The Study on Growth Kinetics of Trichoderma reesei in Batch Fermentation of Cassava Waste and Rice Straws

Pongsri Siwarasak[1] and Penjit Srinophakun[2] and Jarun Chatmanop[2]

Abstract:

The objective of this study is to determine the growth rate of *Trichoderma reesei* from batch fermentation in liquid medium by using single substrate as carbon source which were cassava waste and rice straws. It was found that 12. 5 % and 8.2 % by weight of maximum reducing sugar obtained from 60 g/L of each substrate in liquid medium fremented with 0.41 g/L of *Trichoderma reesei* RMUTT01 at pH 5 and shaking speed of 120 rpm at room temperature (32 °C), for 7 days and 5 days fermentation time of cassava waste and rice straws respectively; the maximum specific growth rate of *Trichoderma reesei* (μmax) in cassava waste and rice straws fermentation is 0.97 per day and 0.16 per day and saturation constant (K_s) is 143.13 and 36.35 gram/liter respectively.

Keyword : cassava waste, rice straws, specific growth rate and *Trichoderma reesei*

1. Introduction

Increasing of petroleum energy price and demand at present are driving force of plans for developing and promoting the need of alternative energy such as gasohol and biodiesel. Ethanol utilization for sustainable energy in this decade is desired especially ethanol produced from agricultural waste. The reason is economic sense and environmental prevention which it can be reduced green house effect. Ethanol production from agricultural waste such as rice straws and cassava waste which are renewable resources is very interesting. This is an alternative energy that can solve environmental impact and value added of solid- waste for agricultural industries, particularly in Thailand. The cassava waste and rice straws are high potential raw materials to produce ethanol from fermentation. Surprisingly, 35 million tons of rice straws produced per year in Thailand. However, the utilization of rice straw in the house hold and industry has been low due to its low bulk density and scattered caused high transportation cost. Then particularly management of this kind of solid waste is necessary. Due to high cellulose content in rice straws, it is good substrate for *Trichoderma reesei* cultivation particularly in submerged culture [1] - [3]. The Cellulase enzyme from this microorganism will be released and hydrolyzed for digest cellulose to reducing sugar. Cassava waste is similar to rice straws which composts of cellulose and carbohydrate that can be also used as a substrate. In

[1] Department of Chemical Engineering, Faculty of Engineering, Rajamangala University of Technology Thanyaburi, Phathumtani 12110 Thailand. Tel. 02-549-3093 Email: pongsri@rmut.ac.th / ajpongsris@hotmail.com

[2] Department of Chemical Engineering, Faculty of Engineering, Kasetsart University Bangkok Thailand.

fact, *Trichoderma reesei* is high potential for cultivation on both substrates to produce reducing sugar due to its ability to hydrolyze polysaccharides such as cellulose and carbohydrate. Reducing sugar from enzyme hydrolysis can be converted to ethanol by Saccharomyces cerevisiae via fermentation [4].

Additional, cassava waste utilization for ethanol batch production had been studied in Thailand, however it was used commercial enzyme hydrolysis for reducing sugar [5] - [7]. Unfortunately particular research of hydrolysis of cassava waste with *Trichoderma reesei* had not been done even through it is agriculture waste from modified starch plants. Because of the growth kinetics of *Trichoderma reesei* on rice straws and cassava waste is significant data for large scale design of enzymatic hydrolysis to produce reducing sugar. The objective of this study is to study growth kinetics of *Trichoderma reesei* in batch fermentation by using individual substrate such as rice straws and cassava waste in batch fermentation with *Trichoderma reesei* cultured in liquid medium on the basis of Monod equation by using Lineweaver-Burk plot to determine rate parameter for Michaelis-Menten type kinetics [8-9].

2 Materials, equipments and methodology

2.1 Materials

Cassava waste from Starch plant Kanjanaburi was dried and grinded. Rice straws from Ampur Klong Luang, Pathumthani were chopped into dimensions about 0.5-1 centimeter, which initially composed of 65.35 % cellulose. It was pretreated to 98.89 % cellulose by using 2 M NaOH [1].

Microorganism: *Trichoderma reesei* RMUTT01 was cultured in potato dextrose agar (PDA) slant and plate.

2.2 Liquid medium

The medium for fungus is called liquid medium, which is compose of mineral salt solution as follows: (g/L) 1.00 magnesium sulfate, 0.05 calcium potassium phosphate, 4.00 ammonium di sulfate, 7.00 corn steep liquor, 5.00 ferrous sulfate, 1.40 zinc sulfate, 1.60 manganese sulfate, 3.60 cobalt chloride, 20.00 mL tween 80 and 1 L distilled water [10]. This solution was adjusted at 5.0 pH. It was sterilized at 121 oC under pressure of 15 psi for 20 minutes.

2.3 Chemicals

There were 3,5-di-nitro salicylic acid, sodium potassium tartate, 2 M sodium hydroxide, pure glucose for reducing sugar analysis, acetyl acetone in sodium carbonate solution, p-dimethylaminobenzadehyde, concentrated hydrochloric acid, 95% ethanol and glucosaminehydrochloride for glucosamine analysis and sodium citrate buffer solution pH 4.8

2.4 Analytical determinations

The concentration of *Trichoderma reesei* RMUTT01 in g/L which measured as initial concentration before inoculated in each of experiment was obtained from gravimetric method by using calibration curve between g/L *Trichoderma reesei* suspended in pure water and absorbance at 600 nm from UV-spectrophotometer [11]. The colorimetric method was applied for glucosamine [12], reducing sugars [13], and cellulase activity (FPA) [10] determination by using uv-spectrophotometer at wave length of 570 nm, 540 nm and 520 nm respectively.

2.5 Methodology

The enzymatic hydrolysis of *Trichoderma reesei* RMTT01 by utilized one-substrate in batch fermentation, the sterilized cassava waste and rice straws were utilized as a substrate. In each experiment; 10 g/L to 80 g/L

cassava waste with increment of 10 g/L and 20 g/L to 100 g/L rice straws with increment of 20 g/L, were cultured with 0.41 g/L *Trichoderma reesei* RMTT01 in 250 mL flask which was compost of 100 mL sterilized liquid medium at pH 5.0 and used shaking speed of 120 rpm at room temperature (32 oC) for 12 days. The samples were collected once a day to analyze for glucosamine, reducing sugars, cellulase activity and also measured pH and temperature.

3 Results

The results from experiments; glucosamine, reducing sugar and cellulase activity obtained from 0.41 g/L *Trichoderma reesei* RMUTT01 culture in 20 g/L to 100 g/L cassava waste and also 20 g/L to 100 g/L rice straws of each batch fermentation for 12 days, as shown in figure 1 to figure 3 respectively. The growth of *Trichoderma reesei* RMUTT01 on cassava waste was better than rice straws thus reducing sugar (g/L) obtained from cassava waste was higher than rice straws batch fermentation for 12 days when compared at equal substrate concentration in liquid medium. However cellulase activity (unit/mL) obtained from rice straws was higher than from cassava waste batch fermentation significantly. Because of pretreated rice straws compost of almost cellulose which was the best substrate for the growth of *Trichoderma reesei*. Then pretreated rice straws can be utilized as carbon source for crude cellulase enzyme production. This crude enzyme will be used for reducing sugar production from cassava waste fermentation further.

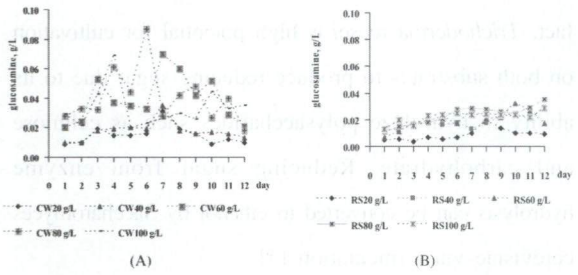

Figure 1 Glucosamine (g/L) obtained from batch fermentation of 20 g/L to 100 g/L cassava waste (A) and also 20 g/L to 100 g/L rice straws (B) for 12 days

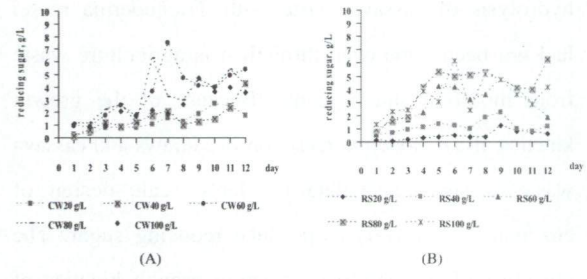

Figure 2 Reducing sugar (g/L) obtained from batch fermentation of 20 g/L to 100 g/L cassava waste (A) and also 20 g/L to 100 g/L rice straws (B) for 12 days

The average concentration of glucosamine, reducing sugar and cellulase activity obtained at cassava waste and rice straws various concentrations of

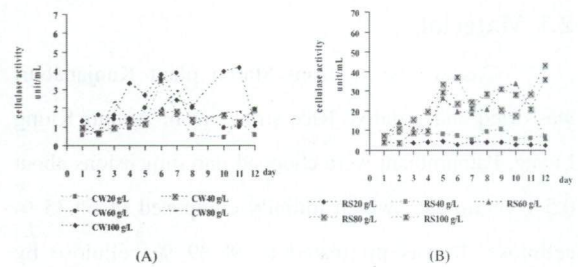

Figure 3 Cellulase activity (unit/mL) obtained from batch fermentation of 20 g/L to 100 g/L cassava waste (A) and also 20 g/L to 100 g/L rice straws (B) for 12 days

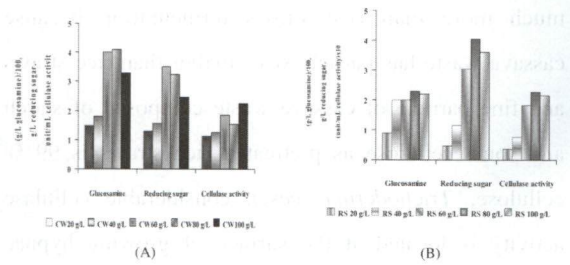

Figure 4 The average glucosamine, reducing sugar and cellulase activity concentration, at various concentrations of cassava waste (A) and rice straws (B)

However from figure 4 there were three concentrations of 60 g/L, 80 g/L and 100 g/L cassava waste and rice straws in liquid medium that had high average value of glucosamine, reducing sugar and cellulase activity with respect to 20 g/L and 40 g/L. The comparable three concentrations data of both substrates were calculated in g/g substrate as shown in figure 5 to figure 7 respectively. Then the maximum values of glucosamine, reducing sugars and cellulase activity at 60 g/L cassava waste and also rice straws in liquid medium were 1.2 x 10^{-3} g/g substrate, 0.13 g/g substrate and 0.65 unit/g substrate for 7 days fermentation of cassava waste and 4.6 x 10^{-4} g/g substrate, 0.08 g/g substrate and 4.4 unit/g substrate for 5 days fermentation of rice straws.

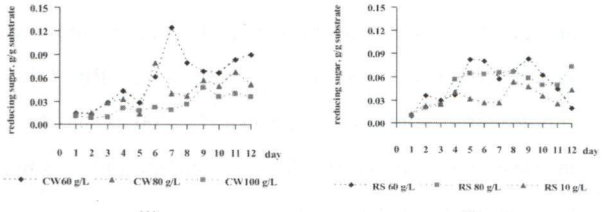

Figure 6 The reducing sugar obtained from 60 g/L, 80 g/L and 100 g/L cassava waste (A) and 60 g/L, 80 g/L and 100 g/L rice straws (B) batch fermentation for 12 days

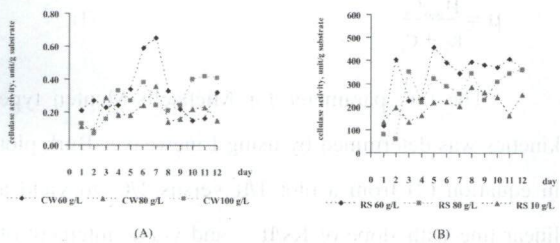

Figure 7 The cellulase activity obtained from 60 g/L, 80 g/L and 100 g/L cassava waste (A) and 60 g/L, 80 g/L and 100 g/L rice straws (B) batch fermentation for 12 days

For the growth kinetics of *Trichoderma reesei* RMUTT01 in one-substrate (cassava waste or rice straws) batch fermentation for 12 days had been determined by plot glucosamine concentrations with time in semi log scale. Base on the exponential first order growth rate, which the growth of *Trichoderma reesei* RMUTT01 depended on active cell mass concentrations, data from figure 1 were used for this calculation. Thus specific growth rate, μ were estimated from slope and also doubling time, t_d of cell mass for each batch were calculated from $\ln 2/\mu$ which was shown in table 1.

Table 1 Specific growth rate and doubling time from graphical calculation of cassava waste (A) and Rice straws (B) for 12 days batch fermentation

(A) cassava waste, g/L	μ_A, d^{-1}	t_d ,d	R^2
20	0.119	5.81	0.66
40	0.216	3.22	0.97
60	0.262	2.65	0.81
80	0.338	2.05	0.89
100	0.439	1.58	0.89

(B) rice straws, g/L	μ_B, d^{-1}	t_d,d	R^2
20	0.026	26.66	0.15
40	0.082	8.48	1.00
60	0.097	7.12	0.97
80	0.109	6.35	0.98
100	0.113	6.13	0.98

Specific growth rate with corresponding substrate concentration from table 1 which were 20, 40, 60, 80, 100 g/L of cassava waste and rice straws respectively which could be used to find the maximum specific growth rate, μ_{max}, and saturation constant, K_s from "Monod equation" in equation (1) where C_s is substrate concentration.

$$\mu = \frac{\mu_{max} C_s}{K_s + C_s} \quad (1)$$

The rate parameter for Michaelis-Menten type kinetics was determined by using Lineweaver-Burk plot in equation (2) from a plot $1/\mu$ versus $1/C_s$ to yield a linear line with slope of Ks/μ_{max} and y-axis intercept of 1/?max as depicted in figure 9 [8].

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \cdot \frac{1}{C_s} + \frac{1}{\mu_{max}} \quad (2)$$

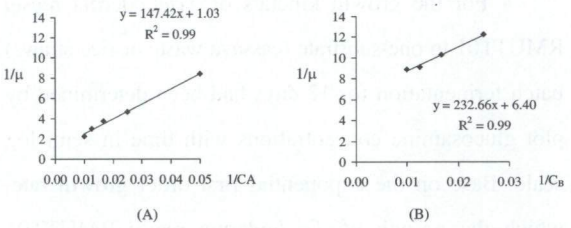

Figure 9 Lineweaver-Burk plots for determination μmax and K_s of cassava waste (A) and rice straws (B) in batch fermentation

From Lineweaver-Burk plots it was found that the estimated value of μ_{max} and K_s were 0.97 d-1 and 143.13 g/L of cassava waste and 0.16 d-1 and 36.35 g/L of rice straws where R^2 of both substrates were 0.99 respectively.

4 Conclusion and discussion

The limitation of cassava waste and rice straws concentration and fermentation time for enzymatic hydrolysis of *Trichoderma reesei* RMUTT01 to produce reducing sugar was found in this study. Fixed 0.41 g/L *Trichoderma reesei* was cultured in 60 g/L cassava waste and 60 g/L rice straws in liquid medium, 12. 5 % and 8.2 % by weight of maximum reducing sugar obtained for 7 days and 5 days fermentation time respectively. Glucosamine, and reducing sugar except for cellulase activity obtained from cassava waste were much more than rice straws fermentation because cassava waste has particle size smaller than rice straws and fine particle of cassava waste composed of starch and some cellulose as pretreated rice straws is 99 % cellulose. *Trichoderma reesei*, considerable cellulase activity is located at the surface of growing hyphae [14]; growth on corncobs has resulted in the formation of biofilm ~30 μm thick which presumably maintains the contact of hyphae with cellulose [15]. During degradation of plant cell walls, fungal hyphae confined in the intercellular spaces of plant cell walls are in close proximity to substrate and in this confined environment, loss of enzyme and hydrolytic product due to diffusion and convection is likely to be limited [16].

The data report here for determination growth kinetics of *Trichoderma reesei* RMUTT01 in batch fermentation of cassava waste and rice straws was shown that it could be used to obtained good fitted that provided R^2 = 0.99 base on Monod equation and Michaelis-Menten model, even through selected data were used. Some experiment data were omitted for determination due to some analysis error. The maximum specific growth rate (μ_{max}) of *Trichoderma reesei* RMUTT01 in cassava waste and rice straws fermentation at pH 5.0 and 32 °C are 0.97 d^{-1} or 0.04 h^{-1} and 0.16 d^{-1} or 0.007 h^{-1} and saturation constant (K_s) are 143.13 and 36.35 gram/liter respectively. The formal study in kinetic parameters for *Trichoderma reesei* Rut C30 cellulose utilization by using Solka floc 200 as substrate in batch cultivation at 28 °C, maximum specific growth rate obtained 0.125 h^{-1} [16].

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