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Kinetic Study of Watermelon *(Citrulluslanatus)* Peel Conversion to Glucose by *Trichoderma reesei*

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ABSTRACT

In this work, the kinetic study of watermelon dry peel powder using crude enzyme from *Trichoderma reseei* in glucose production was investigated. Enzymes from *Trichoderma reseei* were used for the hydrolysis and the effect of mycelium cell loading and substrate concentration were studied. Effect of mycelium mass loading of the range 0.5g to 3g at constant substrate concentration shows an increase in glucose production as mycelium mass increases. Glucose concentration increases from 0.506793 g/l to 0.974228 g/l as substrate concentration increases from 0.5g to 2.5g respectively. The initial rates method was adopted in estimating the kinetic parameters using Michaelis-Menten model. The Three selected models: Michaelis-Menten kinetic model, Lineweaver-Burk, Langmuir and Eadie-Hofstee were used to estimate the values of kinetic parameters. The experiment conducted adequately followed Michaelis-Menten kinetic model where the kinetic parameters r_{max} was found to be 0.00478 g/l.hr while K_m was 0.3174 g/l.

Keywords: Feedstock, Cellulose, Energy demand, Waste, Enzyme, Kinetic Parameters, Pretreated

INTRODUCTION

Rising energy costs and the imminent climate change led to an increased attention to biofuel production (Somerville, 2007; Rubin, 2008). Lignocelluloses, often a major component of different waste from various industries, forestry, agriculture and municipalities is proposed as an alternative renewable feedstock to supply energy demand. 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. (Lee, 2002; Luo *et al.*, 1997; Sun and Cheng, 2002) to more valuable products such as fermentable sugars (Aderemi, *et al.*, 2008).

Poor waste disposal and management are major challenges in Nigeria, especially items such as food, animal waste, vegetable waste, post-harvest waste etc. Such items considered as waste are great reservoir of energy which if well harnessed could contribute to the economic growth of the nation. Watermelon (*Citrullus lanatus*) refers to both the edible fruit and vine-like plant of a climbing and trailing herb belongs to the family *cucurbitaceae* (Shippers 2000; Olumide, 2015). It is a crop that is mostly cultivated in the warmer part of the world.

In Nigeria, the fruit is mostly cultivated in the North eastern part of the country like Maiduguri in a large volume. Because of the high production and consumption rate of the fruit, there is always a high waste generation of the peel which is often regarded as waste with no value and often thrown away littering the environment or fed to animals. In recent times, several efforts have been expended on the conversion of some agricultural waste to glucose hence; a lot of research work on the conversion of watermelon peel to glucose by enzymatic hydrolysis is required.

This paper presents the studies of the processing parameters such as cell loading, substrate concentration (with respect to glucose production), kinetic rate constant, i.e. Michaelis-Menten constant (K_m) and maximum reaction rate (r_{max}).

METHODOLOGY

Effect of Substrate Concentration

The effect of substrate concentration on enzymatic hydrolysis was investigated in a series of batch reactors using different substrate of 0.5g, 1g, 1.5g, 2g and 2.5g, while keeping other reaction conditions such as temperature, pH, cell loading and culture age constant at 30 °C, 4.5, 1g, 2.5g and 5days respectively.

Effect of Cell Loading

Enzymatic hydrolysis of watermelon peel was carried out using different ranges of mycelium mass loading of 0.5g, 1g, 1.5g, 2g, 2.5g and 3g. Other

conditions such as temperature, pH, substrate concentration and culture age were held constant at 30 °C, 4.5, 1g, 5days respectively. The glucose produced was then measured.

Experimental Procedure

Fresh watermelon fruit for the study was procured from Monday market in Maiduguri. The fruit was sliced and the peel was carefully removed, oven dried, mechanically crushed into powder and kept in a sterilized container and in a dry place. The watermelon powder was gently and carefully sieved using 100μm sieve size to obtain fine powder so as to increase the area/volume ratio. The peel powder was then collected and kept in a sterilized container with a cover. The fine peel powder was further pretreated with 20 ml of 10% sulfuric acid (H_2SO_4) in a 1 liter beaker with continuous stirring and allowed to stay for 5 minutes after which the peel was rinsed in muslin clothe to separate it from the acid where it was oven dried at 30 °C for 1 hour.

In a typical run, the temperature of the water bath was set at 45°C. The pH of reactor into which 50 ml of mineral salt medium was poured was set to 4.5 by adding sodium acetate (0.1 M) buffer solution. *T. reseei* mycelium (0.5g) and 2g of treated dry watermelon peel were added. Samples were monitored within one week interval by withdrawing the sample after 24 hours reaction time for analysis. Concentration of glucose produced was determined following the method of Osaribie, (2013), which is using a glucose oxidase-peroxidase labkit (GOD-PAP) and an electrophotometer at 546 nm. The study was conducted at various concentrations of substrate and cell loading.

Kinetic Modeling of Reaction

The following assumptions were made for the model development:

1. The cells used were at their exponential phase
2. Sufficient enzymes were released from the cells making it to be viewed as that of enzyme-substrate kinetics
3. Glucose is the major and target product of interest.

4. There was homogeneous mixing between the enzymes and the available substrate in the reactor and a pseudo homogeneous system is assumed.
5. Only the early stage (initial rate) of reaction is considered in developing the model before possible reversible reactions begin to spring up and start becoming significant

The rate of reaction catalyzed by an enzyme increases linearly with the substrate concentration, up to a point but it soon reaches the maximum rate constant value, $r_{\max}$ beyond which there is no further increase in reaction rate, this is called substrate saturation. The phenomenon of substrate saturation is described by the Michaelis-Menten equation;

$$r = \frac{r_{\max}[S]}{[S]+K_m} \quad - \quad - \quad - \quad - \quad (1)$$

Where,
k_m = Michaelis-Menten
S = Substrate

Eqn. (1) is used to predict the rate of reaction catalyzed by an enzyme at any substrate concentration provided the values of r_{max} and k_m are known. The Kinetic parameters were determined experimentally for the purpose of better understanding of the enzymes activity. This was done by applying three linearized Michaelis-Menten kinetic models as:

$$\frac{1}{r} = \frac{1}{r_{max}} + \frac{K_m}{r_{max}[S]} \quad - \quad - \quad - \quad (2)$$

$$\frac{[S]}{r} = \frac{[S]}{r_{max}} + \frac{K_m}{r_{max}} \quad - \quad - \quad - \quad (3)$$

$$r = r_{max} - k_m \frac{r}{[S]} \quad - \quad - \quad - \quad (4)$$

Where equations (2), (3) and (4) are; Line-weaver-Burk, Langmuir and Eadie-Hofstee respectively. To achieve a rational process design, the kinetic study of enzymatic hydrolysis is very important. Usually, the determination of Michaelis-Menten constant k_m and maximum reaction velocity r_{max} is essential in such study. Presently, there are a number of

procedures that can be used to determine the values of k_m and r_{max} such as linearization and non-linearization methods. However, the direct estimation of k_m and r_{max} is difficult from the Michaelis-Menten plot due to the hyperbolic nature of rate (r) against substrate concentration [S]. Therefore, the determination of the kinetic parameters is usually done by transforming the Michaelis-Menten model equation into linearized plots like the Lineweaver-Burk, Langmuir and Eadie-Hofstee (Guilbault,1976; Lee, 2002; Inamdar, 2009; Rajaram and Kuriacose, 2009; Osarebie, 2013). Michaelis-Menten constant $(k_m)$is the substrate concentration needed to obtain a reaction rate of $\frac{1}{2}r_{max}$.

RESULTS AND DISCUSSIONS

Effect of Cell loading and Substrate Concentration

Figs. 3.1 and 3.2, shows the effect of cell loading and substrate concentration on glucose production.

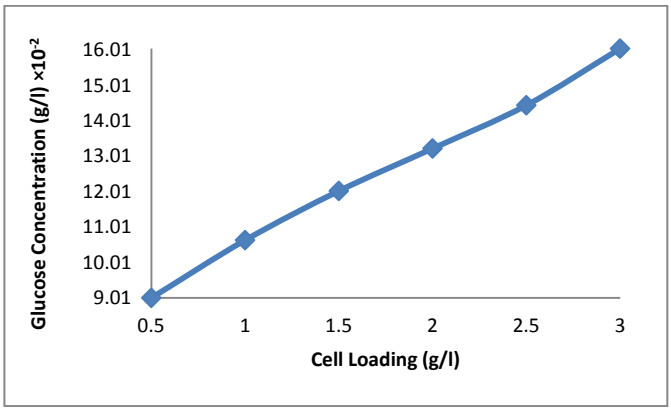

Fig.3.1: Effect of cell loading on Glucose

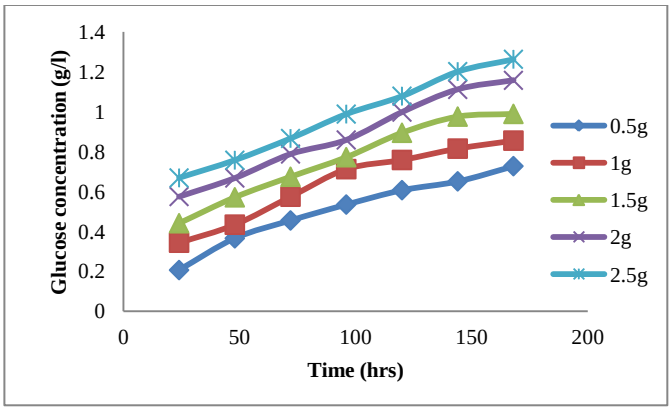

Fig. 3.2: Effect of initial substrate concentration on glucose production

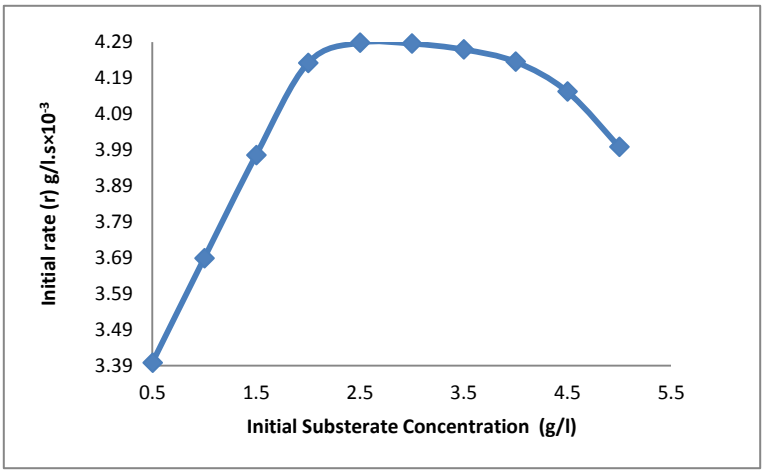

Fig. 3.3: Initial rates of reaction versus initial substrate concentrations

It can be seen that glucose concentration increases with increase in cell loading. This is in agreement with simple Michaelis-Menten equation which describes the behavior of an enzyme-substrate reaction as work done by other researchers; Lee (2002) and Ososanya (2012). Fig. 3.2 shows that glucose concentration increases as substrate concentration increases from 0.5g to 2.5g respectively. High glucose yield in the substrate concentration of 2.5g was possible because there was available substrate for the enzymes to feed upon compared to the reactor with a concentration of 0.5g. Glucose concentration increases from 0.506793 g/l to 0.974228 g/l as substrate concentration increases from 0.5g to 2.5g respectively.

Fig.3.3, is a plot of the initial rates against initial substrate concentration, it shows a functional relationship, where the rate is proportional to the substrate concentration for the substrate concentration. There is a gradual shift from first order to zero order as the substrate concentration exceeds 2g/l. This is typical of Michaelis-Menten reaction where the product appreciates in quantity as the substrate also increases until it reaches it maximum value r_{max} beyond which there is no further increase in reaction rate. Plots of the values of initial rate and initial substrate concentrations for the three afore mentioned linearized methods are presented on Figs.3.4, 3.5 and 3.6 from where the values of the maximum rate and Michaelis-Menten constant were obtained.

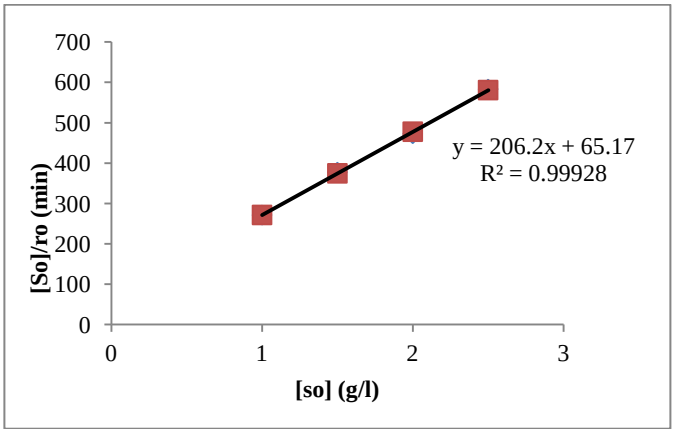

Fig. 3.4: Langmuir plot

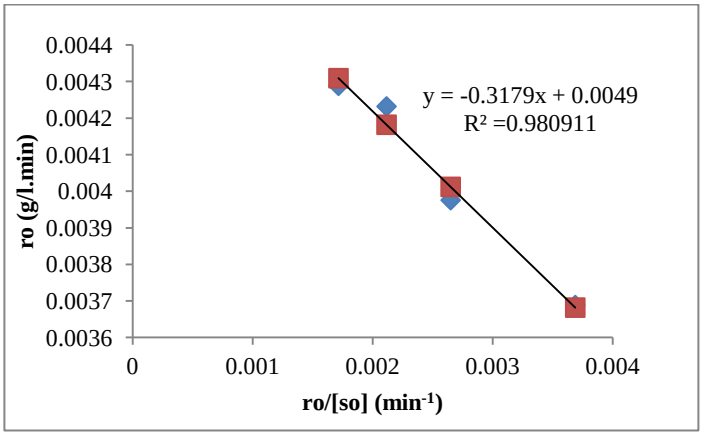

Fig. 3.5: Eadie-Hofstee plot

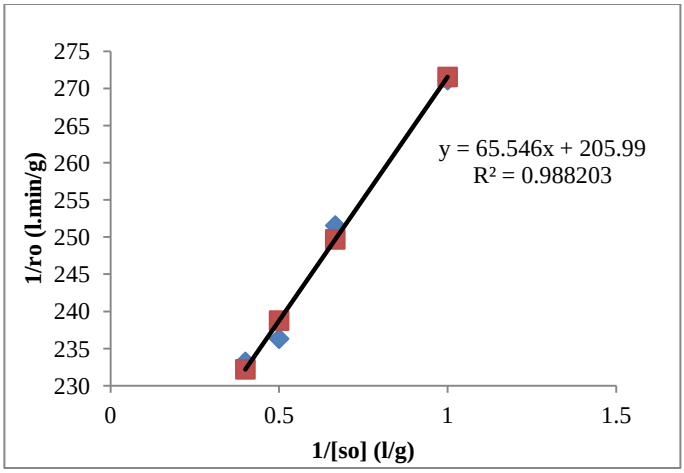

Fig.3.6: Lineweaver-Burk plot

The Lineweaver-Burk, Langmuir and Eadie-Hofstee models were used to estimate the values of the rate constants $r_{\max}$ (maximum rate) and K_m (Michaelis-Menten constant). This was done by comparing the linear equations y = 206.2x + 65.17 (Langmuir), y = -0.3179x + 0.0049 (Eadie-Hofstee) and y = 65.546x + 205.99 (Lineweaver-Burk), which were obtained from Figs. 3.4, 3.5 and 3.6 respectively, with the equation of a straight line $y = mx + c$ where the slopes and the intercepts were obtained for each equation and was used for the evaluation of the constants. From Fig. 3.4, a plot of the ratio of initial substrate concentration to initial rate verses the initial substrate concentration according to Langmuir linearize model gave a regression coefficient of $R^2 = 0.99928$ which depicts the appropriateness of Michaelis-Menten to the study. The values of the constants K_m and r_{max} obtained are presented on Table 4.1.

Table 1: Estimated values of $r_{\max}$ and K_m obtained from linear methods

Method	$r_{\max} \times 10^{-3}(g/l.hr)$	$K_m(g/l)$	R^2
Langmuir plot	4.849	0.3161	0.99928
Lineweaver-Burk plot	4.855	0.3181	0.988203
Eadie-Hofstee plot	4.900	0.3179	0.98091

The kinetic parameters were obtained by taking an average value of r_{max} and K_m. These values of the constants were then substituted into Michaelis-Menten equation which generated the kinetic model for the hydrolysis of watermelon rind as:

$$r = \frac{0.00487[S]}{[S]+0.3174} \quad - \quad - \quad - \quad - \quad (5)$$

The low value of the Michaelis-Menten constant (K_m) indicates that only a small amount of the substrate is needed to saturate the enzyme and high affinity for substrate. It also reveals that the enzyme from *t. reseei* has high efficiency.

CONCLUSION

The study conducted reveals that it is possible to hydrolyze watermelon peel to glucose and to harness the crude enzymes from *Trichoderma reseei* fungus which will serve as an alternative to commercially purified enzymes which are expensive. A linear relationship between cell loading and glucose concentration was observed on the effect of cell loading on glucose production as described by Michaelis-Menten. Glucose concentration increases as substrate concentration increases from 0.5g to 2.5g respectively, high glucose yield in the batch reactor with substrate concentration of 2.5g was possible because there was available substrate for the enzymes to feed upon compared to the reactor with a concentration of 0.5g. Langmuir linearize model gave a regression coefficient of $R^2 = 0.99928$, the kinetic parameters, r_{max} and K_m are 0.00487 (g/l.hr) and 0.3174 (g/l) respectively, which depicts the appropriateness of Michaelis-Menten with this study. The generated kinetic model according to the study for the hydrolysis of watermelon rind is $r = \frac{0.00487[S]}{[S]+0.3174}$.

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