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Kinetic Study of Microbial Growth in Anaerobic Digestion of Solid Waste

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A B S T R A C T

Solid waste when discarded to the environment causes serious environmental problems. The kinetic study of microbial growth in anaerobic digestion process is process specific, yet, there are limited reported studies with regards to the feedstock's (banana peel, chicken dung and waste water treatment sludge). The objective of the study is to carry out the kinetic study of microbial growth, and to know the conditions or environment responsible for the degradation of chicken dung, waste water treatment sludge and banana peel for the production of biogas and biofertilizer. The methodology involves the use of serial dilution, pour plating, cell counting for microbial population and Monod parameters were determined. Microsoft excel 2016 was use to carry out regression analysis, results shows that the maximum, growth rate (μmax) estimated from the basic Monod equation, of microorganism, was 0.098 hr-1 and the half saturation constant, (Ks), 1.2 ×108 mg/l. This pin point the availability of substrate for the survival of microorganisms

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INTRODUCTION

Anaerobic digestion process, is the process in which microorganisms breaks down organic matter in the absence of free oxygen to produce biogas and biofertilizer (Hassan & Abdulsalam, 2017). There are diverse categories of micro-organisms, specifically, Salmonella spp., Escherichia coli (E. coli), and Cryptosporidium, to mention, for the period of anaerobic batch fermentation these microorganisms, grow based on certain assortment of physical, chemical, and nutritional situations (Khan et al., 2021). This happens, by extracting nutrients from the used of various organic waste or combination of two or more organic waste in a single phase and converting them into biological compounds (Abubakar et al., 2023).

LITERATURE REVIEW

The kinetics study of microbial growth can be studied using two methods. Firstly, its involve measurement of the substrate concentrations during an experiment, this procedure is somehow baffling and time consuming. The second method, is at ease. It brings about measuring the microbial population within the slurry (mixture of organic waste and water). Obviously, the growth of microorganisms and substrate consumption rates are two important parameters that kinetic models on anaerobic digestion focuses on (Maier, 2009). In the process of anaerobic digestion, the kinetic study for microbial growth is process specific, yet, there are limited or no kinetic study with regards to the co-digestion of multiple organic waste. This study, tends to explore the kinetic study of anaerobic co-digestion of banana peel, waste water treatment sludge and chicken dung using Monod model. The importance of kinetic models is to assess the growth of microorganisms in environmental conditions, forecast the behaviour of biochemical reactions, support engineers to design and control biological processes and, to define the performance parameters prompting the product yield (Ulukardesler and Atalay, 2018).

METHODOLOGY

Determination of Organic Carbon and Nitrogen

The carbon content was determined using the blank titter, while nitrogen content was determined using the micro kjeldahl method based on ASTM standard as reported by (Abubakar et al., 2022).

The pH and Temperature Readings During Anaerobic Digestion Process

The temperature and pH of the digestion process was recorded three time daily using mercury thermometer and a digital pH meter as reported by (Hassan & Abdulsalam, 2017).

Raw Materials Collection, Pre-treatment, Mixing and Dilution

Banana peels were collected in a delivery bag at Kasuwan Shanu market, Maiduguri, Borno state, Nigeria. Furthermore, it was subjected to pre-treatment such as washing with distilled water to remove impurities. Similarly, the chicken dung was collected at University of Maiduguri, Faculty of Agriculture poultry

farm, Borno state Nigeria. The waste water treatment sludge was collected at Maiduguri bottling company, Borno state Nigeria.

About four (4kg) of chicken dung, 7.5kg of waste water treatment sludge and 0.5 kg of banana peel were mixed which was then, mixed with water in a ratio of 1:1 and then, fed into a ready-made biodigester.

Study of Growth Kinetics of Microbes

The kinetics of microbial growth was studied using Monod model as reported by(Talaiekhozani et al., 2015; Abubakar et al., 2021; Khan et al., 2021).

Estimation of Microbial Population

The estimation of microbial Population in slurry was determined using colony forming unit (X), on nutrient agar. The microbial concentration was determined according to (Abubakar et al., 2021; Khan et al., 2021) by serial dilution using pour method as follows: the dilution was prepared by mixing 1ml of the sample into 9ml of distilled water for first dilution. Then 1 mL of 1st dilution was taken and mixed with 9 mL of distilled water for 2nd dilution. Likewise, the same method was applied to prepare 3rd, 4th, 5th and 9th dilution. Nutrient agar is used as media for bacterial growth. It was prepared by taking 14g of the media agar, mixed thoroughly with 500ml of deionized water. Which was then autoclaved for 50 minute at 121oC and 0.16MPa, 20ml of the mixture was poured into the petri dish. Then, for the growth of microbes, 1 mL of each of the ninth dilution prepared was spread evenly over the petri dishes and allowed to solidify, which was then placed in an incubator at 37oC for 24h, the colony formed was counted using digital colony counter (Olufunmi, 2014).

Material Balance

A well-mixed unsteady state batch biological system (Figure 1.0) where all nutrients were fed initially into the culture and cells produced in the culture grow until one or more nutrient is exhausted, material balance over this process was formed, and it follows by Equation 1.0- 1.5.

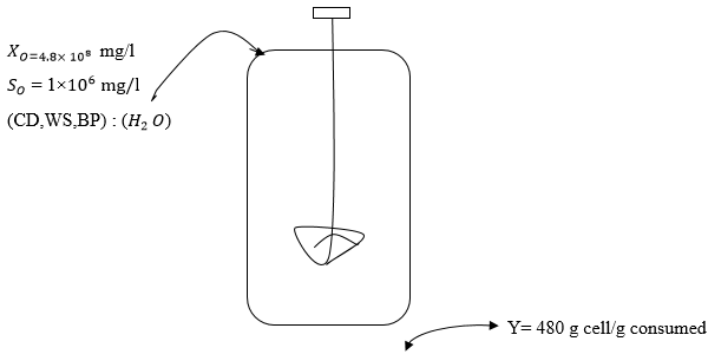

Figure 1. Flows Diagram for the Batch System

Overall balance: Accumulation = Input – Output + Generation 1.0

Cells: $\frac{d(VX)}{dt}$ = $F_{in}X_{in}$ - $F_{out}X_{out}$ + r_xV 1.2

Limiting substrate: $\frac{d(VS)}{dt}$ = $F_{in}S_{in}$ - $F_{out}S_{out}$ + r_sV 1.3

Product: $\frac{d(VP)}{dt}$ = $F_{in}P_{in}$ - $F_{out}P_{out}$ + r_pV 1.4

Water:$\frac{d(VW)}{dt}$ = $F_{in}W_{in}$ - $F_{out}W_{out}$ + r_wV 1.5

Where, the subscript 'in' and 'out' indicates input and output respectively, where F = flow rate, r = rate, S = substrate, X = cell, W = water and P = product. For a batch system, $F_{in} = F_{out} = 0$ and volume, V is constant. The assumption was applied for other possible assumptions which are: (i) concentration of H_2O remains the same ($W_{in} = W_{out}$) and minor amount of water was generated (rw), (ii) biodigester was well-mixed ($P_{out} = P_{in}$; $S_{out} = S_{in}$; $X_{out} = X_{in}$), (iii) no product in feed ($P_{in} = 0$) and (d) cell growth was greater than cell death rate ($rx = \mu X$) to give Equation 3.16 (Osadolor, 2018)

Cells: $\frac{dX}{dt}$ = μX = rx 1.6

Substrate:$\frac{dS}{dt}$ = rs 1.7

Therefore, to generate appropriate data for Monod plot the following Equations (1.8 - 3.0) were used as follows: the colony forming unit (X) was calculated using equation 3.11 as reported by (Abubakar et al., 2022; Khan et al., 2021)

$$\text{CFU (X)} = \frac{\text{Colony Forming Unit} \times \text{Dilution factor}}{\text{volume platted}} \quad 1.8$$

Monod equation is used to study the growth kinetic of microbes, Monod equation is given by Equation 3.19 as reported by (Dueñas et al., 2015; Sakthiselvan et al., 2019)

$$\mu = \frac{\mu_{max} S}{K_{s+S}} \quad 1.9$$

Where μ_{max} maximum growth rate, μ is specific growth rate and X is cell concentration and S is the substrate concentration.

Initial substrate concentration was determine using Equation 3.20 as reported by (Abubakar et al., 2021)

$$S_o = \frac{\text{mass of feedstock}}{\text{volume of water}} \quad 2.0$$

The substrate concentration was determine using experimental values of X using Equation 3.21 given by (Talaiekhozani et al., 2015)

$$S_E = S_o - \frac{(X - X_o)}{Y} \quad 2.1$$

Where, X_o is initial cell concentration, S_E is S experimented and Y is yield (Ghosh et al., 2012; Ulukardesler and Atalay, 2018).

$$Y = \frac{\Delta X}{\Delta S} \quad 2.2$$

Equation 2.3 was used independently for estimating μ without Monod and was combined with Equation 1.6 and integrated to give Equation 2.5 using the following steps (Abubakar et al., 2021; Ghosh et al., 2012)

$$\mu = k\left(1 - \frac{X}{X_\infty}\right) \quad 2.3$$

$$\frac{dt}{dX} = \mu X = \mu = k\left(1 - \frac{X}{X_\infty}\right)X$$

$$\int_{X_0}^{X} \frac{dX}{\left(1 - \frac{X}{X_\infty}\right)X} = \int_0^t k dt \quad 2.4$$

A predictive value of X known as $X_{predicted}$ was obtained using several guess in order to obtain K value using excel solver by regression to find the value that fit X_{Expr} using Equation 2.4 (Abubakar et al., 2021, 2022). K was estimated to be 0.01

$$X_P = \frac{X_o \exp(Kt)}{1 - \frac{X_o}{X_\infty}[1 - \exp(Kt)]} \quad 2.5$$

Where $X_\infty = X_o + YS_o$ 2.6

The value of X predicted from Equation 2.7 was used to obtained the value of S predicted (Abubakar et al., 2021)

$$S_P = S_0 - \frac{X_P - X_0}{Y} \quad 2.7$$

Combining Monod equation given in equation 1.9 and 2.3 as data for equation 3.25 was used to find Ks by finding new set of data known as S_{reg} that fit Xp the value of μ as well as S was determine using Equation 3.28 (Ghosh et al., 2012).

$$\mu = \frac{\mu_{max} S_M}{K_S + S_M} = K\left(1 - \frac{X_P}{X_\infty}\right) \quad 2.8$$

Ks, was guessed repeatedly to give Ks and used together with Y and $X\infty$ mg/l to find $Sreg$ in Equation 2.9 taking $Y = \frac{\mu_{max}}{k}$ as given by Talaiekhozani et al. (2015).

$$S_{reg} = Ks\frac{(X_\infty - X_P)}{X_\infty} = Ks\frac{(X_\infty - X_P)}{X_\infty} \quad 2.9$$
$$\frac{\mu max}{k} - \frac{(X_\infty - X_P)}{X_\infty} \qquad Y - \frac{\mu max}{k}\frac{(X_\infty - X_P)}{X_\infty}$$

Equation 1.9 and 2.3 was compared and concluded that K = μ_{max} that enable us to Customizing S from equation 2.8 so as to compute for S_{Monod}as seen in Equation 3.0

$$S_M = \frac{K_S}{X_P}(X_\infty - X_P) = \frac{\mu k_s}{\mu_{max} - \mu}$$

RESULTS AND DISCUSSIONS

Carbon to Nitrogen Ratio

The carbon to nitrogen ratio (C/N) plays a key vital role in anaerobic digestion process (Tanimu et al., 2021). At higher nitrogen to carbon ration above 30:1 its tends to slow down the digestion process, this is because, at higher carbon to nitrogen ratio there will be increase in the formation of ammonia which will bring about the change in the pH of the digestion process to acidic thereby resulting in destruction of microorganisms (Abubakar et al., 2021). In this study, the C/N was found to be 25:1 which is within the stipulated limit for anaerobic digestion process.

Temperature and pH During Anaerobic Digestion

Temperature and pH the key vital parameters in anaerobic digestion process. The average pH and temperature during the digestion process was found to be 6.8 and 30 OC. Which is within the optimum pH condition for anaerobic digestion; most methanogens are lively between 25-40 oC (mesophilic range) as reported by (Hamouda et al., 2016; Hassan and Abdulsalam, 2017; Abdulkarim et al., 2019; Onuoha et al., 2019; Menta, 2020).

Kinetics of Microbial Growth

Kinetic study of microbial growth was carried out using Monod model Figure 2.0 and 3.0 shows the plot of cell concentration (X) against substrate concentration. For experimental data and the predicted data.

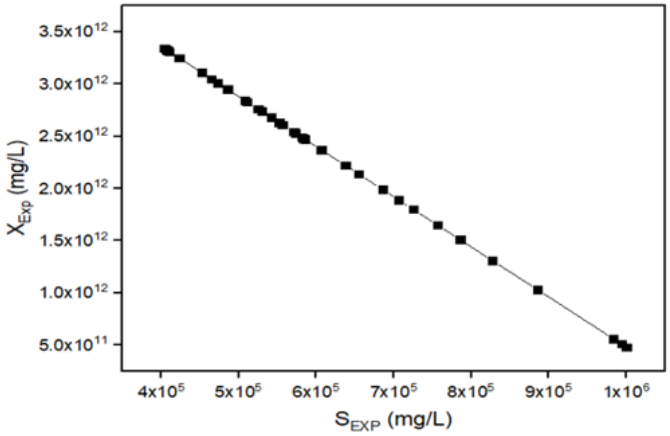

Figure 2. Plot of Cell Concentration (X) Against Substrate Concentration

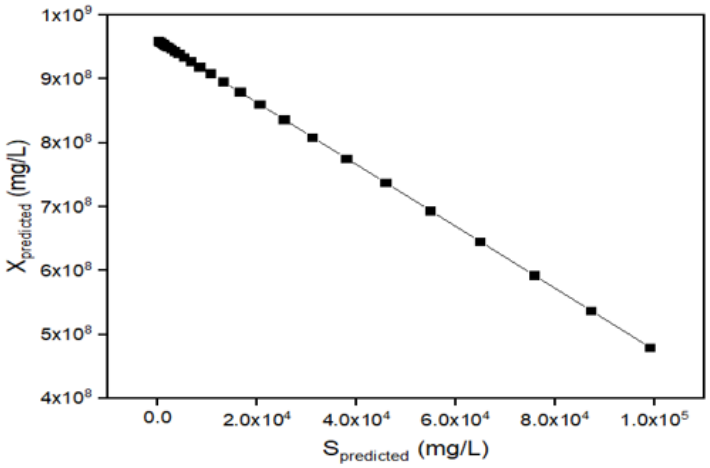

Figure 3. Plot of X Predicted Against S Predicted

Predicted data plot in Figure 3.0 fits properly to the experimental results with both having R2 of 1.0, and hence estimates the parameters k = 0.01hr-1 and Ks = 1.2 × 108mg/l correctly using $X\infty$ = 9.60× 108. The linear relationship between the substrate concentration and the cell concentration better explains this relationship considering it from the bottom right upwards. As the cell concentration increases, substrate concentration decrease. At an initial substrate concentration $S0$ = 1.0×106 mg/l, and decreases to an average constant value 5.8×105 mg/l. Although, Abubakar et al.,(2022) state that, Precision in bacterial population count is right challenging and baffling, nevertheless, this data assumes that single bacteria grows to form a single colony, multiple bacteria forming single colony hides some number of viable cells that were unknowingly counted as one. Values of X for that reason, doesn't word a 100% precise data because it fails to include gain in concentration due to growth in weight/size of the bacteria.

Effect of Substrate Concentration on Specific Growth Rate

The four-sided hyperbola in Figure 4.0 is termed the Monod plot grounded on cell growth at the exponential phase. The Monod model uses a very convenient approximation of the batch growth process, when describing microbial.

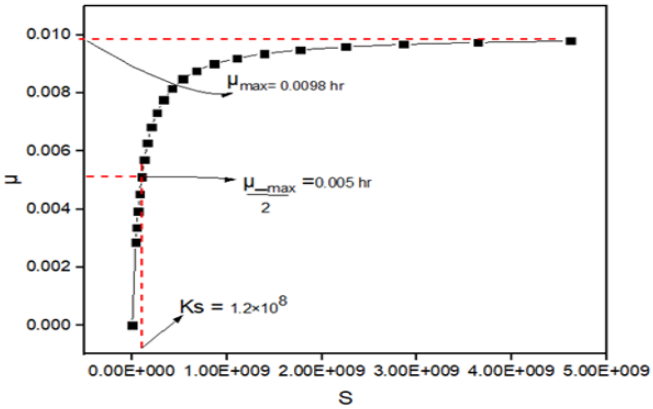

Figure 4. Monod Plot Based on $\boldsymbol{\mu}$ and S Computed from Experimental Values of Microbial Concentration

At an initial value of S = 0 mg/l equivalent to $\mu = 0\ hr\text{-}1$, to a highest value, μmax where S is also high. Dividing the above plot into 3-segments centred on amount of substrate (S). For low S (i.e. $S \ll Ks$), growth have first order dependence on S, that is, growth is highly sensitive to S and the Monod equation reduces to $\mu = \mu maxS/\ Ks$. That is to say, when nutrient is very little, cells had to compete for it. Maier, (2009) stated that, these notions to the fact that amount of substrate limits how fast the cells can grow. So, adding more substrate causes proportional increase in cell growth rate. The centre region is named the mixed order section that satisfies the Monod equation proper. When S is high (i.e. $S \gg Ks$), growth is at μmax and kinetics reduces to a zero-order expression $\mu = \mu max$ as reported by (González-figueredo. et al., 2018). At this juncture, each cell can have as much nutrient or substrate as they so need owing to its abundance because the specific growth rate is high (Abubakar, 2022; González-figueredo et al., 2018). Figure 4.0 is a way for determining μmax and Ks. However, these two parameters can be determined otherwise, using Lineweaver-Burke plot, Hanes-Woolf plot or Eadie-Hofstee plot which are all linear in nature. But then, Equations for these plots were derived from actual Monod equation and offers a considerably tranquil method of estimating the Monod kinetic parameters (Abubakar et al., 2021). Figure 5.0, 6.0 and 7.0 shows the Lineweaver-Burke plot, Hanes-Woolf plot and Eadie-Hofstee plot which are all linear in nature.

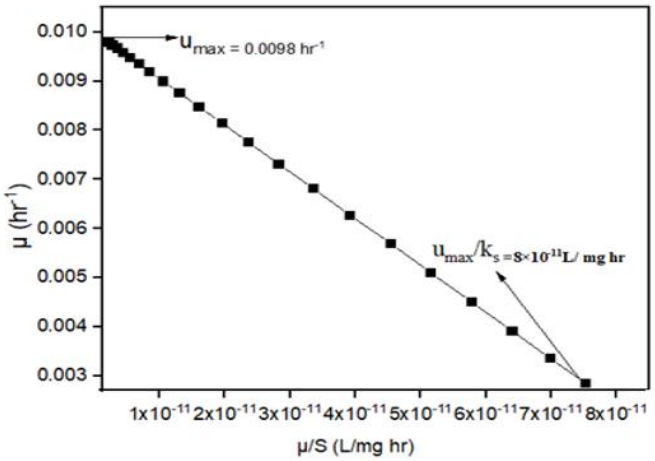

Figure 5. Eadie-Hoftee Plot μ Against μ/s

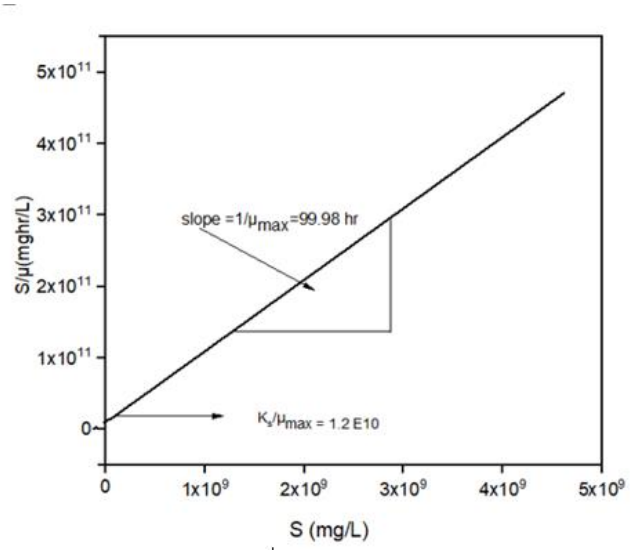

Figure 6. Hanes-Woolf Plot

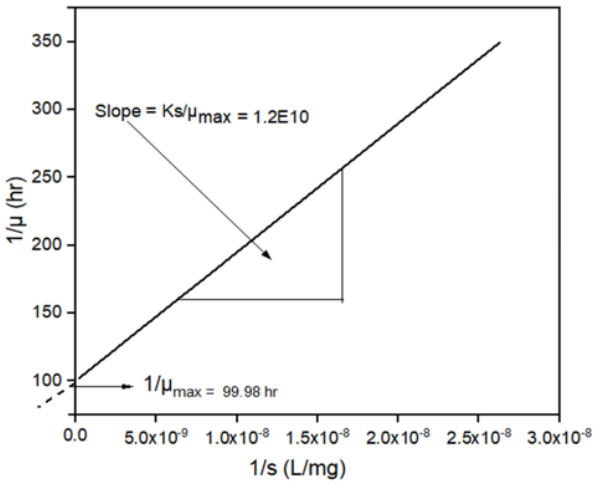

Figure 7. Lineweaver-Burke Plot

The S data used for estimating these parameters excludes toxic S that are capable of inhibiting bacteria growth. These two parameters can be determined alternatively using Lineweaver-Burke plot, Hanes-Woolf pot or Eadie-Hofstee plot (all linear). Equations for these plots were derived from actual Monod equation and provides a much easier approach of estimating the Monod kinetic parameters, as already seen in Figure 12. Equations of the respective Lineweaver-Burke Plot, Hanes-Woolf Plot and the Eadie-Hofstee Plots for results obtained here are given in Equation 3.1 -3.3.

$$\frac{1}{\mu} = \frac{1.2 \times 10^8}{0.0098}\left(\frac{1}{S}\right) + \frac{1}{0.0098} \qquad 3.1$$

$$\frac{S}{\mu} = \frac{1.2 \times 10^8}{0.0098} + \frac{1}{0.0098}\mathrm{S} \qquad 3.2$$

$$\mu = 0.0098 - 1.2 \times 10^8 \frac{\mu}{S} \qquad 3.3$$

The above model equations can therefore, be used for S and μ estimates spanning the period of the experimental growth phase. In his study Abubakar, (2022), the anaerobic digestion of chicken manure the maximum growth rate μ_{max} was found to be 0.0076 hr-1. While, for the 0.0098hr-1 recorded in this study could be attributed to mixed substrates because of more balanced acidogenesis and methanogenesis in mixed substrates than sole substrates (Menta, 2020).

The Contois model was compared with Monod plot obtained from the experimental data in this study. In the literature, Bayen et al., (2018) reported the Contois model given by Equation 3.4

$$\mu = \frac{\mu_{max} S}{K_S X + S} \qquad 3.4$$

The fitting of the Contois model with Monod model is presented in Figure 8.

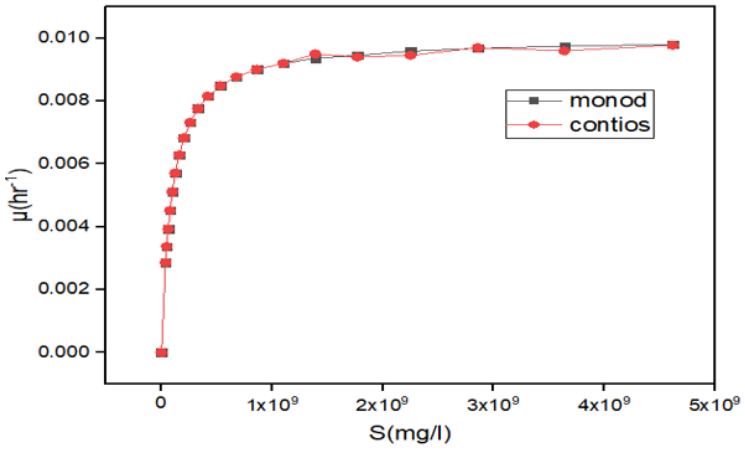

Figure 8. The Fitting of the Contois Model with Monod Model

The Monod plot fitted perfectly or the experimental data obtained to Contois plot as present in figure 4.11 with R2 of 1.0 and the estimated parameters μ = 0.0096 hr-1 and Ks =1.2×108.

CONCLUSIONS AND RECOMMENDATIONS

The kinetic study of the microbial growth using Monod equation showed that the maximum, growth rate (μmax) estimated from the basic Monod equation, of microorganism, is 0.098 hr-1 and the half saturation constant, (Ks), 1.2 ×108 mg/l. Dividing the Monod plot into 3-segments centred on amount of substrate (S). For low S (i.e. $S \ll Ks$), growth have first order dependence on S, that is, growth is highly sensitive to S and the Monod equation reduces to $\mu = \mu maxS/\ Ks$. That is to say, when nutrient is very little, cells had to compete for it. The amount of substrate limits how fast the cells can grow.

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