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Estimation of Kinetic Parameters for Enzyme Catalysed Batch Bioreactor for the Production of Ethanol from Corn

Z. R. Yelebe*, R. J. Samuel** and B. Z. Yelebe***
Department of Chemical Engineering, Niger Delta University, Bayelsa State Nigeria

ABSTRACT
This paper addresses the challenge of estimating various kinetic parameters for the design of an optimized enzyme catalysed batch bioreactor of high efficiency and yield. Mathematical models were developed to describe the batch reaction time in relation to the substrate, enzyme and product concentration. The results obtained from the plots generated were: 35.50gmol/l.hr for the velocity of reaction of the enzymes (V_{max}), 0.10049hr^{-1}for the maximum specific growth rate (μ_{max}) 826.45gmol/l for the Michaelis-Menten constant (K_m), 0.005402577 for maintenance coefficient (M_s), 10.104kgCx/kgCs for yield of cell weight per unit weight of substrate ($Yc_{x/CS}$), 0.05436kgCp/kgCs for yield of product weight per unit weight of substrate utilized ($Yc_{p/CS}$) and 0.01416 for endogenous decay coefficient (K_d) for the design of the batch biochemical reactor. Hence, they are useful parameters for predicting the most appropriate batch reaction conditions and the efficiency of the bioreactor. The mathematical model predictions showed that it can be considered as a good complimentary tool to real system since the simulation results of the mathematical model agrees with experimental data reported in literature.

Keywords **-** Batch Bioreactor, Enzyme, Ethanol, kinetic parameters

I. INTRODUCTION

The production of ethanol is an aged long process that has been in practice for some decades. Irrespective of this fact we are still faced with the problem of designing and installing processes for ethanol production that will give a more efficient yield. As such, we are faced with the task of designing an optimized enzyme catalysed batch bioreactor of high efficiency and yield. To ensure a process of optimum capacity and product yield, the estimation of kinetic parameters for the prediction of such operating conditions must be established. This will improve the rate of ethanol production in the world at large.

The various kinetic parameters to be estimated for the design of an optimized enzyme catalysed batch bioreactor are:

- the maximum rate or velocity of reaction of the enzymes (V_{max}),
- Michaelis-Menten constant (K_m),
- maximum specific growth rate (μ_{max}),
- yield of cell weight per unit weight of substrate ($Yc_{x/CS}$),
- maintenance coefficient (M_s),
- yield of product weight per unit weight of substrate utilized ($Yc_{p/CS}$) and
- endogenous decay coefficient (K_d)

Hisayori et al [1], worked on the direct production of ethanol from raw com starch via fermentation by the use of "Novel surface engineered yeast strain displaying glucoamylase and amylase". Nkechi [2] also worked on the production of ethanol from high yeast molasses gotten from the shredded sugarcane juice. Yeast was used as a source for enzyme used the fermentation was maintained at a pH of 5.3 the end result showed that an ethanol yield concentration 8-10% was produced. Nnnachi [3] also worked on the process synthesis for the industrial production of ethanol from cassava. He used yeast under an anaerobic condition and controlled the pH within the range of 4.5 in conclusion he was able to produce ethanol of concentration 8-12%.

In this fermentation process, enzymes act as the major catalytic agent in the process, the enzymes does not reproduce itself in the process but acts as an ordinary chemical agent. The decomposition of raw organic feed with enzymes acting as the catalyst yields a chemical product. Enzyme fermentation process is given below by the reaction.

(Raw organic feed) $\xrightarrow{Enzyme\ Acting\ as\ Catalyst}$ (Chemical product)

Fermentation occurs in two different stages. There is a primary fermentation stage and a

secondary fermentation stage. During the primary fermentation stage, which lasts about two to three days, 70% of the fermentation activity will occur. Rapid fermentation and growth occurs, resulting in a considerable amount of foaming. To remove the excess carbon dioxide produced, a fermentation lock is implemented allowing for a flow of gas out of the system while preventing an inward flow. Usually during this stage, the bioreactor (fermenter) is in an aerobic environment that allows for the growth of the east cells. As most of the fermentation activity occurs, a lot of yeasts energy is put into reproduction. Yeast cells are capable of multiply my by 100 to 200 times during the first few days alone.

While the secondary stage can last from one to two weeks, depending on the availability of nutrients. During this time the remaining 30% of fermentation activity occurs. This stage normally takes place in an anaerobic environment that results to a slower reaction, as the lack of oxygen forces the yeast to use its energy for alcohol production instead of reproduction

The relevance of this study is tied to the importance of the preferred choice of feedstock (corn) to be used and as well as the uses and importance of the product (ethanol). This study shows the need for large scale com production because the usefulness of com goes beyond its consumption as food, because that it can also be used for the manufacture of a relevant chemical of great importance and uses to man.

Ethanol serves as a solvent for the manufacture of paints, drugs, perfumes, dyes, gums and as a fuel in cars, spirit lamps and store [4]. It is used in the preparation of a large number of organic compounds like ester and as a solvent for sterilization of clinical and laboratory apparatus. It is also used as a preventative for biological specimens and as an intoxicating agent in alcoholic beverages and drinks [5].

II. MODEL DEVELOPMENT FOR KINETIC PARAMETERS

2.1 The Model Assumptions

The mathematical models that describe the batch bio-reactor are developed based on the following assumptions.

(a) There is no mass flow of material in or out of the reactor.
(b) The reaction of the reacting species changes with time.
(c) The reactor is well mixed and there is no spatial variation within the reactor volume.
(d) For most liquid-phase reactions, the density change with the reaction is usually small and can be neglected (i.e. $V=V_0$).
(e) The volume is constant i.e. ($V=V_0$) since it is a closed metal system.
(f) The batch-bioreactor is operated isothermally as most fermentation processes are carried out at either room temperatures or temperatures slightly above room temperatures.
(g) The work term is negligible and the specific heat capacity is constant.
(h) The batch bio-reactor is designed to be a cylindrical vessel with height 50cm (500mm), and diameter 30cm (300mm) since the design is based on a small scale laboratory setup. But the vessel will still give appreciable output and will not occupy much space when fully installed.

Volume of the reactor (V_R) = $\pi r^2 h$
= (3.142 x 15^2 x50) cm^3
= 35, 347.5cm^3

2.2 Modeling of the Enzyme Kinetics in the Batch Bioreactor

The enzyme kinetics in the batch bioreactor is described by two models, the "Michaelis-Menten" model [6] and the "Briggs-Haldane" model [7].

The Michaelis-Menten equation represents the kinetics of many simple enzyme-catalyzed reactions which involves a single substrate.

Also, we have that the concentration of the substrate will always have an effect on the reaction rate ($-r_s$), according to simple Michaelis-Menten Kinetics:

$$(-r_S) = \frac{V_{\max} C_s}{K_m + C_s} \qquad \text{----------} \quad (1)$$

The Briggs-Haldane model is a mathematical description of enzymatic kinetic reaction based on the assumption that, after a short initial startup period, the concentration of the enzyme-substrate complex is in a pseudo-steady state. For a constant volume batch bioreactor operating isothermally the material balance is:

$$(+r_p) = \frac{V_{\max} C_S}{K_m + C_S} \qquad \text{----------} \quad (2)$$

For a constant volume batch bioreactor, combining equations (18) and (19) give a form of an equation that can be linearized to give:

$$\frac{1}{t}\ln\left[\frac{C_{so}}{C_s}\right]=\frac{V_{\max}}{K_m}-\frac{1}{K_m}\left[\frac{C_{so}-C_s}{t}\right] \quad \text{----- (3)}$$

Equation (3) shows $\frac{1}{t}\ln\left[\frac{C_{so}}{C_s}\right]$ as a linear function of $\frac{(C_{so}-C_s)}{t}$. The parameters K_m and V_{max} can be estimated from equation (3), using measured values of C_s as a function of t for a given C_{so}.

Equating (1) and (2) gives:

$$\left(+r_p\right)=\left(-r_s\right)=\frac{dC_s}{dt}=\frac{V_{\max}C_s}{K_m+C_s}\cdot \quad \text{(4)}$$

Re-arranging equation (4) we have;

$$\left(K_m+C_s\right)\frac{dC_s}{dt}=-V_{\max}dt \quad \text{---------- (5)}$$

$$-K_m\frac{dC_s}{C_s}-C_s\frac{dC_s}{C_s}=V_{\max}dt \quad \text{------- (6)}$$

Integrating equation (6) with boundary conditions $C_s = C_{so}$ and t = 0.

$$-K_m\int_{C_{so}}^{C_s}\frac{dC_s}{C_s}-\int_{C_{so}}^{C_s}dC_s=V_{\max}\int_o^t dt \quad \text{---------- (7)}$$

$$-K_m\ln\left[\frac{C_s}{C_{so}}\right]-\left(C_s-C_{so}\right)=V_{\max}dt \quad \text{--------- (8)}$$

Thus re-arranging we have;

$$\frac{1}{t}\ln\left[\frac{C_{so}}{C_s}\right]=\frac{V_{\max}}{K_m}-\frac{1}{K_m}\left[\frac{C_{so}-C_s}{t}\right] \quad \text{--------- (9)}$$

From equation (9), V_{max} and K_m can be obtained by plotting a graph of $\frac{1}{t}\ln\left[\frac{C_{so}}{C_s}\right]$ against $\frac{(C_{so}-C_s)}{t}$ to give K_m as the slope and V_{max} as the intercept

From Fogler [8]:

$$\frac{dC_x}{dt}=\frac{dC_x}{dC_s}\circ\frac{dC_s}{dt} \quad \text{---------- (10)}$$

Where $\frac{dC_x}{dC_s}=-Y_{x/s}$

$$\frac{dC_x}{dt}=Y_{x/s}\cdot\frac{dC_s}{dt}\Rightarrow rc_x=-Y_{x/s}\cdot rc_s \quad \text{--(11)}$$

$$rc_s=\frac{rc_x}{-Y_{x/s}}\Rightarrow\frac{1}{-Y_{x/s}}\cdot rc_x \quad \text{---(12)}$$

Adding the maintenance coefficient of the system we have:

$$rc_s=\frac{1}{-Y_{x/s}}\cdot rc_x+\frac{Ms}{c_x} \quad \text{---------- (13)}$$

Dividing through by C_x

$$\frac{rc_s}{c_x}=\frac{1}{-Y_{x/s}}\cdot\frac{rc_x}{c_x}+Ms \quad \text{----------(14)}$$

$$\frac{rc_s}{c_x}=M_s-\frac{1}{Y_{x/s}}\cdot\frac{rc_x}{c_x} \quad \text{----------(15)}$$

From equation (15), $Yc_{x/CS}$ can be obtained when a graph of rC_s/C_x is plotted against rC_x/C_x to give $Y_{x/S}$ as the slope of the plot, and M_s as the intercept.

$$\frac{dC_P}{dt}=\frac{dC_P}{dC_S}\cdot\frac{dC_S}{dt} \quad \text{----------(16)}$$

$$where\ \frac{dC_P}{dC_S}=-Y_{P/S}$$

$$\frac{dC_P}{dt}=-Y_{P/S}\cdot\frac{dC_S}{dt} \quad \text{----------(17)}$$

$$rc_P=-Y_{P/S}\cdot rc_S$$

$$rc_S=\frac{rc_P}{-Y_{P/S}}\Rightarrow\frac{1}{-Y_{P/S}}\cdot rc_P \quad \text{----------(18)}$$

Adding the maintenance coefficient of the system we have,

$$rc_S=\frac{1}{-Y_{P/S}}\cdot rc_P+\frac{M_S}{c_P} \quad \text{---------- (19)}$$

Dividing through by C_p

$$\frac{rc_S}{c_P}=\frac{1}{-Y_{P/S}}\cdot\frac{rc_P}{c_P}+M_S \quad \text{----------(20)}$$

$$\frac{rc_s}{cp}=M_s-\frac{1}{Y_{P/s}}\cdot\frac{rc_P}{c_P} \quad \text{----------(21)}$$

From equation (21), $Yc_{p/CS}$ can be obtained when a graph of rC_X/C_p is plotted against rC_p/C_p to give as the slope of the plot and M_S the intercept.

From Coker [9]:

$$\frac{dC_x}{dt} = \frac{\mu_{max} \cdot C_s \cdot C_X}{K_s + C_s} \quad \text{---------- (22)}$$

But $rc_x = \frac{dC_x}{dt}$

$$rc_x = \frac{\mu_{max} \cdot C_s \cdot C_x}{K_s + C_s} \Rightarrow \frac{rc_x}{C_x} = \frac{\mu \cdot C_s}{K_s + C_s} \quad .....(23)$$

$$\frac{rc_x}{C_x} = \frac{1}{\mu} = \frac{K_s + C_s}{\mu_{max} \cdot C_s} \quad \text{----------(24)}$$

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max} C_s} + \frac{C_s}{\mu_{max} C_s} \quad \text{----------(25)}$$

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \cdot \frac{1}{C_s} + \frac{1}{\mu_{max}} \quad \text{----------(26)}$$

$$\therefore \frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_s}{\mu_{max}} \cdot \frac{1}{C_s} \quad \text{----------(27)}$$

From equation (27), μ_{max} can be obtained by plotting a graph of $1/\mu$ against $1/C_s$ to give μ_{max} as the slope of the plot. (Line weaver-Burke plot) [6].

III. RESULTS AND DISCUSSION

The purpose of characterization of the kinetic parameters is to identify the most suitable operating conditions of the batch bioreactor based on the underlying assumptions. However, the data are best obtained experimentally, but in the absence of experiment, such data can also be estimation from plot generated using the models developed. Therefore, the kinetic data are obtained from the mathematical models developed for the fermentation of corn starch into ethanol in a batch system using graphically analysis.

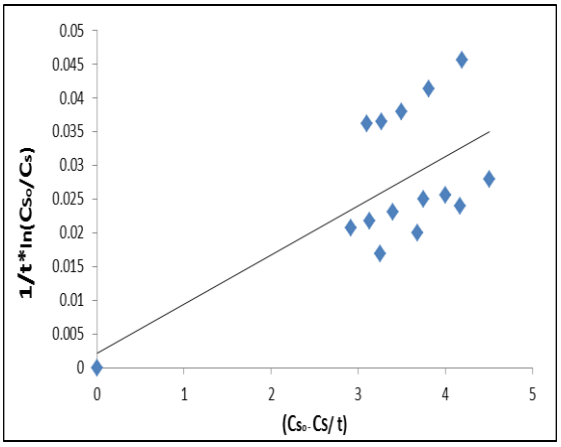

Fig. 1: A plot for the estimation of K_m and V_{max}

Fig. 1 shows a plot for the estimation of the maximum rate or velocity of reaction of the enzymes (V_{max}) and the Michaelis-Menten constant (K_m). These are important kinetic parameters for the estimation of substrate conversion or rate of decomposition per unit time is the batch bioreactor. The velocity of reaction of the enzymes (V_{max}) and the Michaelis-Menten constant (K_m) calculated from slope and intercept of the plot. The estimated values were 35.50gmol/l.hr for the velocity of reaction of the enzymes (V_{max}) and 826.45gmol/l for the Michaelis-Menten constant (K_m).

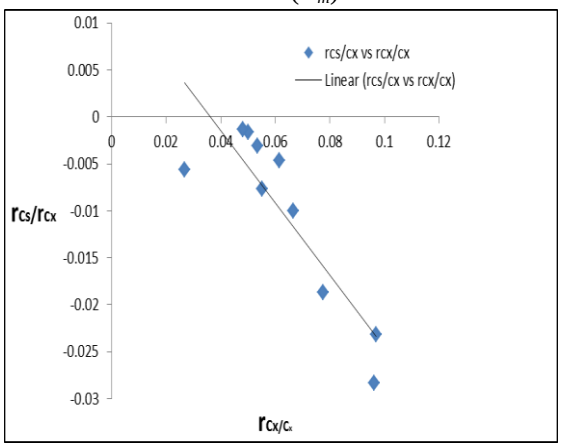

Fig. 2: A plot for the estimation of $Y_{Cx/Cs}$ and M_s

Fig. 2 shows a plot of the rate substrate concentration per unit enzyme concentration versus the rate of enzyme concentration per unit substrate utilized. The yield of cell weight per unit weight of substrate utilized ($Y_{Cx/Cs}$) and maintenance coefficient (M_s) are estimated from the plot as slope and intercept respectively. The result obtained from the calculation was 10.104 kgCp/kgCs and 0.005402577 respectively.

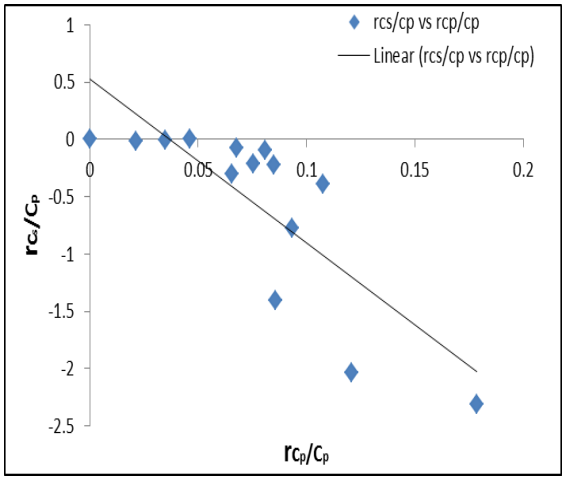

Fig. 3: A plot for the estimation of $Y_{Cp/Cs}$ and M_s

Fig. 3 shows a plot of the rate substrate concentration per unit product concentration versus the rate of product concentration per unit product converted. The yield of product weight per unit weight of substrate utilized ($Y_{Cp/Cs}$) and maintenance coefficient (M_s) are estimated from the plot as slope and intercept respectively. The result obtained from the calculation was 0.05436 kgCp/kgCs and 0.005402577 respectively.

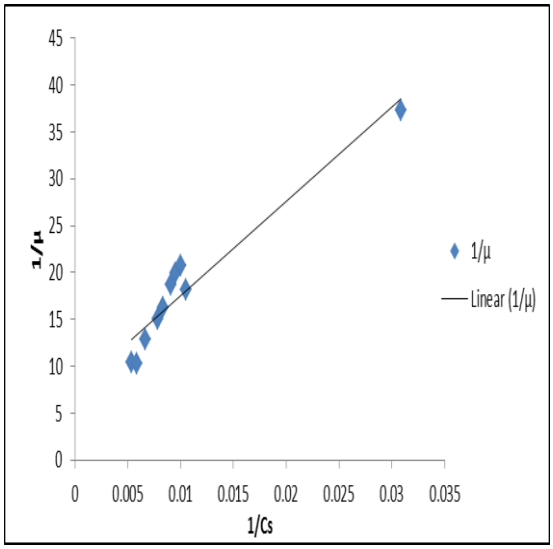

Fig. 4: A plot for the estimation of μ_{max}

Fig. 4 shows a plot of the enzymatic conversion of the substrate into product. The maximum specific growth rate of the enzymes (μ_{max}) increases with corresponding increase in substrate concentration. The estimated value of μ_{max} from the slope of the graph is 0.10049hr^{-1}.

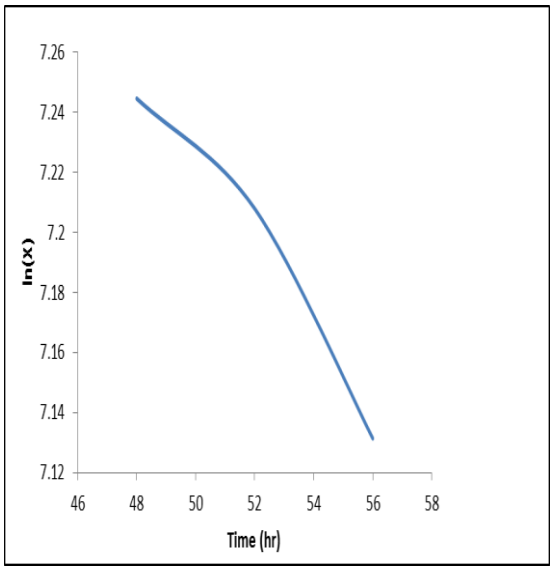

Fig. 5: A plot for the estimation of K_d

Fig. 5 shows a plot of the decay stage of the microbes (48–56hrs). The enzymes starts dying after 48hrs of fermentation which gives a plot such that the value of the endogenous decay constant (K_d) can be calculated as the slope of the curve. The endogenous decay constant (K_d) was estimated to be –0.01416hr^{-1} from the plot.

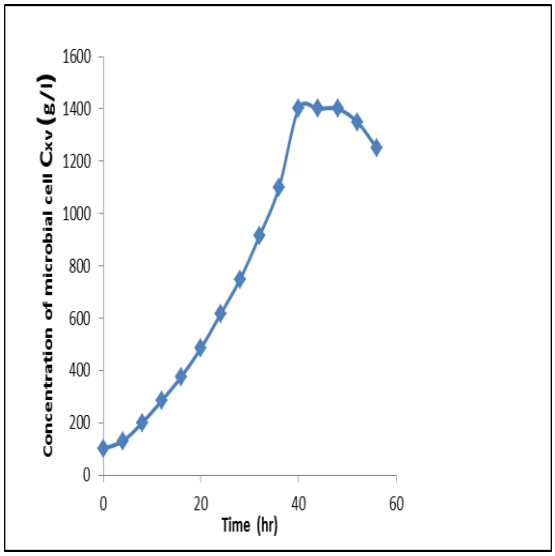

Fig. 6: A plot of microbial cell growth versus time

Fig. 6 describes microbial cell growth, where at time (t=0 – t=4) the lag phase exist i.e. a phase in which the microbial cells learn to adapt to their new environment. From time (t=4 – t=40) shows the exponential growth phase i.e. a phase where the microbial cells starts to feed on the nutrients and hence grow exponentially. Time (t=40 –t=48) shows the stationary phase i.e. a phase where the microbial cells stops to grow due to depletion of the nutrients; this stage is where a large percentage of most fermentation products are formed. Finally time (t=48 to t=56) shows the death phase i.e. where most of the microbial cells starts to die due to no more nutrients in the system.

Table 1 shows a summary of the estimated kinetic parameters.

Table 1: Estimated Kinetic Parameters

PARAMETER	DESCRIPTION	VALUE
V_{max}	Maximum rate or velocity of reaction of the enzymes	$35.50(\frac{gmol}{l.hr})$
K_m	Michaelis-Menten	826.45

	Constant	$gmol/l$ ( )
μ_{max}	Maximum specific growth rate	0.10049 (hr^{-1})
K_d	Endogenous decay coefficient	0.01416 (hr^{-1})
$Yc_{x/cS}$	Yield of cell weight per unit weight of substrate utilized	$10.104 \frac{kg\ Cx}{kg\ Cs}$
M_s	Maintenance coefficient	0.005402577
$Yc_{p/CS}$	Yield of product weight per unit weight of substrate utilized	0.05436 kg Cp/kg Cs

IV. CONCLUSION

This paper covers the characterization of various kinetic parameters for the design of an optimized enzyme catalysed batch bioreactor. The predicted values of various kinetic parameters for the optimum operating conditions of the batch conversion process of substrate (corn) into product (ethanol) showed that; the maximum rate or velocity of reaction (V_{max}) of the enzyme activity promotes a quick and rapid conversion of substrate into product, and the maximum specific growth rate (μ_{max}) of the microbial cells rapidly increases the concentration of the microbial cells at a faster rate and vice versa. The Michaelis-Menten constant (K_m) enhances the conversion rate of substrate. The endogenous decay constant (K_d) predicts the microbial decay rate after decomposition of substrate. Thus, it can be concluded that the parameters V_{max}, μ_{max}, K_m and K_d are key factors in the design of a batch biochemical reactor, because they are useful for predicting the most appropriate batch reaction conditions and the efficiency of the bioreactor.

The results obtained from the plots generated were: 35.50gmol/l.hr for the velocity of reaction of the enzymes (V_{max}), 0.10049$hr^{-1}$for the maximum specific growth rate (μ_{max}) 826.45gmol/l for the Michaelis-Menten constant (K_m), 0.005402577 for maintenance coefficient (M_s), 10.104kgCx/kgCs for yield of cell weight per unit weight of substrate ($Yc_{x/CS}$), 0.05436kgCp/kgCs for yield of product weight per unit weight of substrate utilized ($Yc_{p/CS}$) and 0.01416 for endogenous decay coefficient (K_d) for the design of the batch biochemical reactor.

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