

# Evaluation of Kinetic Parameters of Nitrification Process in Biofilter System to Effluent Liquid Waste of Tofu Industry

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**Abstract:** It is necessary to design a nitrification bioreactor process so that further processing takes place optimally. Performance studies are carried out by evaluating the kinetic parameters that apply specifically to the applied process. The Monod model was applied to determine the value of kinetic parameters in designing and operating a bioreactor. This study aims to determine the value of the kinetic parameters to variations in feed concentration (50, 75, and 100%). The mechanism of the reactor process for the decomposition of pollutants, the influent is fed into the reactor with an up-flow prismatic pump. The decomposition process provides contact time between organic matter and microorganisms, resulting in a good separation from the reactor outlet. The most optimum kinetic parameter value at 100% wastewater concentration with a value of (k) 1.1086 (day<sup>-1</sup>), (K<sub>s</sub>) 1.0564 g l<sup>-1</sup>, (Y) 5.4862 mg MLVSS/mg, (k<sub>d</sub>) 1.7944 (day<sup>-1</sup>), (μ<sub>m</sub>) 6.8372 (day<sup>-1</sup>).

**Keywords:** Biofilter; Effluent; Evaluation; Nitrification; Kinetic parameters

## Introduction

Food industry waste is one of the highest contributors to ammonia or organic nitrogen sources in aquatic systems. Ammonia is a group of nitrogen compounds that are widely contained in wastewater, as toxic to aquatic animals and harmful to aquatic ecosystems. When ammonia dissolves in water as a chemical reaction, it acts as a base which is used to obtain hydrogen ions from H<sub>2</sub>O to produce ammonium ions (NH<sub>4</sub><sup>+</sup>) and hydroxide ions (OH<sup>-</sup>) (Effendi & Sandi, 2018; Feng et al., 2019; Meng et al., 2019).

The tofu industry is one of the industries that contribute organic nitrogen sources in the waters because there is no water treatment system before it is discharged into water bodies. Liquid waste is generated from the process of washing, boiling, pressing, and printing tofu. The characteristics of tofu liquid waste contain a high organic load and a low acidity level of 4-5, under these conditions, the tofu

industrial wastewater is one of the potential sources of pollution if the wastewater produced is directly discharged into water bodies.

The waste characteristics of the tofu industry include two things, namely physical and chemical characteristics. Physical characteristics include total solids, suspended solids, temperature, color, and odor. Chemical characteristics include organic materials, inorganic materials, and gases. Tofu wastewater temperature ranged from 37-45°C, turbidity 535-585 FTU, color 2.225-2250 Pt. Co, ammonia 23.3-23.5 mg/L, BOD<sub>5</sub> 6.000-8.000 mg/L and COD 7500-14.000 mg/L (Herlambang, 2002).

The amount of liquid waste from the tofu industry produced by the tofu manufacturing industry is approximately 15-20 L/kg soybean raw material, while the pollution load is approximately 30 kg Total Suspended Solids (TSS)/kg soybean raw material, Biological Oxygen Demand (BOD) 65 gr/kg soybean raw material and Chemical Oxygen Demand (COD) 130 gr/kg soybean raw material (Potter et al., 1994).

The biological process is the most economical in waste treatment, ammonia is converted into nitrite and nitrate by oxidation by bacteria called the nitrification process. When naturally the nitrification process affects the concentration of dissolved oxygen (DO) in water bodies and can cause eutrophication. When the nitrification process takes place oxic process, it will form nitrate ( $\text{NO}_3^-$ ) and nitrite then in anoxic conditions is reduced to nitrogen gas (Ruiz et al., 2006). Because the wastewater from the tofu industry contains a high organic and protein load, it must be treated technically before being discharged into water bodies. The right process to treat the wastewater of the tofu industry is to use the anaerobic process for the first stage, the effluent from the anaerobic reactor must be reprocessed to remove the nitrogen component, which is continued with the nitrification and denitrification processes.

Aerobic treatment is utilizing microorganisms in wastewater to decompose organic substances. Microorganisms play an important role in the biodegradation process. Therefore, kinetic parameters are an important basis in biological waste treatment. Tofu wastewater treatment is carried out on various kinds of bioreactors used and the expected goals, depending on the variables and parameters used (Gnanapragasam et al., 2017; Lay et al., 2013; Mansouri, 2014; Narra et al., 2014) researched tofu wastewater using a CSTR reactor with efficiency analysis of the conversion of tofu wastewater into hydrogen and ethanol for energy sources in a tofu industry factory evaluated by several types of mud variations with HRT variations of 6-24 hours (Sitoru et al., 2010) investigated the treatment of tofu wastewater for COD, TSS, and Turbidity efficiency using the UASB (3L) reactor with the coagulation-flocculation process. Characteristics pH 3.2, COD 14.744 mg/L, TSS 618 mg/L, turbidity 876 NTU. The results obtained in the treatment of tofu wastewater using UASB and coagulation-flocculation with alum gave removal efficiency of 96%, 93%, and 92% for COD, TSS, and turbidity, respectively. In addition to using PAC as a coagulant, the removal was slightly different, namely 96%, 94%, and 93% for COD, TSS, and Turbidity. Tofu wastewater treatment using a hybrid upflow anaerobic sludge blanket (HUASB) reactor has been carried out, where the processing can remove 86.76% COD (Yanqoritha et al., 2018) and processing at this stage must be reprocessed to remove the nitrogen content, therefore the nitrification process stage becomes a further processing process for the HUASB reactor effluent.

Nitrification is the process of forming nitrate ( $\text{NO}_3^-$ ) from ammonium compounds ( $\text{NH}_4^+$ ) catalyzed by the nitrogenase enzyme owned by nitrate nitrifying

bacteria. Nitrification has a 2 step process, namely nitrification for the process of converting ammonium ions into nitrite ( $\text{NO}_2^-$ ) by nitrifying bacteria such as *Nitrosomonas* and nitrification for the process of converting nitrite to nitrate ( $\text{NO}_3^-$ ) by nitrifying bacteria such as *Nitrobacter* nitrate (Anh., et al., 2006; Bacta-Pur, 2012; Li et al., 2009) stated that to identify the function of functional species the performance was carried out separately by using an aeration system for the nitrification process so that the removal of the  $\text{NH}_3\text{-N}$  component of the effluent from the HUASB reactor process was obtained. During anaerobic processing, the organic components of nitrogen are difficult to degrade. Therefore, the effluent from the anaerobic process must be treated to remove nitrogen (Sousa et al., 2008). Two factors that affect the efficiency of the nitrification process are the biological and physicochemical communities. The nitrification process requires special conditions, namely: temperature 30°C, pH 6-8.5,  $\text{DO} > 3$  mg/L. In the nitrification process, two reactions occur, namely the oxidation of ammonium to nitrite and the oxidation of nitrite to nitrate.

Based on the biochemistry and microbiology of biological processes, kinetic studies provide a rational basis for process analysis, control, and design. A kinetic model is currently being developed to assist in the design and optimization of processes in reactors (Abyar et al., 2017; Gnanapragasam et al., 2017; Jafarzadeh et al., 2009; Mansouri, 2014; Narra et al., 2014). The kinetic model is also considered feasible and suitable for approaches to removal performance, prediction of effluent concentration, and optimization of biological processes (Cho et al., 2020; Mansouri, 2014; Rodziewicz et al., 2019; Wu et al., 2016). The acclimatization process aims to breed bacteria at a certain substrate concentration so that the kinetics rate obtained can be evaluated. Bacteria that have undergone an acclimatization process can then be used to treat liquid waste that contains highly organic materials (Priyono et al., 2012).

Growth kinetics is a characteristic of microbial growth as long as nutrients are available which shows an increase in the number of microbial cells and an increase in biomass. The relationship between substrate concentration and growth rate can be expressed in Equation 1, namely the Monod Equation. The equation as a function of activated sludge is expressed in Equation 2, the equation as a function of the biomass reaction rate is expressed in Equation 3 and the substrate rate equation is in Equation 4 (Tchobanoglous, G., 2003; Wiesmann et al., 2006). Based on this background, this study aims to determine the value of the kinetics of microbial growth parameters in tofu wastewater treatment based on

variations in substrate concentration in the nitrification process.

$$\frac{dX}{dt} = \frac{k_o X S}{K_m + S} \quad (1)$$

$$\left[ \frac{dX}{dt} \right]_{\text{endogeneous}} = -k_d X \quad (2)$$

$$\text{biomass reaction rate} = \frac{k_o X S}{(K_m + S)} - k_d X \quad (3)$$

$$\text{The substrate reaction rate} = \frac{dS}{dt} = \frac{k_o X S}{Y(K_m + S)} \quad (4)$$

Information:

- $K_m$  : substrate saturation constant
- $K_o$  : maximum specific growth rate constant, day<sup>-1</sup>
- $S$  : substrate, mass/vol
- $Y$  : yield coefficient
- $X$  : biomass, mass/vol

The presence of substrates and nutrients for growth is limited to a continuous process, where these nutrients will be depleted and growth will stop. Equation 5 is used for substrate or nutrient limitations.

$$\mu = \mu_m \frac{S}{K_S + S} \quad (5)$$

Information:

- $\mu$  : specific growth, (liter/day)
- $\mu_m$  : maximum specific growth (L/day)
- $S$  : substrate concentration, (mg/L)
- $K_S$  : substrate concentration at half the maximum growth rate, (mg/L)

In general,  $\mu_m/Y$  is replaced with  $k$  (maximum substrate utilization rate/bacterial mass) so that:

$$k = \frac{\mu_m}{Y} \quad (6)$$

## Method

The feed is obtained from the effluent of the anaerobic reactor in the first stage of the tofu industrial wastewater treatment. Fixed variables used in this study include temperature 25-30°C, pH 7.5, and feed volume of 10 liters. While the change variables are the concentration of waste 50.75 and 100%. Data collection was carried out by: sampling from the effluent of the Anaerobic Reactor (Processing stage 1), direct observation of the research object (Nitrification Process), data collection obtained from the initial characteristic test of the sample (COD, TSS, and pH), then analysis of tofu wastewater from the performance of the aerobic reactor (COD, TSS, VSS, and pH). The reactor used in this study is a reactor equipped with a biofilter-attached culture system aerator using a

Bioball, where the function of the biofilter media is so that microorganisms are retained in the media. The series of Nitrification Reactor Equipment is supported by air supply (aeration) which is carried out continuously into the reactor through a Resend 40 type air pump equipped with a Clarivier as a tool to assist the precipitation process. In this aerobic process, Nitrosomonas and Nitrobacter bacteria are responsible for decomposing NH<sub>4</sub>-N in the activated sludge process and the biofilm process. The operating volume of the nitrification reactor is 10 liters with a black bioball as a biofilter. The process begins with seeding and acclimatization is carried out together. The following series of aerobic reactors is shown in Figure 1.

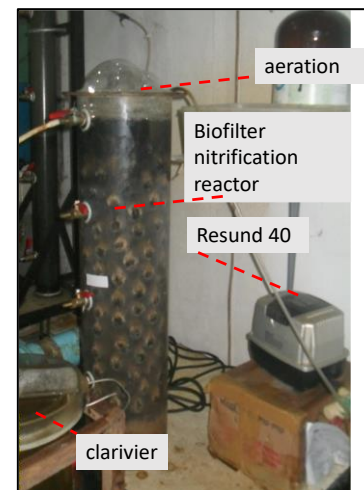


Figure 1. Installation of biofilter nitrification reactor

The seeding and acclimatization process is carried out after one week of the seeding process. Furthermore, seeding and acclimatization were continued, starting with the lowest feed concentration up to 100% feed concentration. The source of microorganisms is fed directly into the reactor up to a volume of 1000 ml for several days continuously and given nutrition 2 tablespoons of sugar until a layer of mucus or biofilm is formed in the reactor. Furthermore, tofu liquid waste is fed starting at a concentration of 40% with the addition of NaHCO<sub>3</sub> to pH 6.5 and 0.5 grams of nutrients in the form of nitrogen and phosphorus, then drained for several days until the acclimatization conditions are stable, then the wastewater as feed is increased to 50% concentration, 75% waste water up to 100%. After the acclimatization process is stable, the reactor is ready to be operated under various conditions. The kinetic parameters were determined using the Monod equation in which the concentration of biomass in the reactor was measured as evaporated suspended solids (VSS).

## Result and Discussion

### Seeding Acclimatization Results

Based on the results of research and analysis that have been carried out on the liquid waste of the tofu industry using an aerobic reactor with process variables, namely, waste concentrations of 50.75 and 100%, the COD (Chemical Oxygen Demand) and TSS (Total Suspended Solid) values can be obtained. In Figure 1 the Nitrification Reactor is seen above, and the integration of aeration is carried out by the Resund 40 aerator.



Figure 2. Seeding process

The seeding and acclimatization process was indicated by a clear color (Figure 2) when the initial seeding stage changed from a cloudy yellow-brown to a dark brown-yellow color (Figure 3). This indicates that the nitrification process is running well.



Figure 3. Acclimatization process

### Evaluation of Kinetic Parameters

To determine the value of microbial growth kinetics using COD data and biomass concentration entered into the monod equation and plotted into a graph. The concentration of biomass in the bioreactor was measured as total suspended solids (Tchobanoglous, G., 2003). The kinetic values of

microbial growth that need to be known are  $k$  (maximum substrate utilization rate),  $K_s$  (semi-saturated constant),  $Y$  (yield coefficient),  $k_d$  (number of cells dying), and  $m$  (maximum growth rate).

The determination of the values of  $K_s$  and  $k$  is shown in Figure 4. where the plot results between  $1/S_e$  and  $\theta \cdot X/S_o - S_e$  produce a straight-line equation  $y = 0.0796x + 1.9849$  so that the ratio of the half-saturation constant to the utilization of the substrate ( $K_s/k$ ) is obtained is slope and  $1/k$  is the intercept of the slope and the straight-line equation. The values of  $k$  and  $K_s$  respectively for each concentration are 0.5038; 1.5686; 1.1086  $gL^{-1}d^{-1}$  and 0.0401; 0.2568; 1.0564  $gL^{-1}$ .

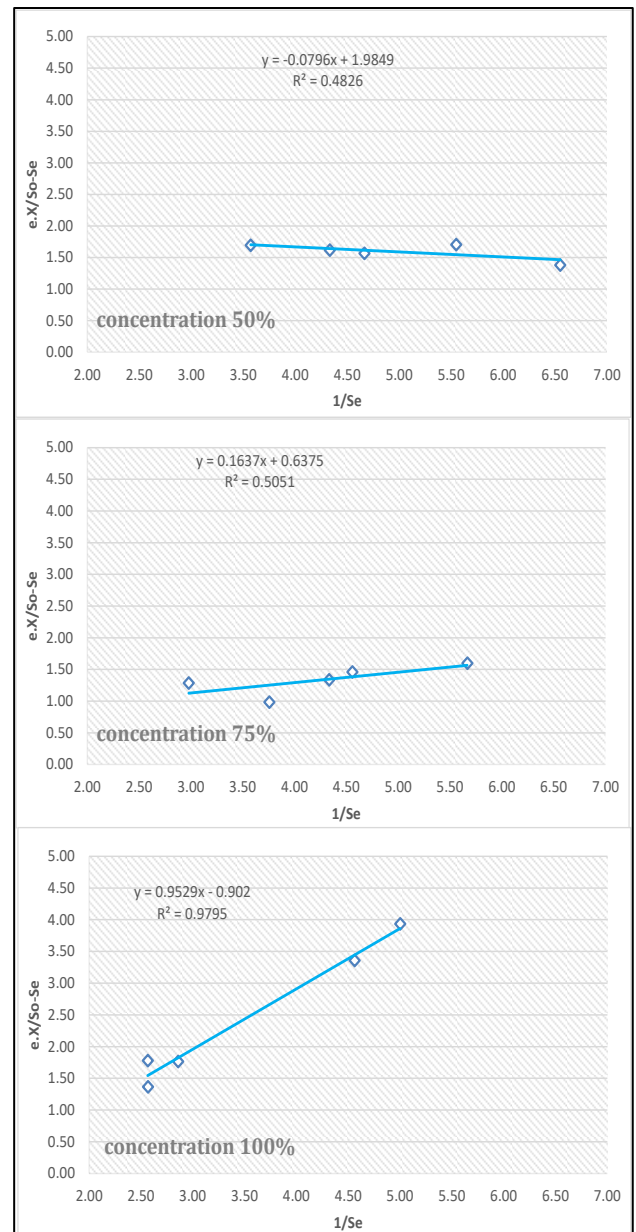
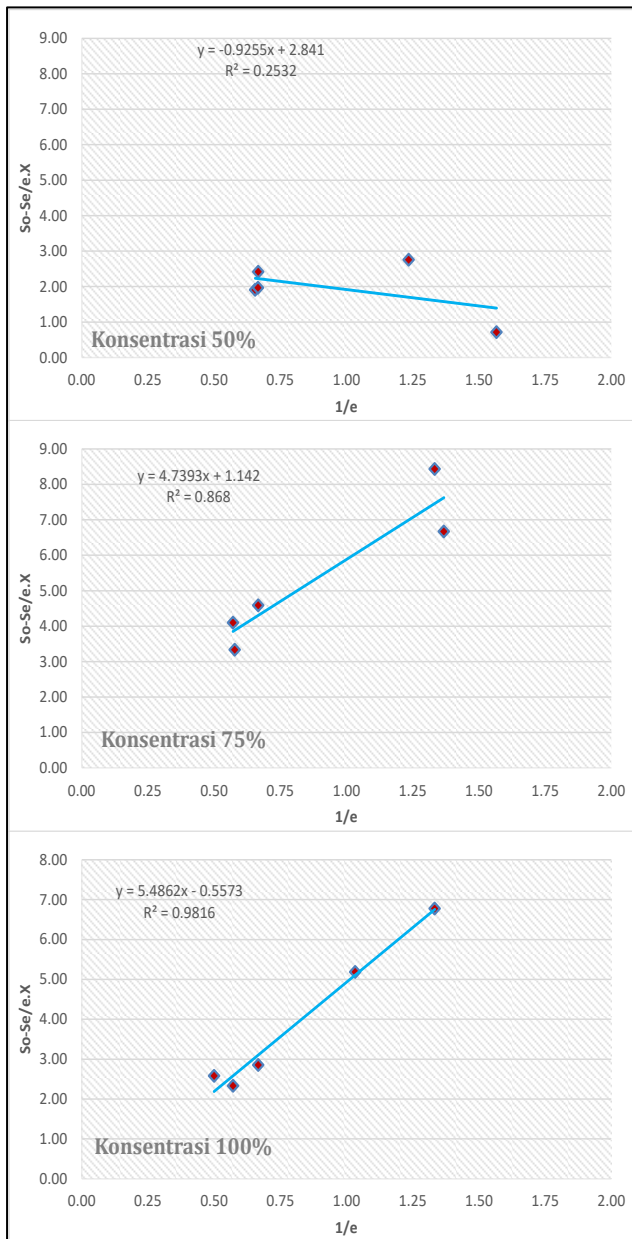


Figure 4. Evaluation of kinetic parameter growth on the determination of  $K_s$  and  $k$  values

Determination of the value of  $Y$ ,  $k_d$ , and  $\mu_m$  is shown in Figure 5. where the plot results between  $1/\theta$  and  $S_0 - S_e/\theta \cdot X$  produces a straightline equation  $y = 0.9255x + 2.841$  so that the value is obtained  $Y = slope$  and  $k_d = intercept$  consecutively is 0.9255; 4.7393; 5.4862 gTSS/gCOD and 0.3519; 0.8757; 1.7944 d<sup>-1</sup>. While the maximum growth rate ( $\mu_m$ ) is calculated using equation 6, so the value obtained is 6.0820; 7.4340; 0.4663 d<sup>-1</sup>.

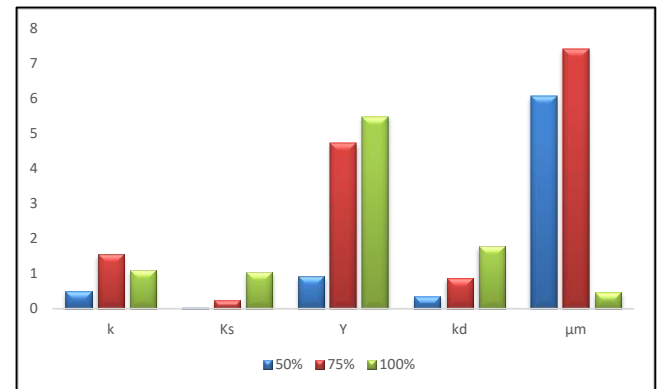
**Table 1.** Monod model kinetic parameters

Concentration	k	K <sub>s</sub>	Y	k <sub>d</sub>	$\mu_m$
50	0.5038	0.0401	0.9255	0.3519	6.0820
75	1.5686	0.2568	4.7393	0.8757	7.4340
100	1.1086	1.0564	5.4862	1.7944	0.4663



**Figure 5.** Evaluation of kinetic parameters growth on determination of  $Y$  and  $K_d$  values

The complete data on the kinetic parameters of the Monod model is in Table 1.



**Figure 6.** Value of kinetic parameters at various concentrations

The  $Y$  value indicates the amount of organic matter converted into new cells. A high  $Y$  value indicates a high content of organic matter that can be degraded by microorganisms. The linear regression of the plot of the relationship between the concentration of MLVSS ( $X$ ) and the difference in the reduction of COD or substrate ( $S_0 - S$ ) resulted in a coefficient of  $Y = 0.9255$  mg MLVSS/mg COD from 50% concentration of anaerobic treatment effluent,  $Y = 4.7393$  mg MLVSS/mg COD for wastewater with a concentration of 75%, and  $Y = 5.4862$  mg MLVSS/mg COD for wastewater with a concentration of 100%. The variation in the  $Y$  value of tofu liquid waste is caused by differences in the three concentrations of tofu liquid waste used. At 100% effluent concentration, the highest  $Y$  value was obtained, and a high  $Y$  value was followed by the highest COD removal efficiency (80%).

The ability of degradation is indicated by a high value of  $Y$  which indicates a better level of degradability, but the kinetics of biodegradation is also influenced by factors of enzyme activity and initial substrate concentration, so it is necessary to control enzyme activity and initial substrate concentration so that biodegradation kinetics run better. The value of the coefficient  $m$  indicates the growth rate of microorganisms. The coefficient of maximum growth rate produced is  $\mu_m = 6.0820$  (day<sup>-1</sup>) for 50% concentration of anaerobic process effluent wastewater,  $\mu_m = 7.4340$  (day<sup>-1</sup>) for 75% concentration,  $\mu_m = 0.4663$  (day<sup>-1</sup>) for 100% concentration. The coefficient value of  $m$  for a concentration of 75% is greater than the value of the coefficient  $m$  for other concentrations. This could be due to the organic load at a concentration of 75% being optimally degraded. The

value of  $m$  is one indicator of the value of the level of biodegradability of wastewater treatment processes. A low  $m$  value indicates a slow growth of microorganisms. On the other hand, a high  $m$  value indicates the rapid growth of microorganisms. The biokinetic variable  $K_s$  indicates the sensitivity of the substrate concentration which is sensitive to biomass growth. If the value of  $K_s$  is large, it means that the substrate concentration range that is sensitive to growth is also large. Concentrations above  $K_s$  indicate a tendency that is less sensitive to biomass growth (Grady, C.P.L., 1980). The results of the linear regression plot of the relationship between  $1/S_e$  and  $X/S_0 - S_e$  resulted in a coefficient of  $K_s = 0.0401$  (g/L) for the 50% concentration of anaerobic process effluent,  $K_s = 0.2568$  (g/L) for a concentration of 75%,  $K_s = 1.0564$  (g/L) for 100% concentration. A high  $K_s$  value is an indicator of a high level of biodegradability as well. This shows that wastewater with a concentration of 100% has a better level of biodegradability than the substrate concentration of 50% and 75%.

Plot the relationship of  $1/e$  to  $s_0 - s_e / \theta \cdot x$  from linear regression  $y$  Plot the relationship of  $1/e$  to yield a coefficient of  $k_d = 0.3519$  (day<sup>-1</sup>) for a concentration of 50%,  $k_d = 0.8757$  (day<sup>-1</sup>) for a concentration of 75%,  $k_d = 1.7944$  (day<sup>-1</sup>) for 100% concentration. The cause of the death phase is usually because microbes consume all the nutrients in the media and are not sufficient for sustaining microbial life. From the results of the linear regression plot, it is known that the  $k_d$  at a concentration of 50% is faster than the  $k_d$  at other concentrations.

This indicates the affinity the organism has for nutrients.  $k$  is the maximum substrate utilization rate/unit of bacterial mass. The results of the linear regression plot of the relationship between  $1/S_e$  and  $\theta \cdot X/S_0 - S_e$  resulted in a coefficient of  $k = 0.5038$ /day for a concentration of 50%,  $k = 1.5686$ /day for a concentration of 75%,  $k = 1.1086$ /day for a concentration of 100%. From the results of the linear regression plot, it is known that the value of  $k$  at a concentration of 50% is faster than the value of  $k$  for a concentration of 75%, 100%. This indicates the affinity the organism has for nutrients.

#### *Evaluation of Monod Parameter Kinetics*

The kinetic model is used to predict the performance of the Aerobic Reactor and evaluate the substrate removal rate. The Monod model describes the rate of microbial growth in a limited substrate (Tchobanoglous, et al., 2003). The accuracy of the Monod model was checked using the regression coefficient ( $R^2$ ). The value ( $R^2$ ) is said to be good if the value ( $R^2$ ) is close to 1 (Halimi et al., 2014; Manache & Melching, 2008; Vijayalakshmi et al., 2018). Based on

Figure 3. and Figure 7. the regression coefficient ( $R^2$ ) of the four concentrations of tofu liquid waste is  $R^2 > 0.75$ , this indicates that the kinetics of the Monod model is suitable for use in the nitrification process for the degradation of tofu liquid waste by using an Aerobic biofilter bioball media reactor for pilot plan scale. The parameter values obtained from the results of this study can be used as a reference in the design of the tofu wastewater treatment unit for determining variations in process variables such as flow rate, reactor volume, and substrate concentration so that low effluent is produced.

## Conclusion

Based on the results of the research conducted, several conclusions were obtained as follows. The most optimum microbial growth kinetics parameter values at 100% concentration were ( $k$ ) 1.1086 (day<sup>-1</sup>), ( $K_s$ ) 1.0564g L<sup>-1</sup>, ( $Y$ ) 5.4862 mg MLVSS/mg, ( $k_d$ ) 1.7944 (day<sup>-1</sup>), ( $\mu_m$ ) 0.4663 (day<sup>-1</sup>). The regression coefficient ( $R^2$ ) of the four tofu liquid waste concentrations  $R^2 > 0.75$  obtained from the results of this study, can be used as a reference in the design of the waste treatment unit in terms of determining variations in process variables such as flow rate, reactor volume, and substrate concentration to get a low effluent. The nitrification process results in optimum nitrite and nitrate production at a concentration of 100% effluent water for the anaerobic tofu industrial waste process, therefore after this nitrification process, a denitrification process must be carried out as a continuation of the nitrification process to remove nitrogen content.

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