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Enzymatic Hydrolysis of Rice Straw to Fermentable Sugar: Kinetic Study

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Abstract

As the gradual up-growing trend of industrialization and urbanization leading steady increment of demand of energy; eco-friendly, bio degradable, cost competitive and promising source of energy with high sustainability is toughly needed for the new era of modern world. Hydrolysis of cellulose by cellulase enzymes is a vital candidate for this option. It is a solid-liquid heterogeneous reaction; strongly affected by the non-reaction resistances caused most notably by the crystalline structure; reaction environment parameters as temperature, p^H, characteristics of enzyme, cell & substrate loading and hence must have to be defined for specific enzyme-substrate amalgamation. In this present investigation, glucose was produced from rice straw using cellulytic enzyme ***pseudomonas sp.***, isolated from municipal solid waste. Glucose yield was found to increase as the rice straw particle size decreased from 0.5 mm to 45 μm, while the optimal temperature and pH were found within the range of 30°C and 7.0 respectively. The concentration and rate of glucose production was observed to depend on pretreatment of rice straw, substrate concentration and enzyme loading. A kinetic model rate expression has been developed for such a process based on the Michaelis – Mentens and Line weaver–Burk approach. Comparison between the experimental data and those predicted from the rate model indicate good agreement with a mean absolute deviation of about 0.304916.

Keywords: Hydrolysis, Rice Straw, ***pseudomonas sp.,*** Michaelis – Mentens, kinetic model

1. INTRODUCTION

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 [1] and hence this increases the demand of an alternative eco friendly, 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 **[2].** Lignocellulosic materials regarded as promising energy source because it is potentially low cost renewable source of mixed sugars for fermentation to fuel ethanol and rice straw is one of the abundant lignocellulosic waste materials in the world [3]. It is annually produced about 731 million tons, which is 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. Rice straw predominantly contains cellulose 32-47%, hemicelluloses 19-27% and lignin 5-24%, and ashes 18.8%.[4,5,6]

Successful development of "third generation" biofuel depends heavily on degradation step of cellulosic biomass processing which is the production of fermentable sugar. There are many technical issues yet to be addressed in the process of converting biomass to convert it to sugar and to get a value added by product from it. Cellulosic biomass is a complex mixture of plant cell wall carbohydrate polymers known as cellulose and hemi cellulose, plus lignin and a smaller amount of other compounds known as extractives. Cellulose contains simple repeating units of glucose, but has a complex structure because of the long chains of glucose subunits joined together by β–1, 4–linkages [7] .Generally a variety of thermal, chemical, and biological processes can be used to produce fermentable sugars from biomass. Generally two basic conventional approaches are followed for converting biomass into fermentable sugars. Saccharification can be carried by acid or enzymatic hydrolysis. 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. Also due to the stringent environmental regulations acid hydrolysis even though well established has poor popularity. Enzymatic hydrolysis is currently the effective, popular energy efficient route for ethanol production. Though the enzymatic hydrolysis requires less or no acid but the price of commercial cellulose is high and thus leads the necessity of an eco low cost, convenient, potential source with easier of production cellulytic enzyme for production of fermentable sugar to meet the up growing demand of word fuel.

In this present study, among the lignocellulosic materials Rice straw (RS) one of main agricultural bi-product of the world, is chosen as substrate for sugar.

The goal of this study was to isolation of the cellulytic Microorganism *pseudomonas sp* and studies the activity of that enzyme 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 Isolation, screening and identification of dye degrading Organism:

The bacterial strain (*pseudomonas sp.*) was isolated in the Research Laboratory of Shahjalal University of Science & Technology, Sylhet, Bangladesh which was able to biodegradation of Rice Straw. The source for the strain was the local waste dumping site at Rickabibazar point of Sylhet district. It was identified on the basis of the standard procedure as by Cappucino JG, Sherman N. [8].

Inoculum preparation and Enzyme extraction:

Spore suspension of bacterial cultures was prepared. Bacterial cultures were refreshed on Nutrient Agar (Merck) in culture bottles. Bacteria were grown in Nutrient Agar and a loop full of bacterial culture was added into the production medium (KH_2PO_4:1 gm/L, K_2HPO_4: 1.145 gm/L,$MgSO_4$: 0.4 gm/L,NH_4SO_4:5.00 gm/L,$CaCl_2$:0.05gm/L,$FeSO_4$: 0.00125 gm/L and CMC: 10 gm/L) which was sterilized by autoclaving at 121ºC for 15 minutes and then 5 ml of production media was transferred into screw cap test tube and treated in shaker incubator at 37^OC for 24 hours at 100rpm and then each of this 5ml medium was transferred in 4 conical flasks (with 20 ml production media in each) and sterilized by autoclaving at 121^OC for 15 minutes and incubated in shaker incubator at 37^OC for 24 hours at 100rpm.

The isolated ***Pseudomonas sp.*** was grown under optimal condition for cellulase production with strain cultured in basal medium containing 1% CMC and incubated for 24 hours on a rotary shaker (100rev/min) at 37^0C and then the culture was centrifuged aseptically for extracellular cellulase preparation in refrigerated centrifuge.

2.2 Pretreatment of rice straw:

In order to expose the cellulose in the lignin-hemicellulose's matrix, the straw was delignified using the treatment with boiling water, H_2SO_4, Nh_4OH and NaOH as described below in order to analyze the relative effectiveness by the current investigations.

i. ***Water treated:*** After vigorously washing the Rice Straw **(RS)** with distilled water to remove the unwanted contaminants such as dirt, mud, debris of another leaf, it was dried in an air oven at 115^OC-120^OC for 15 hours. Then **RS** was crushed into small particles by a blender and fractionated into various sizes by a sieve shaker and stored in airtight glass vassal for further use.

ii. ***H_2SO_4 treated:*** 15 gm of rice straw was dried in air oven at 112^OC -115^OC for 3.5 hours after washing with distilled water followed by enthusiastic mixing in 150 ml of 1 %(v/v) of H_2SO_4 solution and heated at 90^OC in oven for 2 hour. After that, it was washed with continuous charge of distilled water until the neutralization of **RS** sample and then dried in air dryer for 20 hours at 90^OC and was blended to get the desired particle size.

iii. ***NaOH treated:*** ***5*** gm of rice straw was washed in distilled water and then dried in air oven at 102^OC - 104^OC for 2.5 hours and mixed vigorously in 100 ml of 1 %(w/v) of sodium hydroxide solution and heated at 110^OC in oven for 2 hours. Then washed again with distilled water to neutralize the sample and dried in air dryer for 2 hours. The treated straw was blended and particles with desired size were used for further experiment.

iv. ***NH_4OH treated:*** After washing ***5*** gm of **RS** in distilled water, it was dried in air oven at 112^OC - 115^OC for 2.5 hours and mixed vigorously in 100 ml of 1 %(v/v) of ammonium hydroxide solution followed by heating in oven at 110^OC for 1 hour. Then neutralization was done by distilled water and dried in air dryer for 2 hours. The treated straw was blended into particles of desired size.

2.3 Experimental procedure:

The hydrolysis of pre-treated **RS** was performed in rotary flasks shaker (Model : LRD : 750) with working volume of 100 mL in 250 mL flasks. Various amount of treated rice straw was taken a in each flask prior to the experiment and treated with Sodium acetate buffer (0.05 M) to maintain the p^H of the hydrolysis environment. All the components and p^H were assumed to have a uniform distribution in the flask due to continual rotation. All experimental run were conducted with addition of various predetermined amount of enzyme in the degradation environment with different amount of straw, different size of straw, different enzyme loading, different pH and different temperature to delineate the corresponding respect . Samples were taken at every 1 hour interval; boiled for 5 min to destroy the enzyme, thus confirming the ceasing of the reaction. Then the samples were centrifuged, and analyzed for glucose concentration in **UV**-spectrophotometer according to the method by Miller [9].

3. RESULTS AND DISCUSION:

Effect of substrate particle size

In order to avoid the large numbers of projected investigational lope, several preliminary experiment were made to ascertain the practical implication, if any, of delignification on glucose capitulation, and also the particle size of rice straw necessary for optimum production rate with respect to the desired product was

established which are demonstrated by Figures 1 and2. Like any other solid-fluid system, in order to play down the mass transfer restraint inflicted by the existence of hemicellulose and lignin, treatment of the RS sample was done as per the previous section, result of which is shown in Figure1, where the glucose concentration from a preset amount of raw RS mounted by 1.47156 mg/dl as a consequence of first process.

Similarly, the outcome of particle size effect on glucose yield is revealed in Figure 2 where it is observed that as the particle size of the RS straw was abridged from 45µm to 1mm, the glucose concentration build up from 0.5879 mg/dl to 1.0027 mg/dl respectively which may be due to enhancement in the surface area accessible for enzyme attack [10].

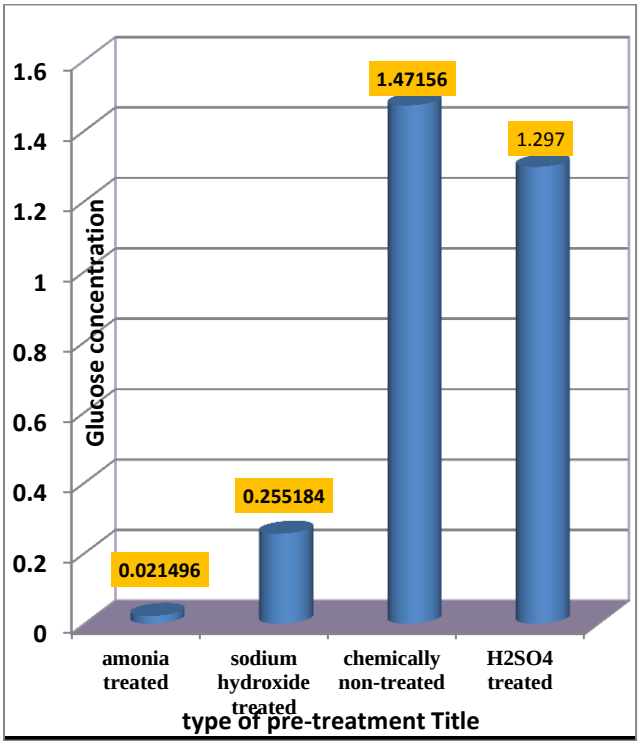

Figure 1: Effect of various types of pretreatment used for substrate (pH: 5.6 (acetate buffer), rice straw: 3.5 gm/L, enzyme: 50ml/ 1L mixture) on glucose yield.

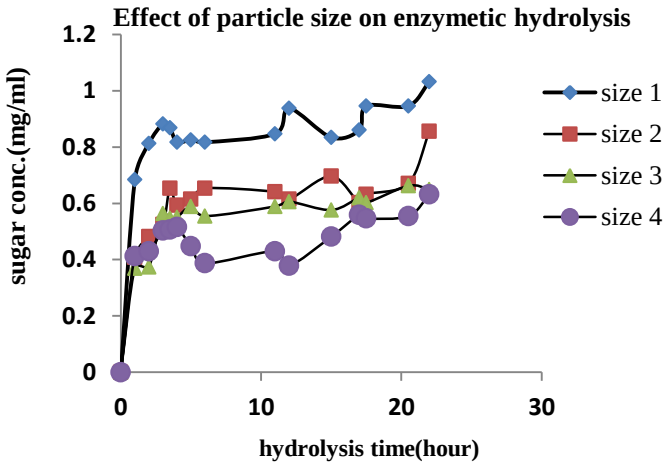

Figure 2: Effect of particle size on sugar production (straw loading: 3 gm/L, enzyme loading: 20 ml/L, p^H: 5.5, size 1= 45µm; size 2=125µm; size 3=500µm; size 4=1mm).

Effect of substrate and Enzyme loading:

As shown in Figures 3 and 4, the experimental data suggests the positive correlation of the maximum glucose production in saccharification environment with the substrate concentration and the enzyme loading. This may be due to higher enzyme-substrate active complex formation due to increased active sites by both charges.

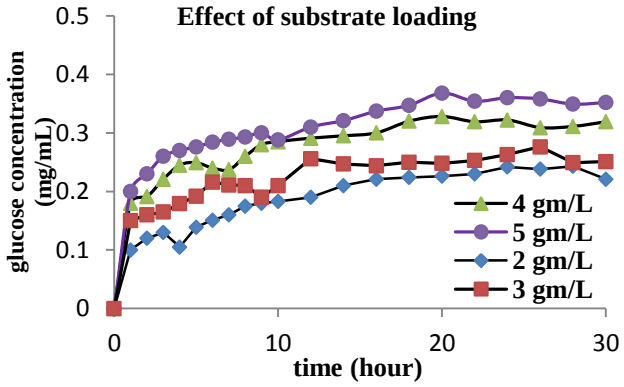

Figure 3: Effect of initial substrate concentration on sugar production.

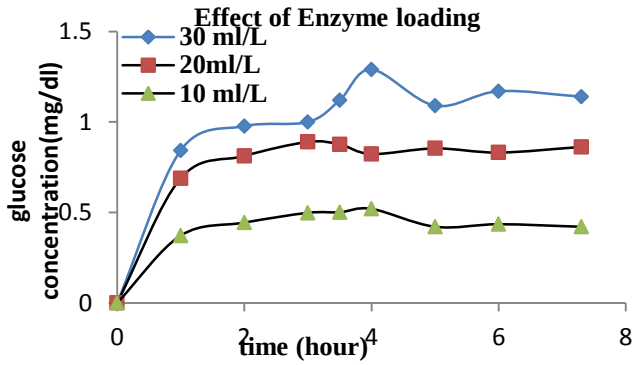

Figure 4: Effect of Enzyme loading (pH: 5.0 & RS loading: 3gm/L)

***Effect of temperature and p^H*:**

Figure 5 represents the outcome of the consequence of temperature on the extent of bioconversion of glucose production where, the alteration of enzymatic efficiency of pseudomonas sp. for glucose production with different temperature is showed and the optimum temperature of 30°C was required to attain the most excellent rice-straw conversion to glucose. The outcome of p^H alteration on glucose liberation is shown in Figure 6. The pH range between 7.0±0.2 gave the optimum yield of glucose.

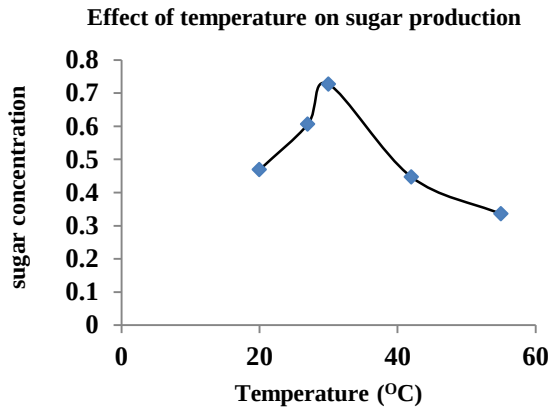

Figure 5: Effect of temperature on sugar production (straw loading: 2gm/L, pH: 5.5, enzyme loading: 20 ml/L).

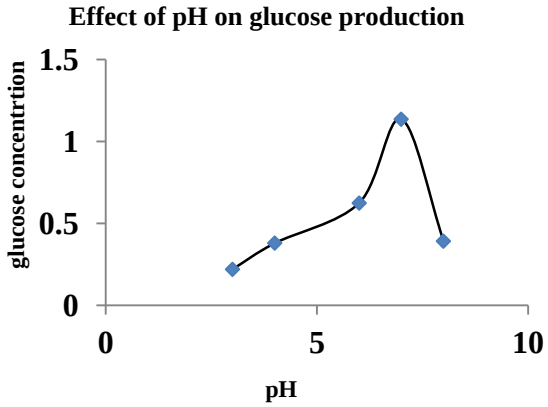

Figure 6: effect of pH on sugar production (enzyme loading 36ml/L, rice straw loading: 2gm/L, temperature: 27 ± 2 °C).

4. DEVELOPMENT OF KINETIC MODEL:

A typical kinetic results by graphical Differentiation of curves using the initial rates method, afforded the plot of initial rates at different levels of substrate initial concentrations as shown in Figure 7.

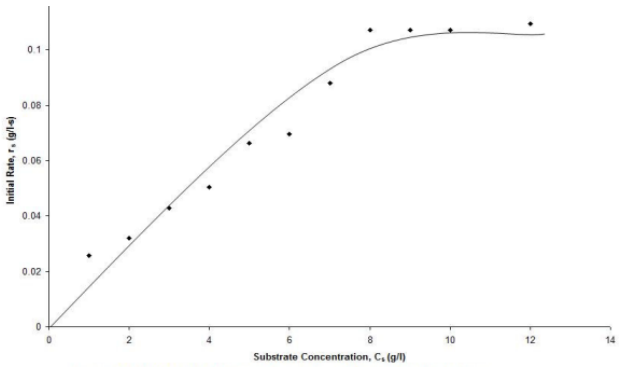

Figure 7: The initial rate as a function of substrate concentration (a typical curve).

From this curve, it can be observed that the reaction rate is proportional to the substrate concentration (that is, first order reaction) when the substrate concentration is in low range is also evident that the rate of reaction move toward a steady value as the substrate concentration becomes elevated. So we can say, the reaction rate alters progressively from first order to zero order as the substrate concentration was amplified. This form of conduct is commonly described by the Michaelis-Menten kinetic expression such as:

$$V_p = \frac{V_{max}\ [S]_o}{K_m + [S]_o} \ldots\ldots\ldots (1)$$

Where, V_{max} (the maximum reaction rate) and K_m (rate constant) are the kinetic parameters, which are needed to be experimentally determined and $[S]_o$ is substrate concentration.

Applying the Line weaver – Burk method to linearize the rate expression by inverting equation (1) yields:

$$\frac{1}{V_p} = \frac{1}{K_m} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]_o} \ldots\ldots\ldots\ldots (2)$$

So, from this straight line plot, the corresponding parameters (K_m & V_{max}) can be determined from intercept $1/V_{max}$ and the slope K_m/V_{max}.

The equation (2) is plotted by the data obtained with differentiation of initial glucose production by time for various initial substrate concentrations. The data best fits a straight line with slope is 0.866 and intercept is 0.7833

From this plot the kinetic parameters (V_{max} and K_m) were estimated as 1.154734 gm/ (L. hour) and 9.102771 gm/L respectively.

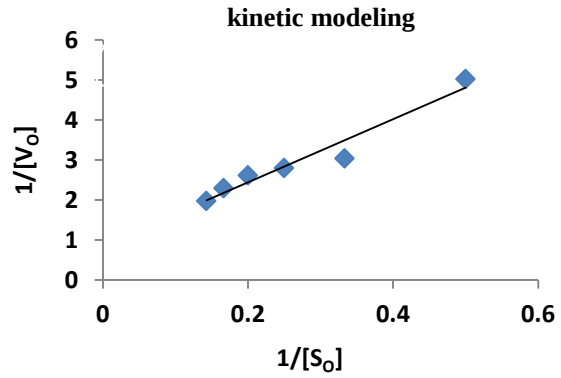

Figure 8: Linearized kinetic modeling plot.

The theoretical data's and experimental data's are plotted in Figure: 9 and the mean Standard deviation are found to be 0.304916**.**

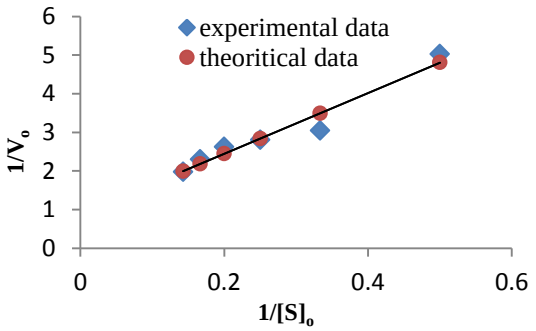

Figure 9: Comparison between theoretical and experimental data.

Based on the evaluated kinetic parameters, the model equation is given as:

$$V_o = \frac{\mathbf{1.4742}[S]_o}{\mathbf{1.2767}+[S]_o} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

5. CONCLUSION:

The present study leads the improvement of biofuel production via the successful illustration of isolation of cellulytic bacteria from abound waste source, capable of efficiently producing a variety of sugars while simultaneously displaying tolerance to high end-product production cost. The substrate rice straw represents an attractive feedstock for sugar production because of its abundance, cost and efficient degradation by cellulolytic organism. Such a process provides the significant environmental benefits terms of reductions in the emission of greenhouse gases, and other pollutants e.g. acid rain, photochemical smog etc and proves its efficiency in fermentable sugar production in large commercial systems based on sustainability, existing resources and residues can help to our natural environment. It can be concluded that pre-treatment of rice straw enhances the rate of glucose production, while particle size of 45µm was found to be more favorable among the analyzed sizes. Operating temperature of 30^{O}C and pH of 7.0 gave the best activity within the range of time investigated. Substrate concentrations, when in low range at a fixed enzyme concentration, favorably affect the glucose concentration. The increment of enzyme concentration gives high glucose concentration and yield. The kinetics parameters of the reaction were obtained as V_{max} = 1.4742 gm/ (L. hour) and K_m =1.2767 gm/L respectively.

This model equation was found to be consistent with the experimental data with the co-relation factor of 0.968 and a mean standard deviation of 0.304916, and also it can be concluded that the present study has promising and practical utility in the production of glucose from rice straw**.**

Acknowledgement

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