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The Rate Equation for Glucose Kinetics from Cellulose Hydrolysis By Trichoderma Reesei

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Abstract

In this research, we evaluate orange mesocarp as a feed stock for production of glucose syrup using *Trichoderma Reesei.* The material was crushed to 100 – 150µm, 200 – 250µm and 300 – 425µm particle sizes and fed into a bioreactor where delignification was carried out. A series of enzymatic hydrolysis was affected at different substrate concentrations of;0.2g/L, 0.4g/L, 0.6g/ ,L 0.8g/L, 1.0g/L, 1.2g/L, 1.4g/L, 1.6g/L, 1.8g/L, and 2.0g/L. While other reaction conditions were kept constant. Additional series of enzymatic hydrolysis was carried out at different cell concentration of 0.01g/L, 0.02g/L, 0.03/g/L, 0.04g/L, 0.05g/L, 0.06g/L, 0.07g/L, 0.08g/L, 0.09g/L, and 0.10g/L. In separate runs, the effect of substance concentration and cell loading on extent of hydrolysis was studied. Our results revealed that from 0.2g/L to 2.0g/L, there was a corresponding increase in glucose concentration from 1.2mmol/L to 2.13mmol/L and 0.4mmol/L to 2.13mmol/L as Cs = 0.6g and 1.2g respectively as time increases. Similarly there was a substantial increase in glucose concentration with increase in cell loading. Hence the reaction was found to follow a simple rate constant, **k** as 0.238 and **n** equal to 1. Hence ($\Upsilon_{p(\mathbf{glucose})} = KC^{n}_{g}$); mmol/Litre.hr.

Hence the rate equation for glucose kinetics is

$$\Upsilon_{P(glucose)} = 0.238C^{n}_{g} \quad \text{------ (1)}$$

Where

Υ_p = rate

0.238 = rate constant

C = Concentration of species C
n = Order of reaction with respect to C

Keyword: Rate equation, Glucose, Kinetics, Trichodarma Reesei

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I. Introduction

In chemistry, the rate equation is an empirical differential mathematical expression for the reaction rate of a given reaction in terms of concentrations of chemical species and constant parameters only as in equation [1] above (**FAO-2004)**. Whereas, glucose is one of a group of carbohydrates known as simple sugar, it has the molecular formula $C_6 H_{12} O_6$. It is found in fruits and honey and is the major free sugar circulating in the blood of higher animals.

Integrated rate equations express the concentration of the reactants in chemical reactions as a function

of time. Therefore, such rate equation can be employed to check how long it would take for a given percentage of the reactants to be consumed in a chemical reaction.

The parameters in the rate equation are used for reactor design and analysis in Chemical Engineering as design tool.

Orange mesocap contains various carbohydrate polymers. Particularly its cellulose content between 30 – 60 %, which makes it an interesting choice for production of metabolites such as fermentable sugars and ethanol by appropriate micro–organisms. The naturally high degree of crystallinity due to the tightly packed crystallites in the cellulose in the orange mesocarp, causes entanglement of the lignin and hemicelluloses in the cellulose–matrix, thus leading to poor glucose yield. (Yabefa, etal; 2014).

Pretreatment is however required to break down the cellulosic complex structure to its simpler components i.e. Cellulose, hemicelluloses and lignin polymer, prior to their conversion to the sugar monomers. **(Ju, et al, 1999)**. It has been discovered that proper treatment of cellulose can change them from liabilities to assets **(Vilikari, et al 2001)**.

Trichoderma reesei has the capacity to secret large amount of cellulolytic enzymes (cellulases and hemicellulases) to glucose.

Recent studies in the biochemistry of cellulose enzymology reveals that the mecharism of cellulose hydrolysis, strain improvement, molecular cloning and process engineering are bringing

T. reesei celluloses closer to being a commercially viable route to cellulose hydrolysis. Major advances have been made in the isolation of *Trichoderma* mutants **(Yabefa, et al, 2014; Aderemi, et al; 2008).**

Novozyme reported studies on enzymatic hydrolysis of cellulose using *Trichoderma reesei* cellulase. Also enzyme and chelating agents in cotton pretreatment has been reported by **Emelia et al; (2001). Waag et al; (2005)** studied the efficient cellulose production from corn straw by *Trichoderma reesei*. Little information, however, exist in literature concerning the effect of substrate concentration and cell loading on hydrolysis of glycosidic bonds of orange mesocarp, with the empirical derivation of a rate equation.

Therefore, it is the goal of this research to study the conversion of chemically treated orange mesocarp to glucose using the cell of *Trichoderma reesei*, for the empirical derivation of a rate equation.

II. Materials and Method

Orange mesocarp was collected in particle sizes (P_1, P_2, P_3). These particle sizes were pretreated by three distinct pretreatment agents, sodium, hydroxide, ammonia and Calcium hydroxide. This was done to increase porosity (**Yabefa, et al (2014).** Applying the method used by **Yakubu et al; (2001);** 4g of orange mesocarp (OMP) was weighed, and pretreated at varying pretreatment conditions (0.1M, 0.2M, 0.3M and 0.4M), Sodium hydroxide, Calcium hydroxide at 100^{o}C and time (15, 20, 25 and 30 mins) in different runs.

Additionally, the ammonia seeping method too was employed to delignify the orange mesocarp. The delignification was treated with 0.3M Hcl acid at 100^{o}C for 1 hour to remove hemi celluloses. The pretreated cellulose was washed with de-ionized water to remove residual acid. All samples were dried in an oven at 50^{o}C for 48 hours and kept in the laboratory stock for further use.

In a typical run, the temperature of the water bath was set at 37^{o}C, a hundred millimeter (100ml) of 0.1M Sodium acetate buffer solution (P^H4.5) was poured into an Erlenmeyer flask filled with stirring mechanism 0.1g of isolated cellulase enzyme and 2.0g of pre-treated orange mesocarp of different particles sizes were added. 40 micro litres of each sample were withdrawn every 4hrs within 58–72hrs reaction time for analysis. The glucose concentration in the sample was determined by using Randox glucose kit and colorimeter (model W.PA, 5001, USA) at 540nm **(Chaudhuri et al; 2003).** Each run was repeated three times and the average was taken to assume accuracy. In separate runs, the effect of substrate concentration and cell loading on extent of hydrolysis was studied. A series of enzymatic hydrolysis was carried out at different substrate ‘concentrations of 0.2g/L, 0.4g/L, 0.6g/,L 0.8g/L, 1.0g/L, 1.2g/L, 1.4g/L, 1.6g/L, 1.8g/L, and 2.0g/L. While other reaction conditions were kept constant.

Additional series of enzymatic hydrolysis was carried out at different cell concentration of 0.01g/L, 0.02g/L, 0.03/g/L, 0.04g/L, 0.05g/L, 0.06g/L, 0.07g/L, 0.08g/L, 0.09g/L, and 0.10g/L other condition were identical to the normal hydrolysis condition. The results were analyzed for glucose concentration produced.

III. Results

Table 1.0: Effect of Substrate Concentration (g/l)

Glucose Concentration (mmo1/L)										
T(h)	0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00
Substrate Concentrating (g/l)										
O	O	O	O	O	O	O	O	O	O	O
4	1.7072	1.4938	1.2808	0.8536	1.4938	0.4268	0.2134	0.2134	0.2134	0.2134
8	1.9206	1,4938	1,9206	1,4938	1,9072	0.8536	1,4938	1.4938	1.0670	1.0670
10	1.9206	1,7072	1,9206	1,7072	1,7072	1,0670	1,4933	1,4938	1.0670	1,0670
24	1.9206	1,9206	2,1340	1,9206	1,9206	2,1340	1,9206	1,7072	2,1349	1,7092
28	1.9206	1,9206	2,1340	1,9206	1,9206	2,1340	1,9206	1,9206	2,1340	1,9206
30	1,7086	1,0906	2,1340	1,9206	1,9206	2,1340	1,9205	1,9206	2,1340	2,1340
34	1,7076	1,7072	2,1340	1,7072	1,9206	2,1340	1,0906	2,134	2,1340	2,1340
48	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	2,134	1.7072	2,1340
52	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	2.1340	1.7072	2,1340
58	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	2.1340	1.7072	2,1340
72	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	1,7076	2.1340	1.7072	2,1340

Substrate $\leq$ 2g/L

Enzyme = 0.1g/L

Tempt = 37^{o}C

P^{H} = 4.5

IV. Results and Discussion Effects of Substrate Concentration:

The results are presented in the Table 1.0. From the results of effect of substrate concentration, it is deduced that as the substrate concentration increases from 0.2g/L to 2.0g/L; there was a corresponding increase in glucose concentration from 1.2 mmo1/L to 2.13mmol and 0.4 mmo1/L to 2.13mmo1/L at Cs= 0.6g and 1.2g respectively as time increase. This trend is not unusual because similar results were reported on the hydrolysis of rice straw using *Aspergiluis niger*, animal manure, soft wood, weeds and bagase **(Wen; et al; 2004).** This can be further explained from the point of view, that increasing the substrate concentration without a corresponding increase in enzyme concentration amounts to availabity of more cellulose in the bioreactor for hydrolysis.

Thus, the activity of endoglucanase and cellobio hydrolase will be reduced since there is more cellulose for it to hydrolyze. Also there will be sugar depletion from the substrate into the medium and possible use of glucose by fungi to supply its internal energy (metabolic) requirement (Lee, J.M; 1992).

Table 2.0: Effect of Cell Loading (g/l)

Glucose Concentration (mmo1/L)										
Cell loading (G/L)	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
0	0	0	0	0	0	0	0	0	0	0
4	1.7072	1.0670	1.9206	1.7072	1.2804	2.1340	1.4938	1.2804	1.4938	1.4938
8	1.9206	1.4934	2.1340	1.9206	1.4938	2.1340	1.7072	1.4938	1.7072	1.7072
10	2.3474	2.3474	2.5608	1.4938	1.4938	1.9206	1.7072	1.4938	2.3474	1.7072
24	2.3474	1.9206	1.7072	1.7072	1.7072	1.7072	1.7072	1.4938	2.5608	2.3474
28	1.7072	1.9206	1.7072	1.9206	1.7072	1.7072	1.9206	1.7072	2.5608	2.5608
30	1.7072	1.9206	1.7072	1.9206	1.7072	1.7072	1.9206	1.7072	2.3474	2.5608
34	1.7072	1.9206	1.9206	1.9206	1.7072	1.7072	1.9206	1.7072	2.3474	2.5608
48	1.7072	1.4938	1.9206	1.9206	1.7072	1.7072	1.9206	1.7072	2.3474	2.5608
52	1.7072	1.4938	1.9206	1.9206	1.7072	1.7072	1.9206	1.7072	2.3474	2.5608
58	1.7072	1.4938	1.9206	1.9206	1.7072	1.7072	1.9206	1.7072	2.3474	2.5608
72	1.7072	1.4938	1.9206	1.9206	1.7072	1.7072	1.9206	1.7072	2.3474	2.5608

Cell = 0.1g/L
Substrate = 1g/L
Tempt = $37^{o}C$
P^{H} = 4.5

V. Effect of Cell Loading

Cellulose concentration in the degration of orange mesocarp by *T. reesei* studied under different cell loading and at fixed substrate concentration. The result is presented in Table 2.0. The deductions from these results are as follows: For each cell loading, there is an increase in glucose concentration. That is, there was a substantial increase in glucose concentration with increase loading. Similar results on bioconversion of forest product for ethanol production have been reported (Chapma, et al; 2000). An exponential growth phase (the progressive doubling of cell numbers) resulting in a continually increasing rate of growth in the population (**Abia, et al; 2002)** is seen from Table 2.0.

VI. Rate Equation Determination

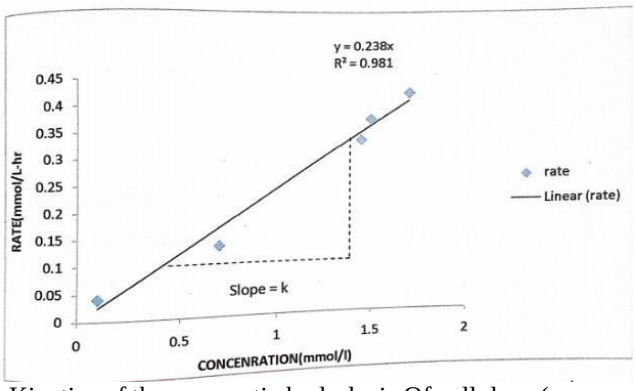

Fig I: Kinetics of the enzymatic hydrolysis Of cellulose (orange mesocarp)

Using the differential method in determining the reaction rate constant, for **Figure I** with a good fit of R–Squared = 0.981, the kinetics of the reaction can be described as first order with a rate constant K = 0.238. This means that the rate equation is consistent with the data. Hence the rate equation is given as

- $\Upsilon_A = \frac{KC_A}{dt} = \frac{KCp}{dt} = KC_A^n$

Therefore the rate of glucose formation is

- $\boxed{\Upsilon_{P\,(glucose)} = 0.238C^n{}_g}$ mmo1/L.hr)

Where C_g = concentration of glucose and
K = 0.238/hr is the first order reaction rate constant

VII. Validation of Result

From **Figure II,** the model rates were predicted from the first order rate equation of glucose production, while the data rates are the experimental values. From the **Figure II,** it shows that the predicted rate values and the experimental rate values are consistent. Therefore the rate equation and kinetic parameters are useful in engineering, design and analysis.

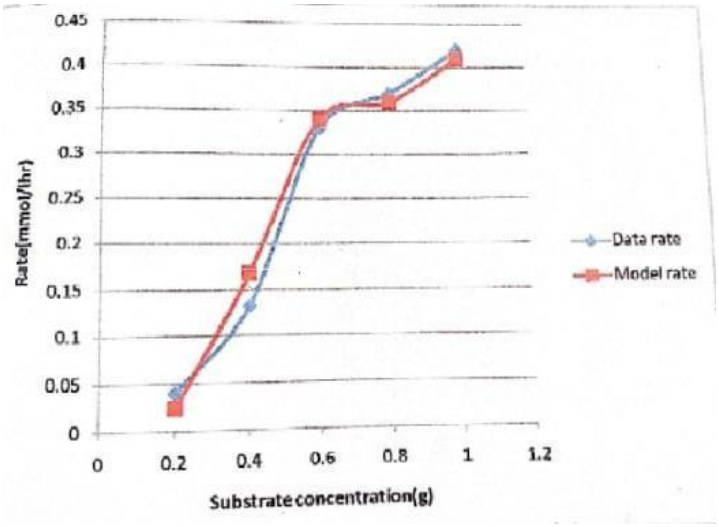

Fig II: Comparism between Experimental and Predicted Values

Table 3.0: Data Rate and model Predicted Rate.

Glucose Concentration	0.10	0.70	1.45	1.50	1.70
Data Rate	0.04	0.13	0.033	0.37	0.42
Model Rate	0.02	0.16	0.34	0.36	0.41
Substrate Concentration	0.20	0.40	0.60	0.80	1.00

Explanation of T-test

From the analysis in Table 3.0; T-test shows an acceptable result (0.986≥0.05) meaning P- Calculated is greater than P-alpha level of significance. Therefore, there is no significant difference between the data (experimental) rate and the model predicted rate. Therefore, the data is useful and the rate equation can be used to predict the extent of reaction at a particular time.

VIII. Conclusion

Therefore, in conclusion, as substrate concentration increases from 0.2 g/L to 2g/L, there was a corresponding increase in glucose concentration from 1.7mmo1/L to 2.3mmol/L. It was also observed as well that, as the substrate concentration increases at a constant (fixed cell concentration, there was a corresponding increase in glucose concentration.

Hence the rate equation for glucose kinetics is first order and represented as:

$\Upsilon_{P\,(glucose)} = 0.238C_g$ mmo1/Litre.hr.

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