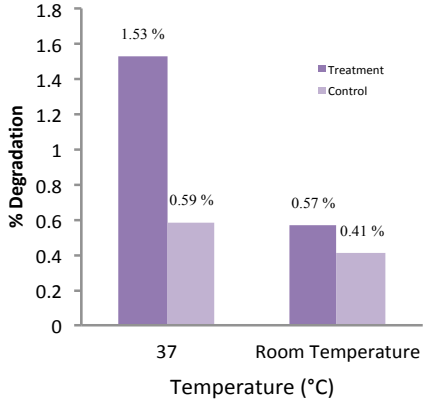

Figure 4. Degradation percentage of bagasse by immobilized *P. chrysosporium* on variation of temperature (weight of bagasse= 4g, dry weight of *P. chrysosporium* = ± 0.065 g, pH = 5, time = 7 days; erlenmeyer 500 mL).

Figure 4 shows that the degradation ability of immobilized *P. chrysosporium* was much higher at 37°C than at room temperature. The 37°C is indeed an optimum growth temperature for *P. chrysosporium*. It is estimated that the expression of lignocellulose degrading enzymes produced by *P. chrysosporium* at 37 °C was also higher than at room temperature.

CONCLUSION

The yield of bagasse degradation by the immobilized *P. chrysosporium* without or with the rejuvenation was 1.78% and 1.07% respectively, with bagasse as much as 2g, for 7 days incubated, at 37°C, and shaking rate of 50 rpm. The optimum condition of bagasse degradation was performed by the immobilized stationary phase *P. chrysosporium* (0.065 g dry weight) in 3.3% agar without rejuvenation for as much as 4 g of bagasse, the incubation temperature of 37°C, 7 days, which showing degradation rate of 1.045%.

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