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Full Length Research Paper

The kinetics of glucose production from rice straw by *Aspergillus niger*

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In this investigation, glucose was produced from rice straw using cells of *Aspergillus niger*, isolated from maize grain. Glucose yield was found to increase from 43 to 87% as the rice straw particle size decreased from 425 to 75 µm, while the optimal temperature and pH were found within the range of 45 - 50°C and 4.5 - 5 respectively. The concentration and rate of glucose production was observed to depend on pretreatment of rice straw, substrate concentration and cell loading. A kinetic model rate expression has been developed for such a process based on the Michaelis – Mentens and Lineweaver – Burk approach. Comparison between the experimental data and those predicted from the rate model indicate good agreement with a mean absolute deviation of about 0.2.

Key words: Rice straw, cellulose, glucose, *Aspergillus niger*, enzyme loading, kinetic parameters, kinetic model.

INTRODUCTION

The world population has continued to grow steadily, necessitating an increase in the demand for the production of affordable food (Anderson, 2006). Rice happens to be the most consumed staple food in all the continents, resulting in the generation of enormous amounts of waste such as straw and husks (Sharma et al., 2001). It is common knowledge that the cost of rice production is billed wholly on the grains alone.

Recent studies have shown that researchers in this field have successfully converted many cellulosic materials such as saw dust, solid animal wastes, crop residues etc (Solomon et al., 1990; Lee, 1992; Olsson and Hann-Haggerdahl, 1996; Luo et al., 1997; Cao et al., 1997 and Sun and Cheng, 2002) to more valuable products such as fermentable sugars.

Rice straw contains between 25 – 45% of cellulose, 20 – 30% hemi-cellulose and 10 – 15% lignin (Sharma et al., 2001; Sun and Cheng, 2002; Lequerica et al, 1984; Vlesenko et al., 1997). As is the case in most existing biological fermentation processes, the major setbacks in the development of cellulosic conversion technology are associated primarily with the complex nature of cellulose structure and it's resistant to degradation. Other difficulties include the limited number of commercially available enzymes capable of digesting cellulose and the high requirement of enzyme loading coupled with the long period usually needed to attain an appreciable conversion level (Luo et al., 1997). These factors make it difficult to run biological systems in a continuous mode.

Attempts at addressing the aforementioned problems have led investigators to use soluble cellulose, filter paper, sawdust and animal solid wastes with impressive results (Duff et al., 1996; Wen et al., 2004; Gregg and Sadder, 1996). Kaur et al. (1998), reported studies on enzymatic hydrolysis of rice straw using *Trichoderma reseei* cellulase. Also, the effect of cross-linked *Aspergillus niger* cellulase on the hydrolysis of rice hull has been reported by Sharma et al. (2001). Vlesenko et al. (1997) studied the enzymatic hydrolysis of pretreated rice straw.

However, little information exists in the literature concerning reaction rate parameters for rice straw hydrolysis. The generation of such kinetic parameters is an integral part of the current study. Furthermore, shifting from the traditional batch fermenter systems to flow processes will require a drastic reduction in the incubation period (reaction time). To achieve a significant conversion within such a reaction time may require unreasonably high en-

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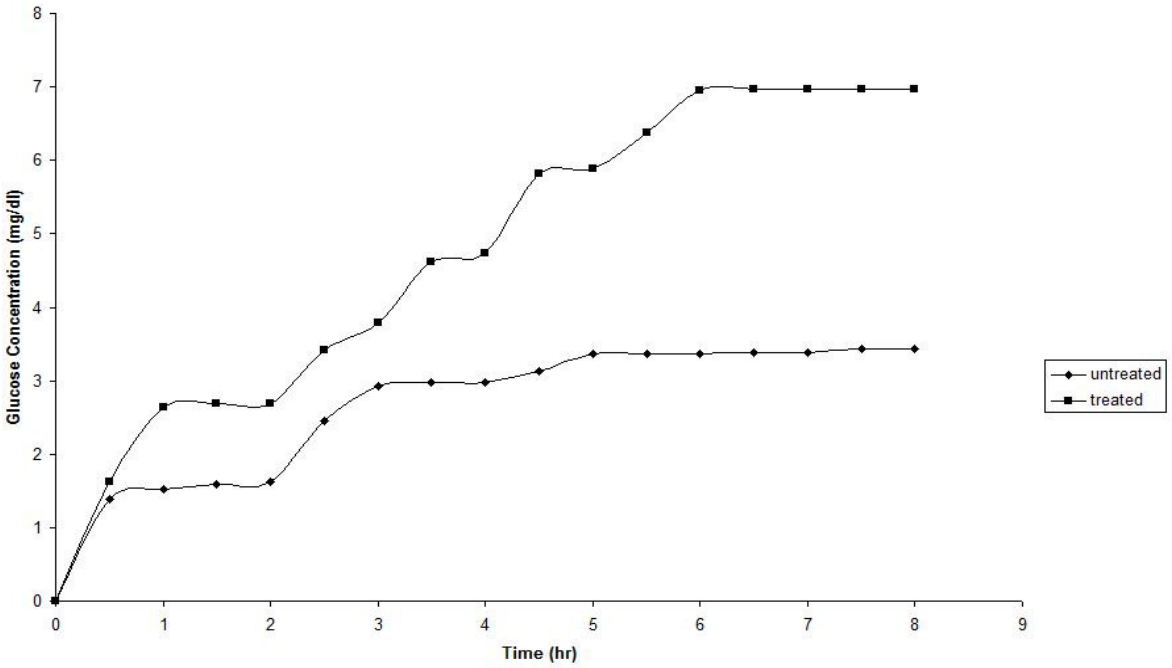

Figure 1. Effect of ammonia pretreatment of rice straw on glucose yield.

zyme loading and cost. Perhaps, a better alternative lies in direct cell utilization, which has the capacity to supply fresh enzymes to the system via secretion; this should be possible at a lower cost compared to systems that utilize pure enzymes.

Hence, it is the goal of this paper to study the conversion of an ammonia treated rice straw to glucose using cells of *A. niger*. This will enable the development of a descriptive kinetic model useful for analysis and design of continuous flow fermentation systems.

MATERIALS AND METHODS

Isolation of Organism

Potato Dextrose Agar (PDA) medium as detailed by Alexoponlus and Beneke (1984) was used for the isolation of the organism. The organism was identified as *A. niger* using an Olympus Venox –T microscope based on the standard structure of *A. niger* given by Robert and Ellen (1988). Subculturing from the parent culture was done to obtain pure colonies. A wire loop was sterilized using flame sterilization to kill surface bacterial in order to avoid contamination. The wire loop was used to take a portion of the growing fungal cultures from the edge of the culture plates. This was transferred to a sterilized bottle containing fresh PDA medium. Sub culturing was repeated for about ten times so as to obtain a fairly pure colony before storing in a refrigerator for subsequent exploitation.

Pretreatment of rice straw

In order to expose the cellulose in the lignin-hemicellulose's matrix, the straw was delignified using the ammonia steeping method described by Cao et al. (1996) having established its merit through preliminary investigations.

Experimental procedure

In a typical run, the temperature of the water bath (Sharmond model) was set at 45°C. A hundred milliliters (100 ml) of 0.1 M sodium acetate buffer solution (pH 4.5) was introduced into an Erlenmeyer flask fitted with stirring mechanism along with 0.1 g of *A. niger* species and 2.0 g of ammonia treated rice straw were added. Samples were withdrawn after every 30 min within 8 h reaction time for analyses. The concentration of glucose in the sample was determined by following the method of Lee and Fan, (1982) and Lee, (1992) which uses a Randox glucose kit and colorimeter (Model WPA 5001, U.S.A.) at 520 nm. The experiment and glucose analysis was carried out using varying substrate concentrations, particle sizes, cell loadings, temperature and pH. Each run was repeated three times and the mean value of each set of runs was reported.

RESULTS AND DISCUSION

Preliminary investigation

Due to the large number of anticipated experimental runs, some preliminary studies were done to ascertain the practical significance, if any, of delignification on glucose yield. Similarly, the particle size of rice straw required for optimum reaction rate in favor of the targeted product was also determined. Figures 1 and 2 presence these findings. Since the present study is a typical solid-fluid system, it was deemed necessary to minimize the mass transfer constraints imposed by the presence of hemicellulose and lignin, via chemical treatment of the sample. The effect of this pretreatment is seen in Figure 1, where the glucose concentration from a fixed quantity

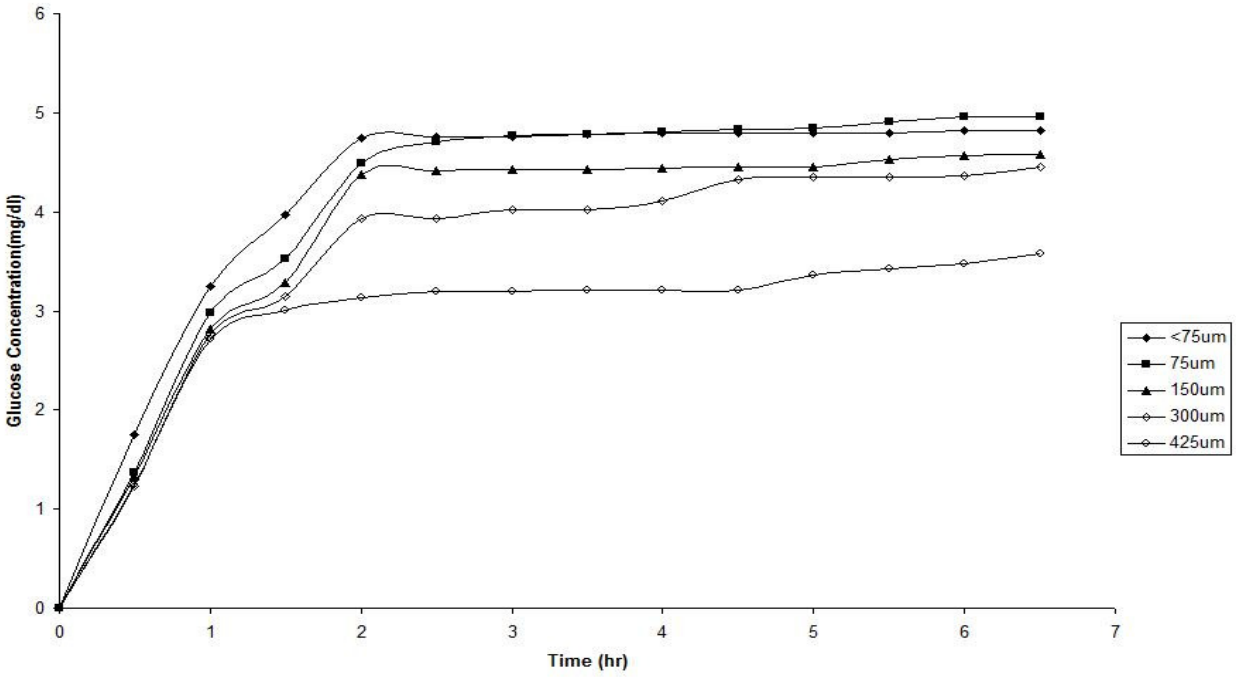

Figure 2. Effect of particle size on glucose yield.

of raw rice straw rose from about 3.4 to about 7.4 mg/dl as a result of ammonia steeping step. This satisfactorily agrees with Chosdu et al. (1993).

Similarly, the result of particle size effect on glucose yield is shown in Figure 2. It is observed that as the particle size of the raw rice straw was reduced from 425 to 75 μ m, the glucose concentration rose from 3.2 mg/dl to $\cong$ 5.0mg/dl presumably due to increase in the surface area available for enzyme attack. However, further reduction in the particle size below 75 μ m has no practical influence on glucose yield. This result conforms to the work reported by Wen et al. (2004).

Effect of substrate and cell loading

It is clearly seen from Figure 3 that the maximum glucose concentration in solution varied positively with the substrate concentration. Similarly, as the cell loading increased, the glucose concentration increased proportionally as shown in Figure 4. This may be due to continuous excretion of enzymes by the cells into the solution.

Effect of temperature and pH

Figure 5 shows the result of the effect of temperature on glucose concentration. This result shows the optimum temperature range of 45 - 50℃ is needed to achieve the best rice-straw conversion to glucose. This is in agreement with the result obtained from the hydrolysis of steam pretreated softwood reported by Tengborg et al. (2001) and animal manure lignocellulosics reported by Wen et al. (2004) which was found to be optimum at 50℃. The effect of pH on glucose concentration is shown in Figure 6. The pH range between 4.5 and 5.0 gave the optimum yield of glucose. This corroborates the results of Tenborg et al. (2001) at which the optimum glucose yields were obtained at a pH of 4.8.

Development of kinetic model

The modeling in this section is based on the following observations and assumptions:

1. The cells were introduced at their exponential growth phase.
2. The model is targeted to capture only the first segment (initial rate) of the concentration-time curve.
3. Glucose is the main product of interest.
4. A pseudo homogeneous system is assumed throughout based on solid concentration of ≤ 10 g/l
5. 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.

Consequently, treatment of 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.

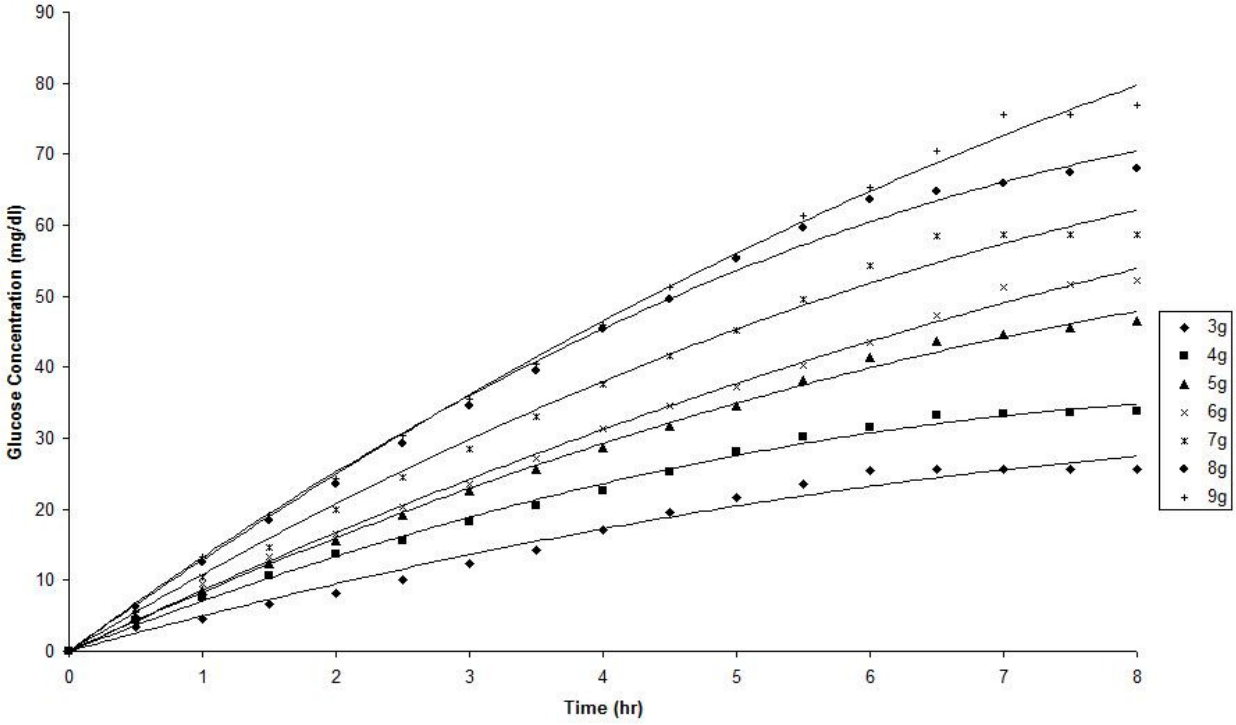

Figure 3. Effect of substrate concentration and reaction time on glucose yield.

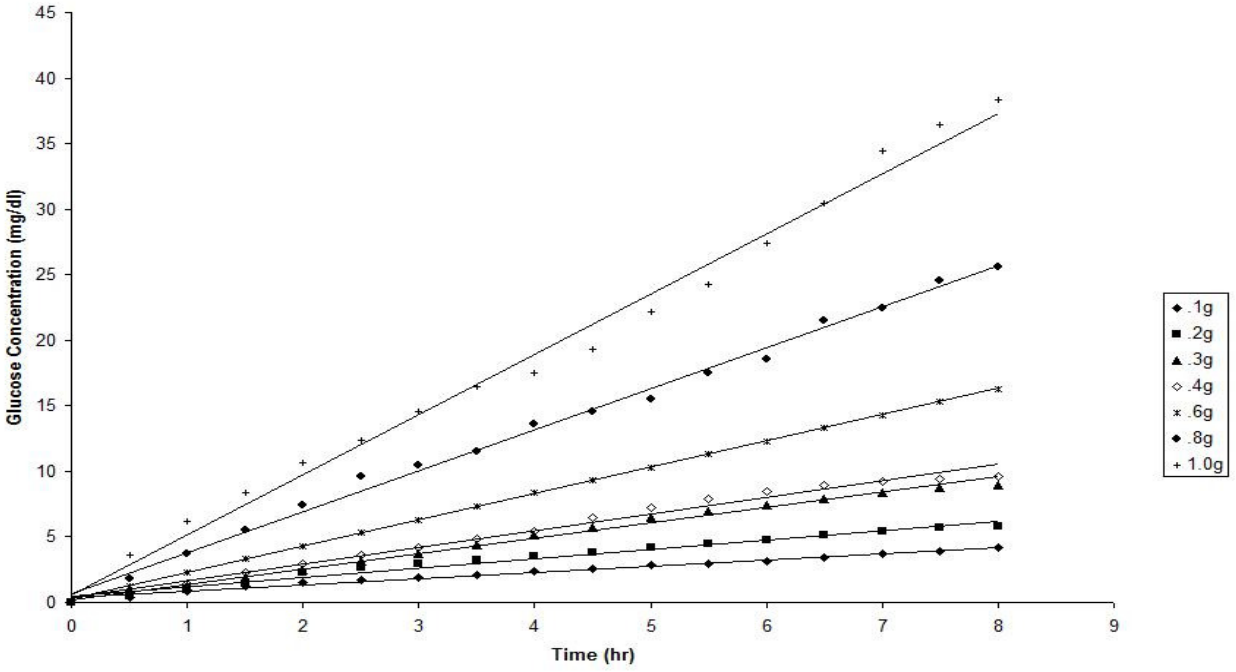

Figure 4. Effect of cell concentration on glucose yeild.

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. Also from the curve it can be seen that the rate of reaction approaches a constant value as the substrate concentration becomes high. That is, the reaction rate

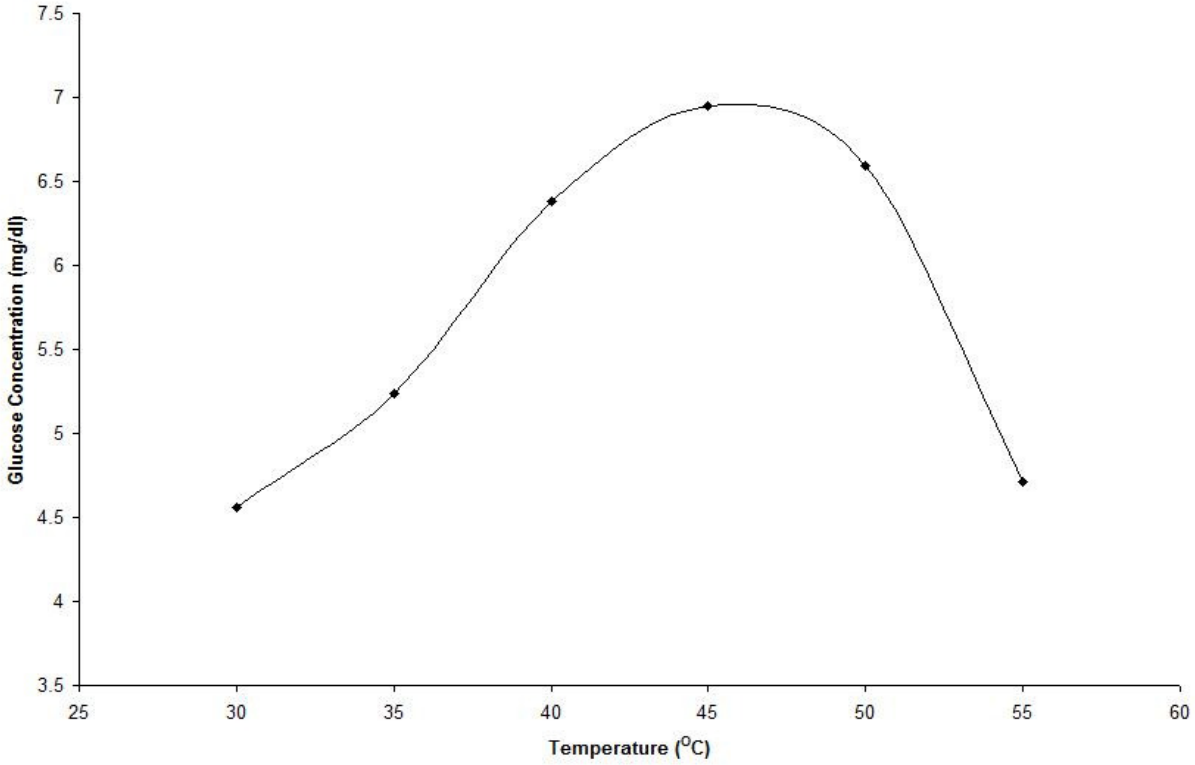

Figure 5. Effect of temperature on glucose yield from rice straw.

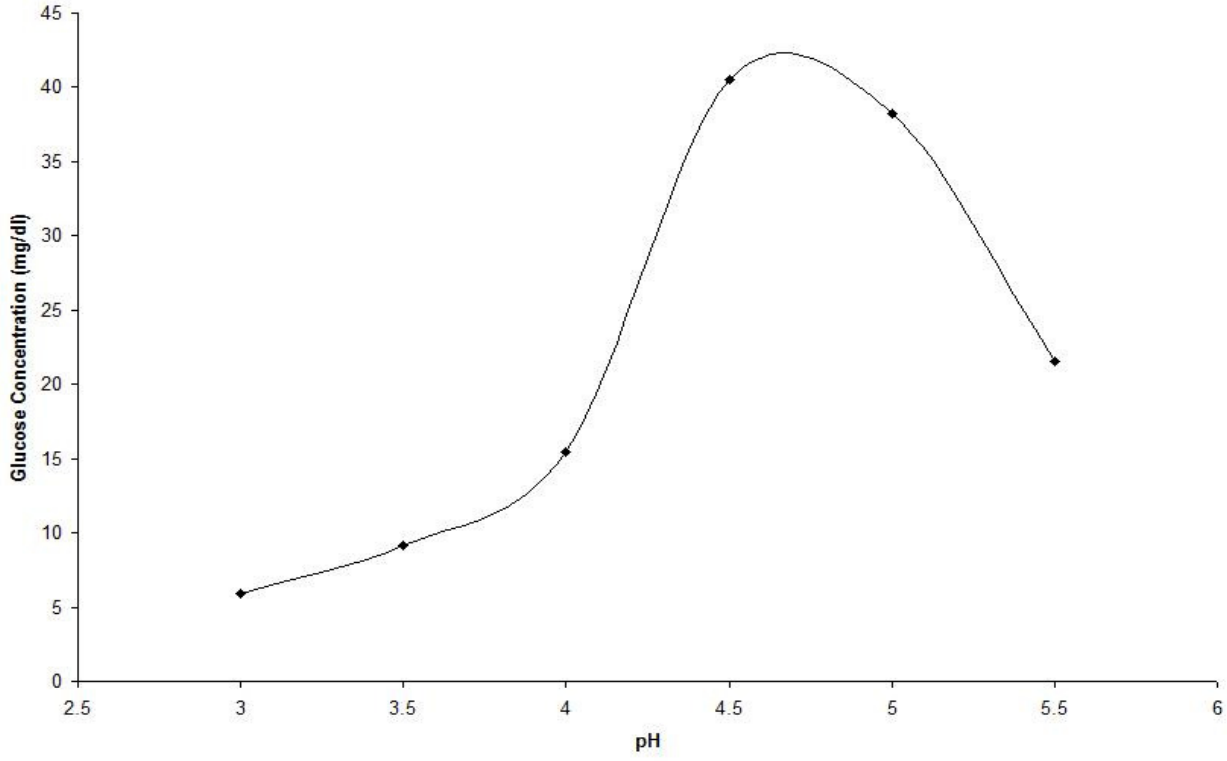

Figure 6. Effect of pH on *A. niger* cell activity on rice straw hydrolysis.

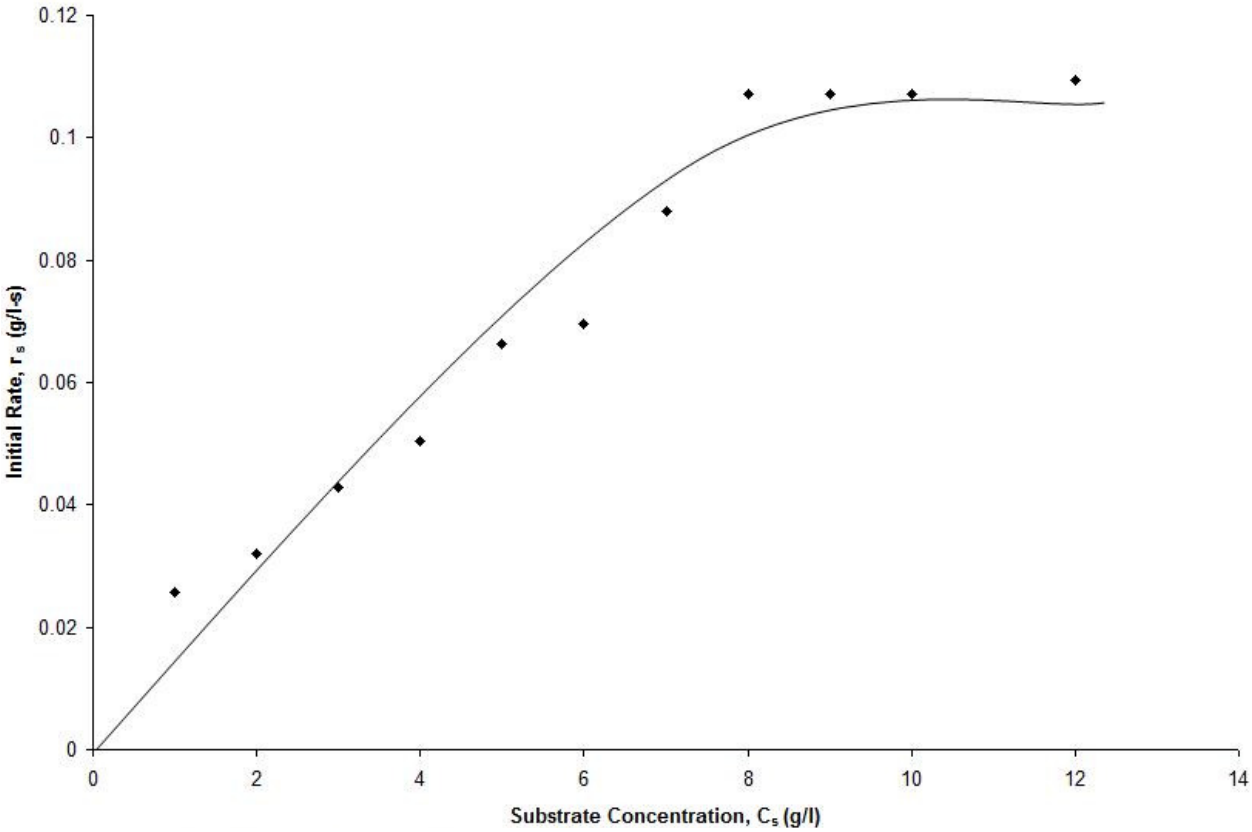

Figure 7. A typical initial rate as a function of substrate concentration.

changes gradually from first order to zero order as the substrate concentration was increased.

This form of behavior is commonly described by the Michaelis-Menten kinetic expression such as:

$$r_p = \frac{r_{\max} C_s}{k_m + C_s} \quad (1)$$

Where r_{max} (the maximum reaction rate) and k_m (rate constant) are the kinetic parameters, which are needed to be experimentally determined and C_s is substrate concentration.

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

$$\frac{1}{r} = \frac{1}{r_{\max}} + \frac{k_m}{r_{\max}} \frac{1}{C_s} \quad (2)$$

A plot of the reciprocal of the initial rate r versus the reciprocal of the initial substrate concentration C_s is expected to yield a straight line with an intercept $1/r_{max}$ and the slope k_m/r_{max}. A plot of this using the generated experimental data in this work is shown in Figure 8 from which the kinetic parameters (r_{max} and k_m) were estimated as $1.5288*10^{-4}$ g/l s and 33.7 g/l, respectively. The maximum reaction rate r_{max} ($1.5288*10^{-4}$ g/l s) was found comparable to the values 0.1910 - $1.77666*10^{-4}$ g/l s reported by Imai et al. (2004) for hydrolysis of a soluble cellulose (CMC) using the synergetic effects of combined cellulases from *Trichoderma viride* and *A. niger.* It is believed that this comparative value despite the use of real substrate (insoluble cellulose) in this work can be attributed to the continuous excretion of enzymes into the system by the cells, which was able to mask effectively the often reported glucose inhibitory effect on cellulase from *A. niger.*

The high value of k_m, 33.7 g/l obtained in this work is not unconnected with the portion of the substrate utilized by the cells to supply its internal energy (metabolic) requirement.

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

$$r_p = \frac{1.5288 * 10^{-4} C_s}{33.65 + C_s} \quad (3)$$

The consistency of this model equation was tested with the generated data to statistically evaluate its reliability.

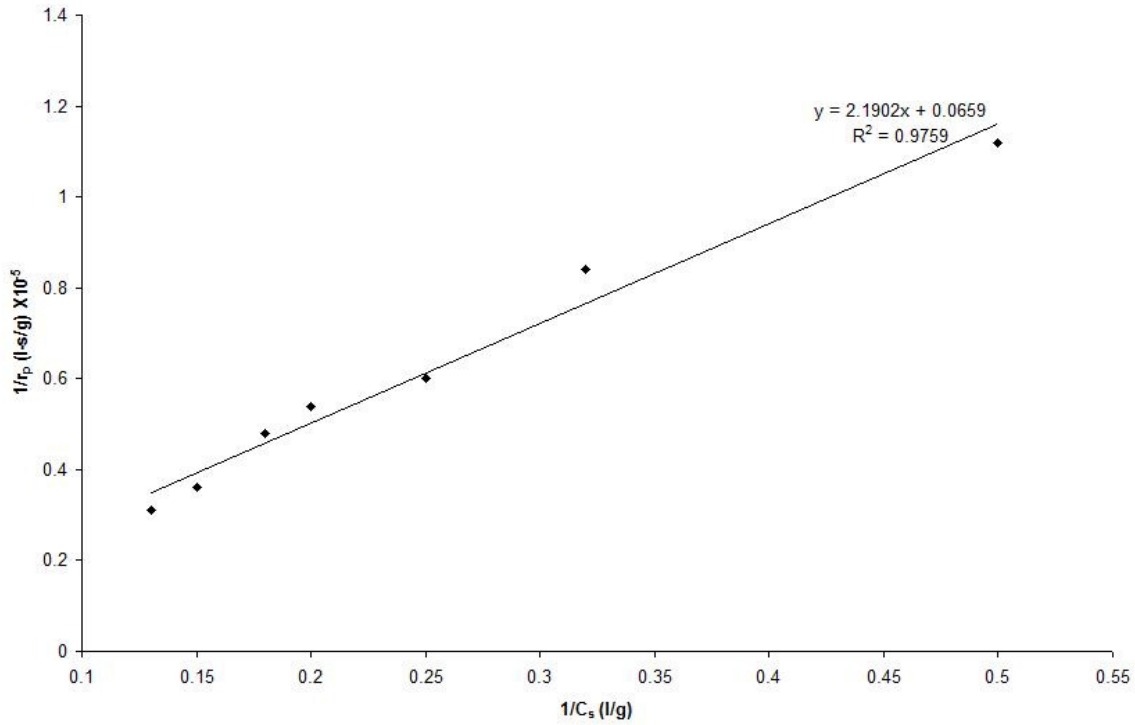

Figure 8. Line Weaver-Burk plot of the Michealis Mentens' kinetics.

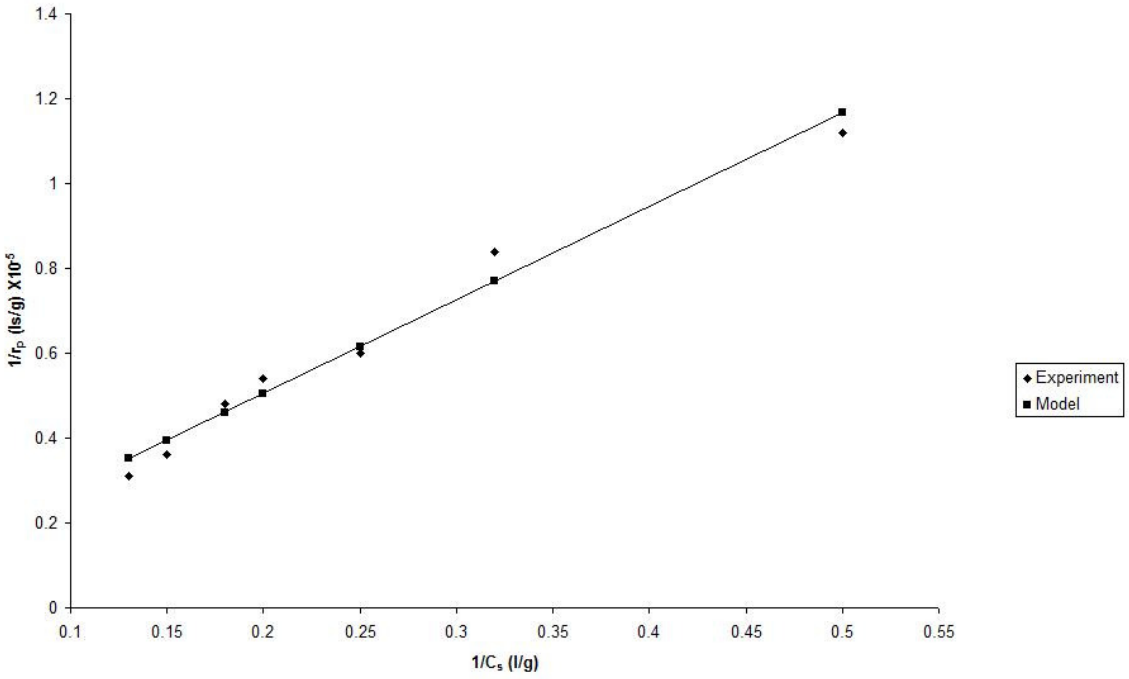

Figure 9. Comparison off model predicted rate against experimentally obtained rate.

The result of the consistency test as presented in Figure 9 shows the model equation is consistent with the experimental data with the mean standard deviation of 0.2. Considering the low cost of cellulose, increased activity shown by the cell system and relatively low cost of cell procurement in comparison to pure enzyme system it

can be concluded that the present study has promising and practical utility in the production of glucose from rice straw.

Conclusion

Rice straw hydrolysis provides an alternative source of fermentable sugar (glucose), which can be further converted into value added product such as ethanol. It can be concluded that pre-treatment of rice straw enhances the rate of glucose production, while particle size of 75μm was found to be optimal. Operating temperature of 45 - 50^{O}C for 8 h and pH of 4.5 - 5.0 gave the best cell activity within the range of time investigated. Substrate concentrations, when in low range at a fixed cell concentration, favorably affect the glucose concentration. Cell loading increases both glucose concentration and yield.

The kinetics parameters of the reaction were obtained as r_{max} = 1.5288*10^{-4} g/ls and k_m = 33.7 g/l. The kinetic model for the process was given as:

$$r_p = \frac{1.5288 * 10^{-4} C_S}{33.7 + C_S}$$

This model equation was found to be consistent with the experimental data with the mean standard deviation of 0.2

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