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Design of an Optimized Enzyme Catalysed Batch Bioreactor for the Production of Ethanol from Corn

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Abstract: - This paper addresses the challenge of designing an optimized enzyme catalysed batch bioreactor of high efficiency and yield for the production ethanol from raw corn starch. Mathematical models developed predicted the batch reaction time of 25, 25 and 41hrs in relation to the substrate, enzyme and product concentration respectively compared with experimental batch time of 56hrs in all cases. The results obtained also showed that the velocity of reaction of the enzymes (V_{max}) and the maximum specific growth rate (μ_{max}) are key parameters in the design of the batch bioreactor with higher values of each predicting shorter batch reaction time profile. Hence, they are useful 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, Corn, Enzyme, Ethanol, Fermentation*

I. INTRODUCTION

Ethanol production 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 that the process that is to be designed will be efficient in terms of capacity and product yield the reactor will be stirred. This will improve the rate of ethanol production in the world at large.

Hisayoriet 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%. Brink [4] also explored ethanol production from cotton gin waste based on approximation of the composition of the cotton plant. He developed a general design for 2-4million gallons per year ethanol production plant. The idealized design considered simultaneous methane production, as well as avenues for recycling energy. The general outlook for cotton gin waste usage that he presented is very optimistic.

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 showsthe 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.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. DEVELOPMENT OF MATHEMATICAL MODEL

The mathematical model for the batch bioreactor is developed based on the general mole balance design equation for reactors, which gives that the rate of mass inflow of reacting specie minus the rate of mass outflow of the reacting specie plus rate of generation of specie by chemical or biochemical reaction within the system is equal to rate of accumulation of mass within the system.A mole balance on specie A at any point in time "t" yields the following equation [6]:

Rate of mass Inflow of A into the system (moles/time)	+	Rate of generation of A by chemical or biochemical reaction within the system (moles/time)	−	Rate of mass outflow of A from the system (moles/time)	=	Rate of Accumulation within the system (moles/time)

Inflow + generation – outflow = Accumulation

$$F_{A0} + G_A - F_A = \frac{dN_A}{dt} \quad \text{---------- (1)}$$

Where N_A represents the number of moles of A in the system

The total rate of generation within the system volume is then sum of all the rates of generation in each of the sub-volumes.By taking appropriate limits and also making use of the definition of an integral, we can write the following equation for the total rate of generation:

$$G_A = \int_O^V r_A d_V \quad \text{---------- (2)}$$

From this equation, we see that r_A is in an indirect function, since the properties of the reacting materials (e.g. concentration, temperature) can have different values at different locations in the reactor.So then, rearranging equation (1) and replacing G_A into it with now yield:

$$F_{Ao} - F_A + \int_o^v - r_A d_V = \frac{dN_A}{dt} \quad \text{---------- (3)}$$

Equation (3) is therefore the general mole balance equation from which we can develop the design equations for various types of industrial or laboratory scale reactors. The batch time and reactor volume (continuous flow) necessary to convert a specified amount of the reactants into products can also be determined from it. But for the purpose of this work we are only interested in developing models that describe the design of an enzyme catalyzed batch bioreactor.

2.1 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 MODEL EQUATIONS

C_X
C_S
$C_P V$

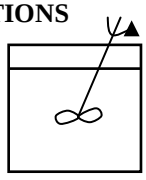

Fig. 1: Flow sheet for a batch bioreactor

General Mass Balance:

$F_{A0} - F_A + G_A - C_A = \frac{dN_A}{dt}$ ---------- (4)

Where:

F_{A0}= Mass inflow of reacting specie into the system.

F_A = Mass outflow of reacting specie out of the reactor.

G_A = Rate of generation of specie within the system.

C_A = Rate of consumption of specie within the system.

$\frac{dN_A}{dt}$ = Rate of accumulation of mass within the system per unit time.

Thus, applying the underlying assumptions, and integrating gives the batch time (t_b) required for enzymatic conversion of substrate into product in the batch bio-reactor as:

$$t_b = \frac{K_m}{V_{\max}} ln \frac{C_{so}}{C_s} + \frac{C_{so} - C_s}{V_{\max}} \quad \text{---------- (5)}$$

However, enzymes are subject to deactivation, thus the concentration of active enzyme in the reactor, and therefore the value of V_{max} may change during reaction when deactivation is significant, variation of V_{max} with time can be expressed by:

$V_{max} = V_{max0}e^{-kdt}$ ---------- (6)

Thus, equation (5) becomes:

$$t_b = -\frac{1}{kd} \ln \left[1 - Kd \left(\frac{K_m}{V_{\max 0}} \ln \frac{C_{so}}{C_s} + \frac{C_{so} - C_s}{V_{\max 0}} \right) \right] \quad \text{---------- (7)}$$

Similarly, we can also carry out a mass balance on the cell culture in the batch bioreactor. Starting from inoculums, C_{xo} at t=0, and an initial quantity of limiting substrate C_{so} at t = 0, the biomass will grow after a short lag phase and will consume substrate. The growth rate slows as the substrate concentration decreases and becomes zero when all the substrate has been consumed.

Based on the model assumptions, we can write mass balance for the constant volume zero feed batch fermentation, using the generalized mass balance equation:

$$\frac{1}{V} \frac{d(y.v)}{dt} = \Sigma r_{gen} - \Sigma r_{con} + Dy_i - rDy \quad \text{---------- (8)}$$

Where y is a general extensive property

Therefore the mass balance equations for the state variables (viable cells, non-viable cells, substrate and product) are given below as follows [7]:

Viable cells: $\frac{dc_{xv}}{dt} = \mu C_{xv} - K_d C_{xv}$ ---------- (9)

Non-viable cells: $\frac{dc_{xd}}{dt} = K_d C_{xv}$ ---------- (10)

Substrate: $\frac{dC_s}{dt} = -\left\{ \frac{\mu C_{xv}}{Y_{x/s}} + M_s C_{xv} + \frac{\alpha \mu C_{xv} + \beta C_{xv}}{Y_{p/s}} \right\}$ ---------- (11)

Product: $\frac{dC_p}{dt} = \alpha \mu C_{xv} + \beta C_{xv}$ ---------- (12)

Including a set of initial conditions at the time of inoculation, the profiles of these variables with time can be determined by integration to yield the following equations for viable cells, substrate and product respectively:

$$t_b = \frac{1}{\mu_{\max}} Ln \left(\frac{C_{xv}}{C_{xvo}} \right) \quad \text{---------- (13)}$$

$$t_b = \frac{1}{\mu_{\max}} Ln \left\{ 1 + \frac{C_{so} - C_s}{\left(\frac{1}{Y_{x/s}} + \frac{M_s}{\mu_{\max}} \right) C_{xvo}} \right\} \quad \text{----------(14)}$$

$$t_b = \frac{1}{\mu_{\max}} Ln \left[\left(1 + \frac{\mu_{\max}}{q_p C_{xvo}} \right) (C_p - C_{po}) \right] \quad \text{---------- (15)}$$

Therefore the total downtime (t_{dn}) and operation time T_t are given below as:

$t_{dn} = t_p + t_1 + t_{hv}$ ----------(16)

and

$T_t = t_b + t_{dn}$ ----------(17)

Where; t_b is the time required for enzymatic conversion or the time required to achieve a given microbial cell concentration, substrate concentration and product concentrations in a batch culture or system.
t_{hv}isthe time taken to harvest the contents inside the batch bioreactor.
t_p is the time needed to clean, sterilize and otherwise prepare the bioreactor for the next operation.
t_1 isa lag time of duration that occurs after inoculation during there is no growth or product formation

2.3 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 [8] and the "Briggs-Haldane" model [9].
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:

$$(-r_S) = \frac{V_{\max} C_s}{K_m + C_s} \qquad \text{---------- (18)}$$

TheBriggs-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 isothermallythe material balance is:

$$(+r_p) = \frac{V_{\max} C_S}{K_m + C_S} \qquad \text{----------(19)}$$

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

$$\frac{1}{t} Ln\left[\frac{C_{so}}{C_s}\right] = \frac{V_{\max}}{K_m} - \frac{1}{K_m}\left[\frac{C_{so} - C_s}{t}\right] \qquad \text{---------- (20)}$$

Equation (20) 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 (20), using measured values of C_s as a function of t for a given C_{so}.

III. RESULTS AND DISCUSSION

The purpose of simulation is to make a comparison with real experimental data presented for a real process and check the adequacy of the models and the underlying assumptions. However, the data are best obtained experimentally, but in the absence of experiment, such data can also be gotten from Journals and literatures. Therefore, the kinetic data for simulation and validation of the mathematical modelsfor the fermentation of corn starch into ethanol in a batch system were obtained from Manikandan et.al [10].The values of the parameters used to simulate the mathematical models are presented in Table 1.

Table 1:Parameters for Model Validation

PARAMETER	DESCRIPTION	VALUE	SOURCE
V_{max}	Maximum rate or velocity of reaction of the enzymes	35.50($\frac{gmol}{l.hr}$)	Calculated
K_m	Michaelis-Menten Constant	826.45 ($^{gmol}/_{l}$)	Calculated
α	Growth related product formation coefficient	2.67$\frac{g\ product}{g\ of\ biomass\ -hr}$	[10]
β	Non-growth related product formation coefficient.	0.062$\frac{g\ product}{g\ biomass\ -hr}$	[10]
μ_{max}	Maximum specific growth rate	0.10049 (hr^{-1})	Calculated
K_d	Endogenous decay coefficient	0.01416 (hr^{-1})	Calculated
$Yc_{x/cS}$	Yield of cell weight per unit weight of substrate utilized	10.104$\frac{kg\ Cx}{kg\ Cs}$	Calculated
M_s	Maintenance coefficient	0.005402577	Calculated
$Yc_{p/CS}$	Yield of product weight per unit weight of substrate utilized	0.05436 kg Cp/kg Cs	Calculated

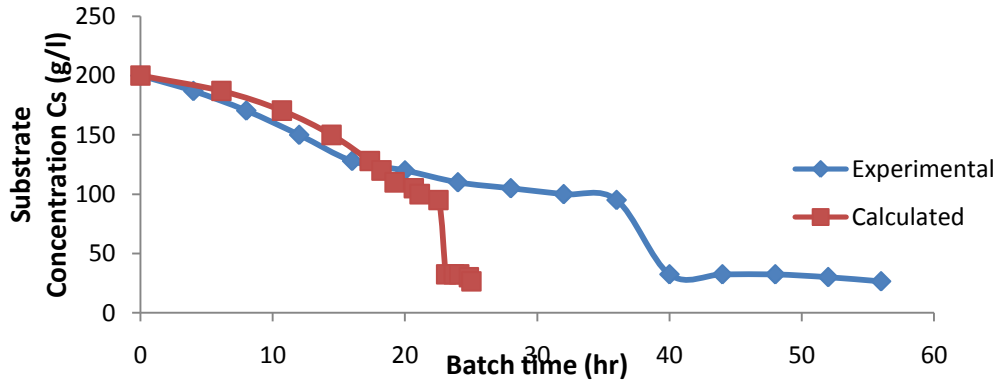

Fig. 2: Substrate Concentration versus Batch Reaction Time

Fig. 2 shows that the concentration of the substrate decreases as the batch reaction time increases, and the calculated batch reaction time gives the same substrate concentration profile but at a shorter time frame of 25hrs when compared with the experimental batch reaction time of 56hrs. The behavior of the plot graphically confirms that the mathematical model predicted an optimized fermentation process for the substrate compared with the experimental values.

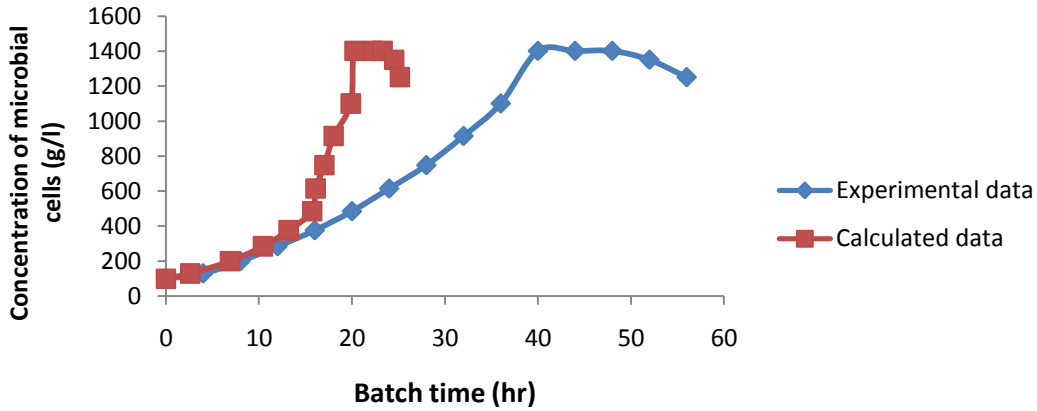

Fig. 3: Concentration of Microbial Cells versus Batch Reaction Time

Fig. 3 shows that the concentration of the microbial cells increase as the batch reaction time increases and the calculated batch reaction time gives the same microbial cell concentration profile but in an acceleratedtime frame of 25hrs when compared with the experimental batch reaction time 56hrs. Thus, the plot graphically confirms that the mathematical model predicted an accelerated microbial growth rate and fermentation for the substrate compared with the experimental values.

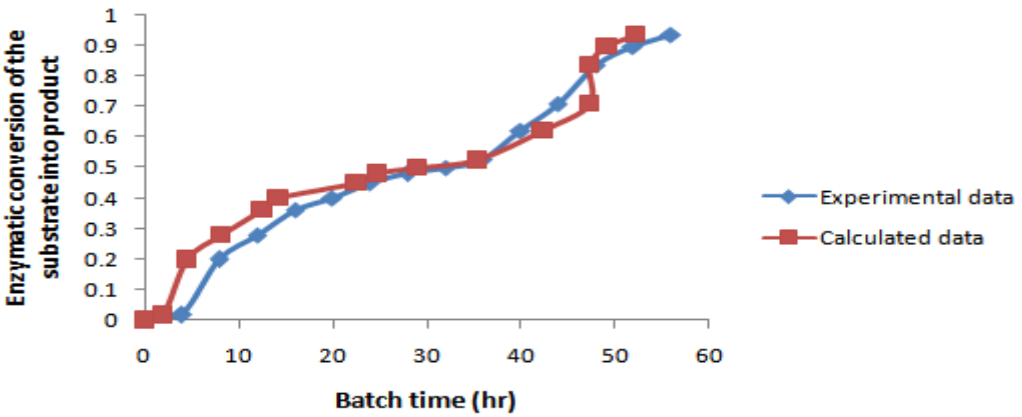

Fig. 4: Enzymatic Conversion of Substrate into Product versus Batch Reaction Time

Fig. 4 shows that the enzymatic conversion of the substrate into product (ethanol) increase as the batch reaction time increases.The calculated batch reaction time predicted the same profile compared with the experimental batch reaction time. Thus, the mathematical model prediction agrees with the experimental value as the calculated values werealmost the same with the experimental values.

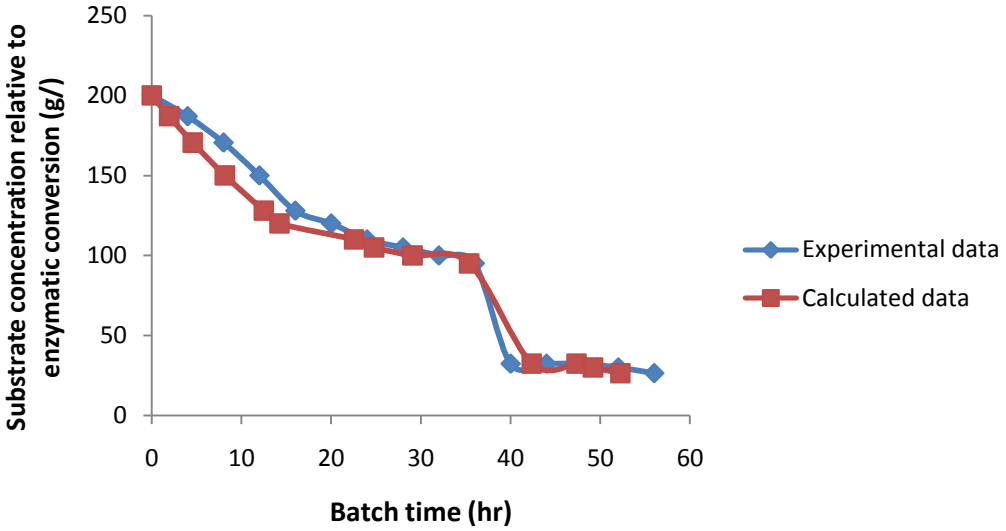

Fig. 5: Substrate Concentration relative to Enzymatic Conversion versus Batch Reaction Time

Fig. 5 shows that the concentration of the substrate (corn) relative to enzymatic conversion of the substrate into product (ethanol) decreases with increase in batch reaction time.The simulated batch reaction time predicted almost the same substrate concentration profile when compared with the experimental batch reaction time. Thus, the mathematical model prediction agrees with the experimental values showinguniform rate of substrate decomposition.

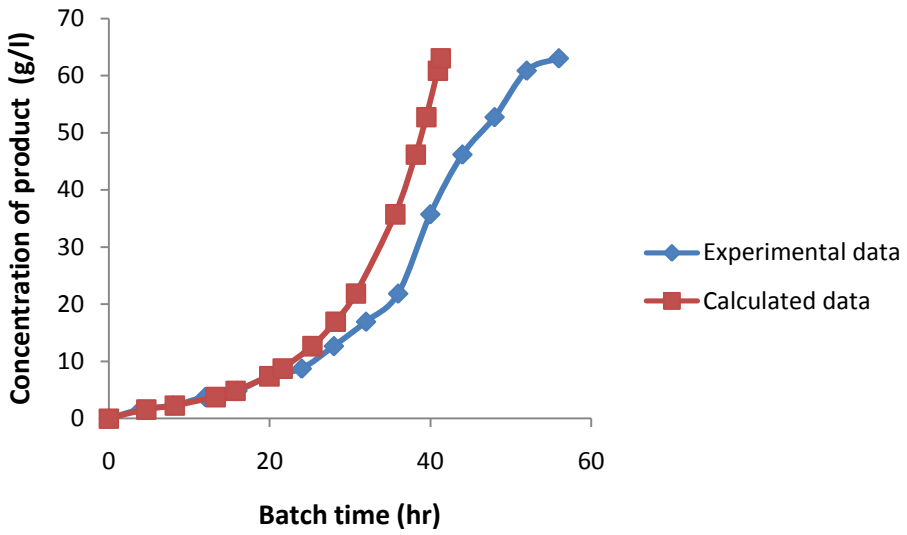

Fig. 6: Concentration of Product versus Batch Reaction Time

Fig. 6 shows that the concentration of the product increaseswith batch reaction time and the calculated batch reaction time predicteda faster product concentration profile of 41hrs when compared with the experimental batch reaction time 56hrs. Thus,the simulated result showed enhanced reactor performance and efficiency in product formation. The mathematical model prediction showed an optimized compliance with the experimental values.

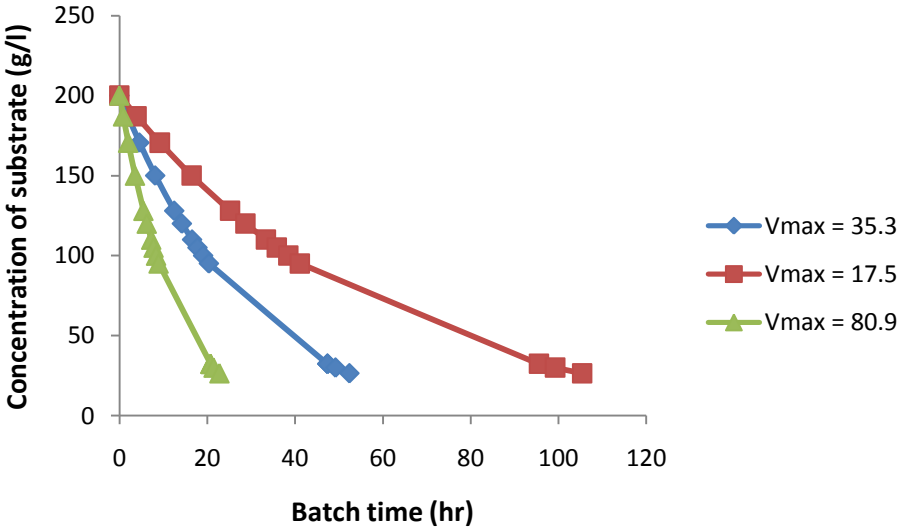

Fig. 7: Substrate Concentration versus Batch Reaction Time at different Velocities of Reaction

Fig. 7 shows that the maximum rate or velocity of reaction of the enzymes when varied has an effect on the batch reaction time required to achieve a given substrate concentration relative to enzymatic conversion of the substrate into product.The higher the maximum rate or velocity of reaction of the enzymes, the quicker the batch reaction time required to achieve a given substrate concentration relative to enzymatic conversion of the substrate into product and vice-versa.

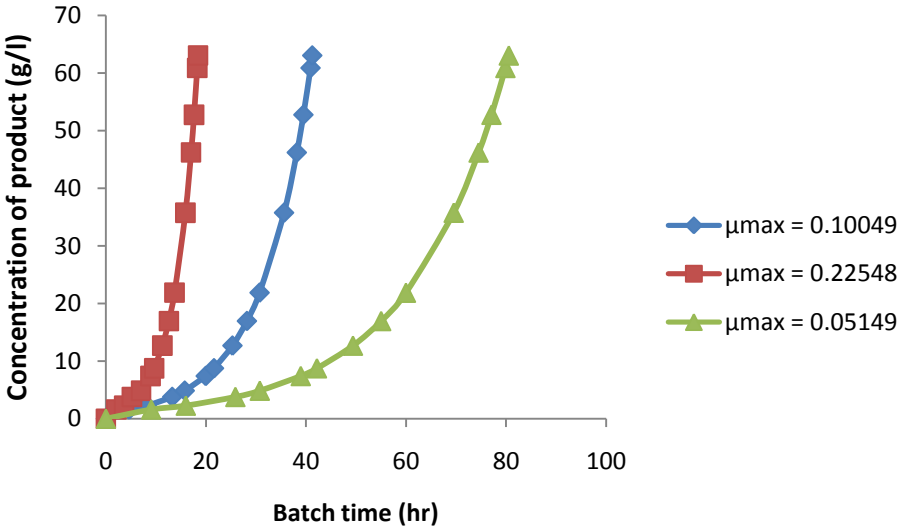

Fig. 8: Concentration of Product versus Batch Reaction Time at different Values ofμ_{max}

Fig. 8 shows that the effect of the maximum specific growth rate (μ_{max}) of the microbial cells on the batch reaction time required to achieve a given product concentration profile. The higher the maximum specific growth rate of the microbial cells the quicker the batch reaction time required to achieve a given product concentration profile, and vice-versa. Thus, operating the reactor with higher values of μ_{max} will yield more products in a short time frame.

IV. CONCLUSION

Thisstudy predicted the effects of various operating kinetic parameters on the batch time profile for the whole conversion process of the substrate (corn) into product (ethanol) with the following observations;The simulated data showed an optimized profile for the batch time profile of 25, 25, 56 and 41hrs required to achieve a given concentration of substrate, concentration of the microbial cells, enzymatic conversion of the substrate

into product and the concentration of the product respectively, compare with experimental batch time of 56hrs for all cases. An increase in the maximum rate or velocity of reaction (V_{max}) of the enzyme activity promotes a quick and rapid conversion of the substrate into product, and an increase in the maximum specific growth rate (μ_{max}) of the microbial cells rapidly increases the concentration of the microbial cells at a fasterrate and vice versa. An increase in the maximum specific growth rate (μ_{max}) of the microbial cells also increases the concentration of the product. Thus, it can be concluded that the parameters (V_{max}) and (μ_{max}) 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 adequacy of the mathematical model predictions showed that it can be considered as a goodcomplimentary tool for the real system since the simulation results of the mathematical models agree with experimental values reported in literature. The simulation results however, predicted higherperformance efficiency for the bioreactor than the experimental results due to the fact that experiments are most times prone to experimental errors.

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