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Kinematic study of reducing sugar production from rice straw by raw wood-rotting enzyme strain

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Abstract: In this study, a cost effective, simple, environment friendly way of fermentable reducing sugar production from rice straw has been carried out by using naturally grown raw wood rotting enzyme. The rotten bark of three trees was collected as the source of enzyme, called enzyme strain. The yield of reducing sugar from rice straw by enzyme strain at various operating condition was studied. A kinetic model expression has been developed for the enzymatic hydrolysis process based on the Michaelis – Mentens approach. Comparison between the experimental data and theoretical data predicted from the rate model render sound accord with a mean deviation of about 0.679. Strain collected from the Rain tree (Samanea Saman) showed the maximum production of reducing sugar. The absence of light gives 36.36% higher production than that of the presence of light. The optimum pH is found as 5. Strain concentration at 0.0233g/ml shows the maximum sugar production as 0.09854 mg/ml in 10 days. Substrate concentration at .0143mg/ml gives maximum production of 0 .15991 mg/ml in 16 days. From this study the optimum condition was found as 0.157 mg/ml fermentable reducing sugar in 11 days. This study provides an alternative and attractive cost effective source of fermentable sugar which can be further converted to valuable product such as bio-ethanol to meet the worlds increasing energy demand.

1. INTRODUCTION

With world energy consumption predicted to increase 54 % between 2001 and 2025, considerable focus is being directed towards the development of sustainable and carbon neutral energy sources to meet future needs[1].At present, fossil oil is the most widely used fuel in a great majority of activities; however, it is a finite and scarce resource, with exceptionally high operation costs in addition to major environmental impacts[2]. Those consequencesincreases the demand of an alternative ecofriendly, abound, low cost source of energy. Lignocellulosic biomass, the most abundant carbohydrate source on earth, is a vital candidate for energy in this prospect for in the form of charcoal, hydrogen, ethanol and biogas; the last three requiring hydrolysis of the lignocellulosic material[3]. Lignocellulosic materials regarded as promising energy source because of its potential low cost renewable source of mixed sugars for bio-ethanol production and rice straw is one of the abundant lignocellulosic waste materials in the world[4].Fermentation of these sugars gives the production of fuel as bio-ethanol.
Stability of biomass, the variety of feedstock, the choice of suitable catalysts converting biomass into sugar and the cost of collection and storage of a low-density biomass makes the Sugar production difficult.

Rice is considered as most common staple food through the globe with leading the formation of huge amount of straw and husk and hence a great bio resource. It is annually produced about 731 million tons, distributed in Africa 20.9 million tons, Asia 667.6 million tons and Europe 3.9 million. Rice straw can potentially produce 205 billion liters bioethanol per year, which is about 5% of total of consumption.This is the largest amount of fuel from a single biomass feedstock. Rice straw predominantly contains cellulose 32-47%, hemicelluloses 19-27% and lignin 5-24%, and ashes 18.8%. The pentose are dominant in hemicelluloses which xylose is the most important sugar followed by arabinose and hexoses. The carbohydrate of rice straw involves glucose 41-43.4%, xylose 14.8-20.2%, arabinose 2.7-4.5%, mannose 1.8% and galactose 0.4%[5, 6]. Recent studies have shown that researchers in this field have successfully converted many cellulosic materials such as saw dust, solid animal wastes, and crop residues etc.[7, 8] to more valuable products such as fermentable sugarsinitialstep to bio-ethanol production.

Successful development of “third generation” biofuel depends heavily on degradation of cellulose, the one of most important stage of fermentable sugar, is carried out generally by acid or enzymatic hydrolysis to produce fermentable sugar. Generally two basic conventional approaches are followed for converting biomass into

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fermentable sugars. Among them, the high reactor cost, use of costly acid and post-processing of the acid makes the acid hydrolysis and so overall production process too costly compared with the conventional petrochemical counterparts. Though the enzymatic hydrolysis requires less or no acid but the price of commercial cellulose is high. This leads to the necessity of an alternative way of producing an ecofriendly, low cost, convenient, potential process of production of fermentable sugar to meet the up growing demand of fuel.

In this present study, among the lignocellulosic materials Rice straw (RS) is chosen as substrate for sugar production .The goal of this study was to analyze activity of various naturally grown wood rotting enzymes on the scarification of RS as source of cellulose in various conditions. Also a kinetic model is developed base on the Michaelis – Mentens approach with considering the glucose is the only product of interest.

2. MATERIALS AND METHODS

2.1 Wood rotting enzyme strain

Naturally grown wood rotting enzyme was collected from rotten part of some tree bark as Jarul(Lagerstroemia speciosa), Akasmoni (Acacia auriculacformis), Rain tree(Samanea Saman) from Shahjalal University residence area .After collection, various amount ofthe tree bark were blended and mixed with 350 ml distilled water followed by kept in anaerobic condition until floc formation was identified by visual inspection. Then the mixture was re-dispersed blended by a blender and filtered. This filtrate solution was treated as raw enzyme strain.

2.2 Preparation of substrate

The rice straw was chosen as natural substrate and collected from field of Shahjalal university locality and washed with distilled water to remove objectionable dirt. Washed RSwas boiled in distilled water for 3 minutes and dried in an air oven for 48 hour at 90^{o}C. The particle size of < 0.6 mm was separated by a sieve shaker and feed to the bioreactor.

2.3 Construction of bio-reactor

The reactor was a dark colored 2.5 L bottle attached with a cork in it the mouth. Assembled with a thermometer, a vent tube made of Pyrex glass, and a sample collector tube.

The pre measured amount of rice straw and raw enzyme strain was feed in the bio-reactor and kept in dry and dark box.The cellulosic enzymatic hydrolysis experiments were performed at room temperature in different pH, substrate loading, strain concentration, various enzyme sources to determine an optimum. Determination of glucose production was done by DNS method[9] with one day interval.

3. RESULT AND DISCUSSION

3.1 Preliminary investigation

The 350ml of strain solution of three sources, 28.56 gm/L, was treated with 12gm of **RS** in absence of light and the reducing sugar production was analyzed. The obtained data is plotted in Fig. 1. the same procedure was followed in presence of light and the result is shown in Fig. 2. the strain solution from rain tree was selected as of maximum productivity in absence of light. Further experiment was carried outwith prepared strain from rotten rain tree bark in absence of light.

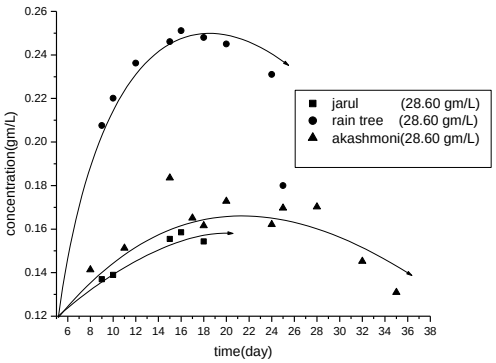

Fig. 1: Reducing sugar production by analyzed tree bark in absence of light.

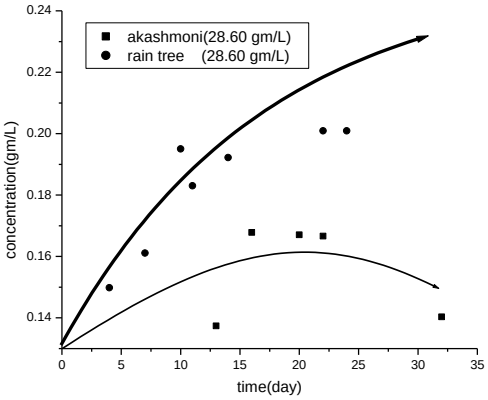

Fig. 2: Glucose production in presence of light.

3.2 Effect of substrate loading

To increase the production of fermentable sugar high solid loading is required [10]. From Fig. 3 it is clear that the maximum glucose concentration in solution varies with the substrate concentration. Here the maximum sugar formation rate is associated with 14.3gm/L substrate loading.

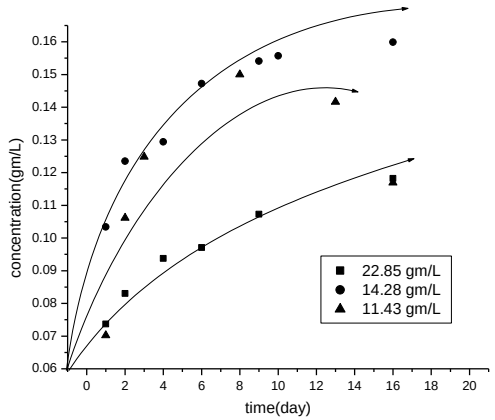

Fig. 3: Reducing sugar production under different substrate (RS) loading.

3.3 Effect of cell loading

As the cell loading increased, the glucose concentration increases as shown in Figure 4. This may be due to continuous excretion of enzymes by the cells into the solution [8].From figure 4the optimum strain loading is 22.85 gm/L.Fermentable sugar production rate shows a positive change against the increasing concentration of cell also reportedby Lisa G. A et al. (2012)[11]

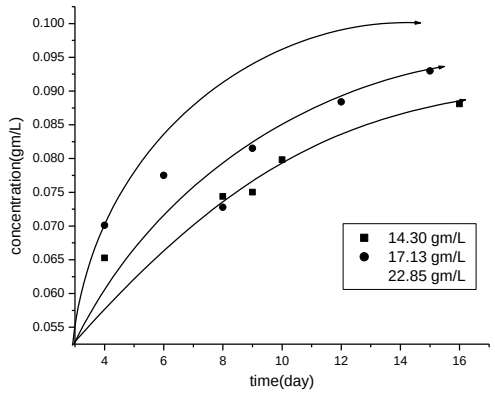

Fig. 4: Variation of concentration of reducing sugar strain concentration.

3.4. Effect of pH and temperature

Optimal pH is very important for growth of the microorganism and its metabolic activities. As the metabolic activities of the microorganism are very sensitive to changes in pH, cellulase production by cellulases was affected by varying pH of the medium. Our findings are comparable to previously reported results from literature. Gomes et al. (2006)[12] found that cellulases work better in more acidic (4.5-5.0), also Yang et al. (2004)[13] found that maximum production of cellulase at pH 4.5Figure: 5show the result of the effect of pH on glucose concentration. The pH around 4.5and room temperature gave the optimum yield of glucose as referred optimum pH and temperature in different experiment [14]. The temperature for the whole process was at room temperature.

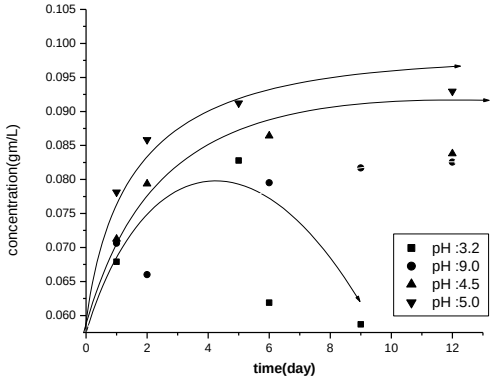

Fig. 5: Glucose production at various p^H at room temperature.

3.5. Optimum condition

By these studies the optimum condition was defined as of substrate concentration 14.28 gm/L, p^H: 4.5, 22.86 gm/L of strain loading at room temperature. The Glucose production at this condition is given in figure:

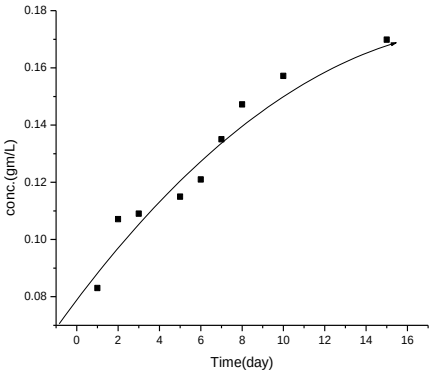

Fig. 6: reducing sugar production on optimum condition.

4. DEVELOPMENT OF KINETIC MODEL

The modeling in this section is done by considering only glucose is the product of interest. The assumptions are:
1. When the strain is introduced, the enzymes were in exponential growth phase.
2. The model is targeted to capture only the first segment (initial rate) of the concentration-time curve.
3. The reaction was viewed as enzymes been excreted by the cells into the solution, enabling the overall system to be treated as that of enzyme-substrate kinetics [7].
4. Development of kinetic model is done by initial rate method. The initial rate with different initial substrate loading was determined by graphical differentiation method.

The scarification generally expressed as:

$$E + S \leftrightarrow [ES] \rightarrow E + P$$

For such process the saccharification mode is described by Michaelis – Mentens approach as follows:

$$v = \frac{V_{max} \times [S]_0}{K_m + [S]_0}$$

The linearized form of this model is given by Line-waver bark equation as:

$$\frac{1}{V} = \frac{1}{V_{max} \times [S]_0} + \frac{K_m}{V_{max}}$$

So, a plot of the reciprocal of the initialrate (v) versus the reciprocal of the initial substrate concentration $[S]_o$ is expected to yield a straight line with an intercept $1/v_{max}$ and the slope km/v_{max}. A plot of this using the generated experimental data in this work is shown in Figure: 7 .The evaluated kinetic parameters were: $\mathbf{v_{max}}$ = 0.867378 gm/ (L.day) and $\mathbf{k_m}$ =156.5617 gm/L respectively.

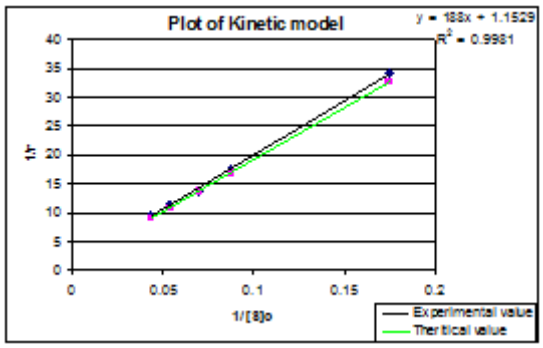

Fig. 7: Plot of Line-waver bark plot of kinetic model

So, with the determined kinetic parameters, the model equation is given as:

$$v = \frac{0.867378 \times [S]_0}{156.5617 + [S]_0}$$

The consistency of this model equation was tested with the generated data to statistically evaluate its reliability.The result of the consistency test as presented in Fig. 7 shows the model equation is consistent with the experimental data with the mean standard deviation of 0.679.

5. CONCLUSION

The obtained kinetics parameters of the saccharification were as $\mathbf{V_{max}}$ = 0.867378 gm/(L.day)and $\mathbf{K_m}$ =156.5617 gm/L. The kinetic model for the process was given as: This model equation was found to be consistent with the experimental data with the mean standard deviation of 0.697.

Due to the low cost and availability of **RS** and good activity of scarification by naturally grown enzyme, it can be concluded that the present study has promising and practical utility in the production of glucose from rice straw.

6. ACKNOWLEDGEMENT

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