NITRIFICATION KINETICS IN AQUACULTURE WASTEWATER TREATMENT USING BATCH REACTOR

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Abstract: Accumulation of aquaculture wastewater in pond that contains ammonium can disturb the metabolism of the body and cause death in freshwater prawns. Therefore, ammonium should be removed through nitrification on the suspended growth bioreactor such as batch reactor. The objective of this study was to evaluate the removal efficiencies of ammonium, to determine kinetics of biomassa growth, and to determine nitrification rate through kinetic constant and reaction order. Five laboratory scale batch reactor with working volume of each reactor 12,5 L were fed with synthetic wastewater that already contains mixed culture bacteria. This research was carried out with different ammonia nitrogen concentration : 1, 2, 3, 4 and 5 mg NH_3-N/l. The parameter observed during the process were temperature, pH, DO (Dissolved Oxygen), COD (Chemical Oxygen Demand), VSS (Volatile Suspended Solids),TAN (Total Ammonia Nitrogen), nitrite, and nitrate. The results showed that ammonia nitrogenremoval only occured on the first four days of operation reactor with the greatest removal efficiencyin R2(2 mgNH_3-N/l) wasabout 25.74%. Nitrite accumulation did not occur during reactoroperation. In addition, from this research obtained μ_m was 0.038 day^{-1} and Ks was 0.189 mg/l.
keywords: aquaculture wastewater, ammonium, nitrification, batch reactor, kinetics

INTRODUCTION

Fish farming(aquaculture) is a food production system whose development is fastest in the world. Such activities are not only performed by the system cages, floating net and embankment., but can also be done with the pond system. Based on statistical data of marine and fisheries (2013), the result of an aquaculture production in Indonesia in 2012 reached1,433,820 tons. The production has increased 27.2% compared with production in 2011, was1,127,127tons.One of the freshwater fishery commodities with the potential to be cultivated commercially is prawns, known as the giant fresh water prawn.

Prawns (*Macrobrachium rosenbergii*) including freshwater shrimp native to Indonesia because there are19 species of the genus Macrobrachium. Prawns are the most popular freshwater shrimp because of its large size and has a high economic value in both the domestic and overseas markets. Water quality is very important forprawns rearing. In Indonesia, many prawns rearing using static systems (rearing without water exchange). One of the problems on static system is a decrease water quality. It is due to aqua culture wastes derived from metabolic processes excretedthrough the gills in the form of ammonium ion(NH_4^+) (Salim and Sadafule, 2013). In addition, ammonium is generated through organic matter decomposition by microorganisms in the water (Fernandes etal., 2010). Research carried out by Gunawan(2007), showed that the rearing of prawns on a static system having an ammonium concentration between0.56 to 3.03mg/L, while the recirculation system between0.07 to 0.38mg/L. Prawns can tolerate ammonium levels of up to1mg/L. Ammonium levels above 1mg/L can disrupt the body's metabolism and cause death in prawn(Barajas et al., 2006). Therefore, ammonium removal from aqua culture wastewater is necessary to improve water quality.

One of the alternatives for ammonium removal is biological treatment. The treatment is environmentally friendly, economical, effective, and efficient and has the potential for wide applications. Ammonium removal can be achieved through nitrification. Nitrification is the process of converting ammonium into nitrate by microbes (Bitton, 2011). Nitrification can held on the suspended growth reactor, the microorganisms that are responsible for wastewater treatment is maintained in a suspended condition in a liquid such as in a batch reactor.

Batch reactorisassumed to be perfectlymixedso that the entire reactor content is homogenous at any given time. Based on research that has been carried out by Escobaretal (2002), a batch reactor can be used for ammonium removal in waste milk with ammonium initial concentration of 11.7mg/L and the final concentration of ammonium is 0mg/L. Batch reactor is often used to estimate the kinetic parameters because it required operating time is relatively short (Jih et al., 2008). However, information on the value of kinetic parameters for aqua culture wastewater treatment with batch systems have not been found. Based on this, it is necessary to research about aqua culture wastewater treatment using a batch reactor so that ammonia nitrogen can be remove through nitrification and treated wastewater can be used for aquaculture.

RESEARCH METHODOLOGY

This research was conducted in several stages beginning with problem identification and study of literature so that the research can be designed in appropriate action base on literature.In this research, there are 5 units batch reactors and each reactor was used for every variation of concentration. That reactor was a laboratory-scale with 15 liter total volume and 12.5 liter working volume. Batch reactor made of polypropylene (PP), which has a size of 23x23x33.8cm. Air supplycomes froman aquarium pump that supplied through air diffusers tone located at the bottom of the reactor thus enabling stirring. During operation, dissolved oxygen (DO) was maintained above 2mg/l. Schematic diagram of bacth reactor used in this research is shown in **Figure1**.

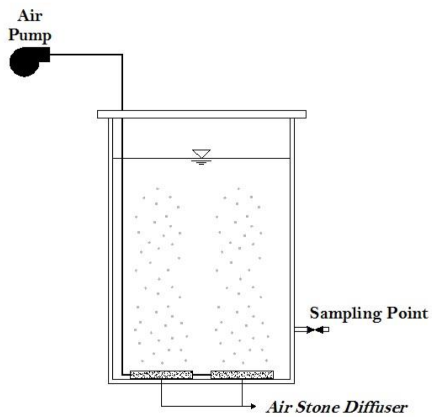


Figure1. Schematicdiagram of bacth reactor

Furthermore, synthetic wastewater used in this experiment to avoid fluctuations in influent concentration and ensure the availability of wastewater to be treated. The composition ofthe synthetic wastewater refers to Harwanto et al(2011) which consists of glucose, ammonium sulfate, sodium bicarbonate, sodium phosphate, and manganese(II) sulfate whereas concentration refers to Hai et al (2015). All the ingredients are mixed with aquadest as a solvent. In this research, synthetic wastewater for ammonia nitrogen concentration of 5 mg NH_3-N/l requires chemicals with certain mass is shown in **Table1**.

Table1. Composition of synthetic wastewater

Composition	**Mass (gram)**
$C_6H_{12}O_6$	0.0055
$(NH_4)_2SO_4$	0.041
$NaHCO_3$	0.0704
Na_2HPO_4	0.0045
$MnSO_4$	0.0006

Each reactor filled with 8 liters of bacterial culture with a biomass concentration of 3603 mg/l was settling for 2 hours so it will separated between the supernatant and sludge.Then, the supernatant was discarded and synthetic wastewater with various concentrations of ammonia nitrogen (TAN), which is 1, 2, 3, 4and5mgNH_3-N/L was fed into each reactor so that the volume to 12.5liters. The reactor was operated in aerobic conditions to keep the dissolved oxygen(DO) above 2mg/l. At this stage, samples were taken every day with grab sampling method until it reaches a steady state and that samples were analyzed for TAN, nitrite, nitrate etc. The parameters and methods of analysis used in this research is shown in **Table2**.

Table 2. Procedure and analysis methode

Parameter	Procedure	Analysis Methode
Temperature	SMEWW-2550	Termometer
pH	SMEWW-4500-H^+B	Electrometric Method
DO	SMEWW-4500-O-G	Membrane elektrode method
COD	SMEWW-5220-C	Closed reflux
VSS	SMEWW-2540-E	Gravimetri
TAN	SMEWW-4500-NH_3-F	Phenate method
Nitrite	SMEWW-4500-NO_2-B	Colorimetric method
Nitrate	SMEWW-4500-NO_3-B	Brucine method

RESULTS AND DISCUSSION

Effect of Ammonia Nitrogen Concentration Variation on Temperature

Temperature is an important factor influencing the growth of bacteria because bacteria can live at certain temperatures. The results of temperature measurements during reactor operation is shown in **Figure2**.

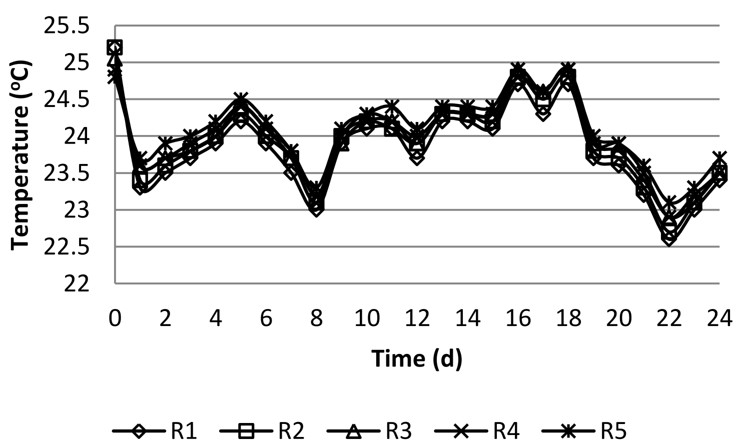


Figure 2. Temperature conditions in batch reactors

Based on **Figure2**,shows that the value of the temperature fluctuated during the24 days of running. This can be caused temperature during reactor operation did not control so water temperature will change according to air temperature. Temperature values range between 22.6-25,2˚CinR1; 22.7-25,2˚CinR2; 22.9-25,1˚CinR3; 22.9-24,9˚CinR4; and23.1-24,8˚CinR5. Optimum temperature for nitrification bacteria growth is15-30˚C(WEF, 2008). The value of the temperature during reactor operation was still in the range of15-30˚C. This indicated that the temperature inside the reactor support nitrification.

Effect of Ammonia Nitrogen Concentration Variation on pH

The degree of acidity(pH) defined as hydrogen ions(H^+) concentration in water. In biological treatment unit, pH is environmental parameters that influence bacteria activities in the substrate utilization. The results of pH measurements during reactor operation is shown in **Figure3**.

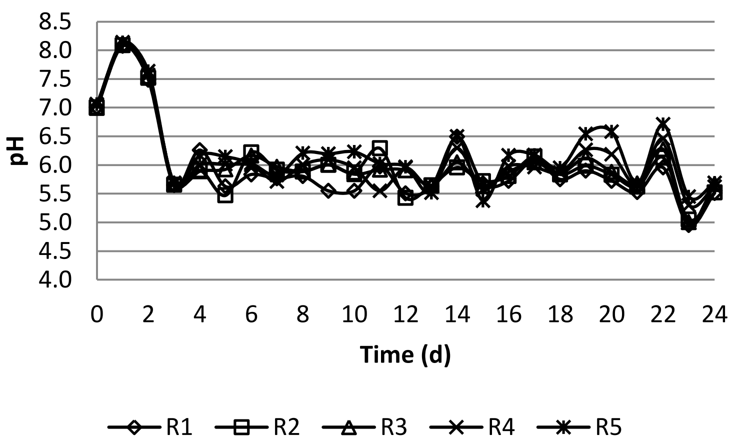


Figure 3.pH conditions in batch reactors

Based on **Figure3**, shows that the pH value in each reactor has decreased on the second day. This was due to the presence of organic matter (glucose) derived from bacterial cultures and synthetic wastewater. Glucose is a carbon source for the bacteria that very easily biodegradable. Metabolism occurred during reactor operation that causes glucose is oxidized to carbon dioxide(CO_2).Then, carbon dioxide(CO_2) reacts with water to form carbonic acid.In addition, ammonium derived from synthetic wastewater. Nitrification causes oxidation of ammonium to nitrateand generated hydrogen ions(H^+).

The presence of carbonic acid(H_2CO_3) and hydrogen ions(H^+) in water cancauses decreased ofpH values that resulted in acidic water. This can lead to inhibition nitrification in reactor such as the oxidation of ammonia nitrogen. According to Fumasoli et al(2015), the oxidation of ammonia nitrogenusually stops when the pH value drops below pH 6.

Effect of Ammonia Nitrogen Concentration Variation on Dissolved Oxygen (DO)

Dissolved oxygen needed by bacteria for aerobic respiration (Metcalf and Eddy, 2003). The results of dissolved oxygen(DO) measurements during reactor operation is shown in **Figure4**.

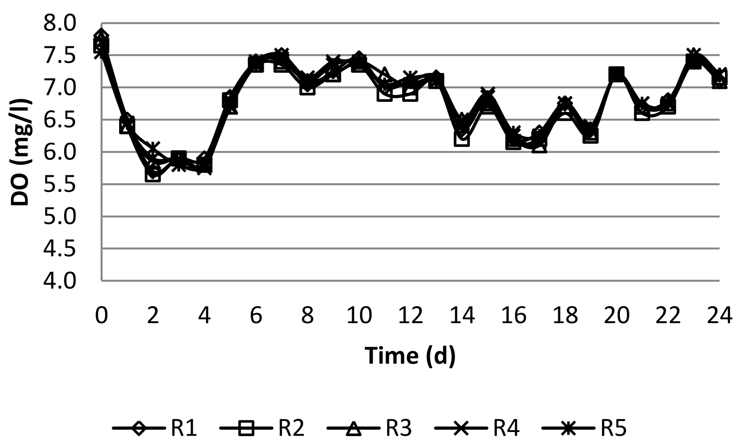
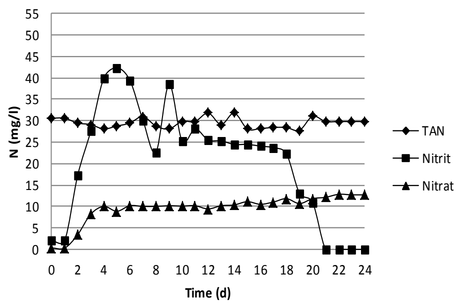


Figure 4. Dissolved oxygen (DO) conditions in batch reactors

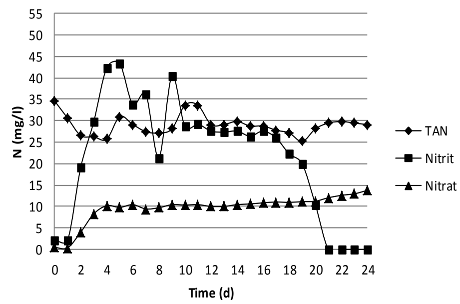
Based on **Figure4**, it was known that the concentration of dissolved oxygeninR1, R2, R3, R4, andR5 in sequence, range between5.7 to 7.8mg/l; 5.6 to 7.7mg/l; 5.8 to 7.8mg/l; 5.7 to 7.8mg/l; and5.8 to 7.6mg/l. Then, dissolved oxygen concentration decreased until a second day in each reactor. On the 3rd day until the 8th day, dissolved oxygen concentration has increased an dissolved oxygen concentration tends to be stable on the 8th day to 24thday. Decreased of dissolved oxygen concentration caused due toon aerobic biological treatment, bacteriause oxygen for oxidation organic and ammonia nitrogen resulting decreased of dissolved oxygen concentration. Increased of dissolved oxygen concentration that occurred on the 3rd day until the 8th day because most organic and ammonia nitrogen was oxidized on the previous day so that the organic compounds and ammonia nitrogen that was oxidized on the 3rd day until the 8thhave lower concentration that causedecreased oxygen required by bacteriafor oxidized organic and ammonia nitrogen. Although there has been decreased dissolved oxygen concentration during reactor operation, but it was not below 2mg/l. Thus, oxygenwas not a limiting factor in oxidation of organic and ammonia nitrogen.

Effect of Ammonia Nitrogen Concentration Variation on TAN, Nitrite, and Nitrate

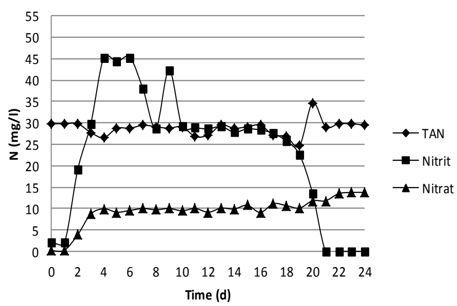
The pH value of each reactor has decreased below pH 7 during reactor operation. According to Murti et al(2013), ammonia nitrogen at pH5-7 is in the form of ammonium ion($NH4^+$). The results of ammonia nitrogen, nitrite, and nitrate measurements during reactor operation is shown in **Figure5**.



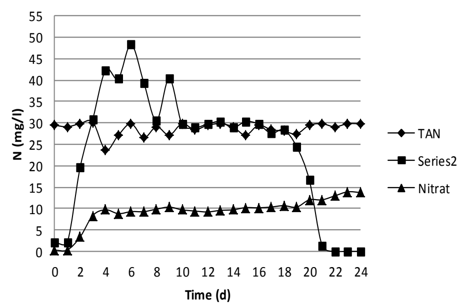
a. R1 (1 mg NH_3-N/l)



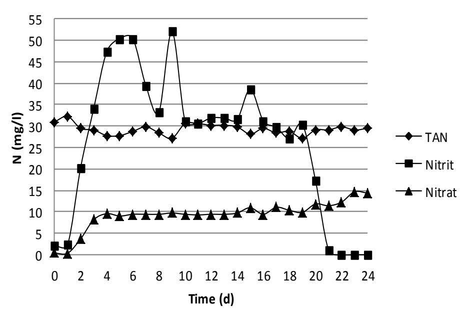
b. R2 (2 mg NH_3-N/l)



c. R3 (3 mg NH_3-N/l)



d. R4 (4 mg NH_3-N/l)



e. R5 (5 mg NH_3-N/l)

Figure 5. Inorganic nitrogen concentration in batch reactors

Based on **Figure5**, it can be seen that ammonia nitrogen concentration decreased until the 4th day was 8.19% in R1; 25.74% in R2; 10.97% in R3; 19.68% in R4; and10.36% in R5. Then, ammonia nitrogen concentration tend to fluctuated until the 20th day and tend to stable on21thday to 24thday. Decreased of ammonia nitrogen concentration can be caused by oxidation of ammonia nitrogen to nitrite. This indicated that nitrification occurred in reactor. From Figure5, shows that ammonia nitrogen concentration of each reactoron the 5thday to the 24thday still high which indicated that oxidation of ammonia nitrogen was inhibited. This was due to the decreased of pH value below pH 6 on the 3rd day. According to Fumasoli et al(2015), the oxidation of ammonia nitrogen usually stops when the pH value drops below pH 6.

Increased of nitrite concentrations began on the 2nd day was 88.21% in R1; 88.66% in R2; 88.31% in R3; 88.65% in R4; and 88.53% in R5. This indicated that ammonia nitrogen was oxidized to nitrite. In each reactor, nitrite concentration began to decreased on the 6thday. Decreased of nitrite concentration followed by increased nitrate concentration because nitrite will be oxidized to nitrate. Based on **Figure5**, it was known that there is no accumulation of nitrite during reactor operation. This was due dissolved oxygen concentration in the reactor more than 2mg/l so that oxidation of nitrite to nitrate run well.

Nitrate concentration increased on the 2ndday was90.61% inR1; 90.26% in R2; 91.24% in R3; 89.43% inR4; and89.93% inR5. This indicated that nitrification occured in the reactor because the end product from nitrification was nitrate which is formed as a result from nitrite oxidation.

In autotrophic nitrification, *Nitrobacter* play a role in oxidation of nitrite to nitrate. According to the EPA(2002), the optimum pH for *Nitrobacter* growth is7.5-8. However, oxidation of nitrite to nitraterun well despite the pH values was decreased. This indicated that oxidation of nitrite to nitrate in the reactor did not involve autotrophic bacteria (*Nitrobacter*), but it involved heterotrophic bacteria. According to Zhang et al(2015), heterotrophic nitrification play apredominant role in nitrate production. In addition, heterotrophic nitrification is carried out by bacteria that tolerant to acid (Allison and Prosser, 1993). So, mixed culture used in this researchwasheterotrophic bacteria.

Effect of Ammonia Nitrogen Concentration Variationon COD

COD(Chemical Oxygen Demand) defined as amount of oxygen required to oxidize organic compounds. The results of COD measurements during reactor operation is shown in **Figure6**.

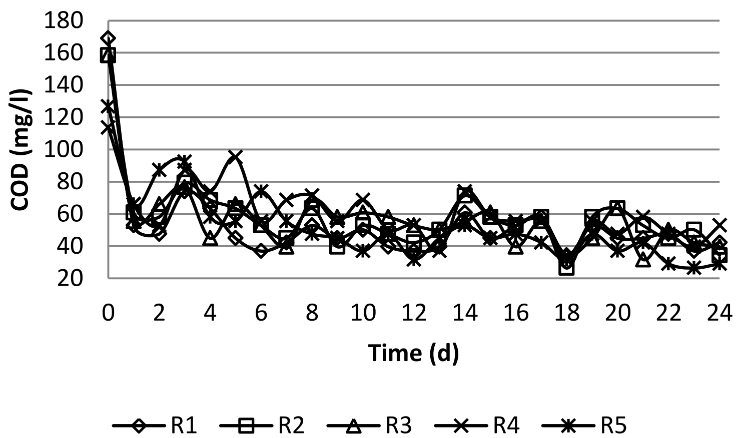


Figure 6.COD concentrationin batch reactors

Based on **Figure 6**, shows that organic compounds concentration measured as COD decreased until 24thday was 75% in R1; 78.33% in R2; 75% inR3; 53.49% inR4; and77.08% inR5. This indicated, bacteria utilized organic compounds(glucose) as a substratethat occured oxidation of organic compounds and result indecreased organic compounds concentrations. Concentration of substrate was the less that lead to bacteria death. According to Mulyani et al (2012), the death of bacteria can increase soluble COD concentration.

When reactor operation from the 1stday to 24thday, organic compounds in each reactor concentration, range between 31.68 to 79.2 mg/l inR1; 21.12 to 79.2 mg/l in R2; 26.4 to 79.2mg/l in R3; 31.68 to 100.32mg/l in R4; and 21.12 to 95.04 mg/l in R5. COD concentration greater than 60mg/l will influence nitrification (Celenza, 2000). However, the process that occurred in the reactor was heterotrophic nitrification. Research conducted by Zhao et al (2012), showed that conditions with glucose and NH_4^+as an organic carbon source and nitrogen source cause the growth of *Alcaligenesfaecalis* (heterotrophic nitrifier). While the condition absence of an organic carbon source or in the presence of carbonate as the sole carbon source cause failed and no nitrite or nitrate could be detected. Thus, the presence of organic compounds required in heterotrophic nitrification.

Effect of Ammonia Nitrogen Concentration Variation on VSS

Biomass concentration during reactor operation can be measured by VSS (Volatile Suspended Solid). That parameter defined as many organic compounds that suspended in water. The results of VSS measurements during reactor operation is shown in **Figure7**.

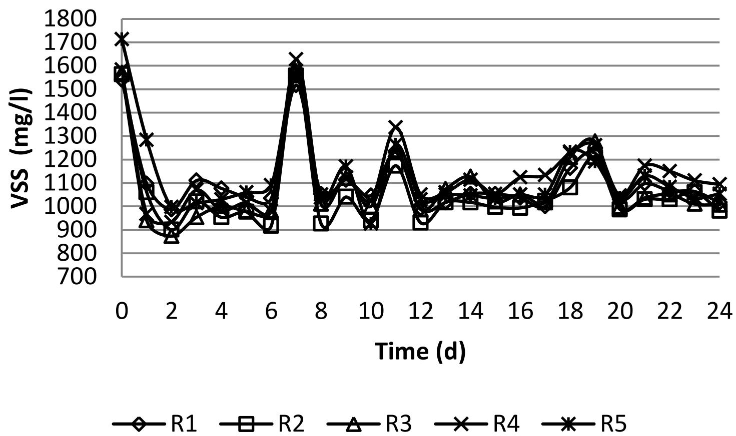


Figure 7.VSS concentrationin batch reactors

VSS concentration at the beginning was 1540mg/l inR1, 1563mg/l in R2, 1570mg/l in R3, 1583mg/l in R4, 1713mg/l inR5. According to Bitton (2011), nitrification will perform optimally when biomass concentration between1200 to 2500mg/l. VSS concentration at the beginning of reactor operation support nitrification, but during reactor operation VSS concentration tend to fluctuated. Based on **Figure7**, it can be seen that VSS concentration decreaseduntil the 2ndday was 35.93% in R1; 42,43% in R2; 44.37% in R3; 41.05% in R4; and41.83% in R5. This can be caused bacteria did not get enough substrate to support of bacteria growth and resulted death of bacteria then bacteria cells lysis. According to Sudol et al(2010), lysis of bacterial cells can increase soluble COD. Furthermore, VSS concentration of each reactor increased on the 7th, 9th, 11th, 18th, and 21th day.This was due to bacteria utilized organic compounds so that the growth of bacteria measured as VSS has increased while the concentration of organic compounds measured as COD has decreased on the 7th, 9th, 11th, 18th, and 21th day. Although VSS concentration during reactor operation had been below 1200mg/l, but nitrification can be accomplished

Bacterial Growth Kinetic

Kinetics of bacterial grow this determined by observing bacteria growth versus time on a log phase. Analysis of bacterial growth kinetic in batch system was described by Monod model is shown in **Figure8**.

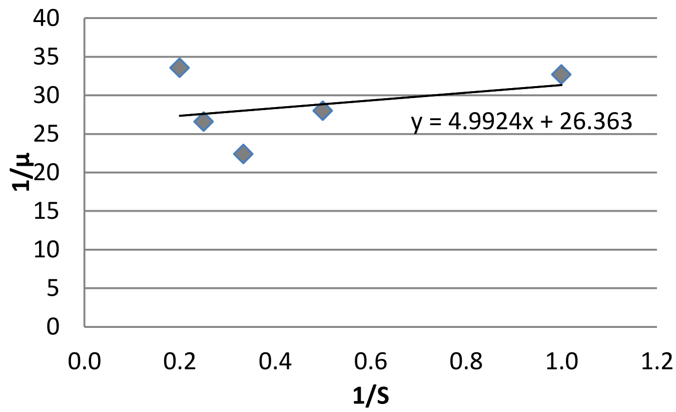


Figure 8. Lineweaver-Burk plot for determination of $\mu_m$and Ks

Based on **Figure8**, the value of the maximum specific growth rate (μ_m) was 0.038day^{-1}and a half-saturation constant value(Ks) was 0.189mg/l. According toward et al(2011),the maximum specific growth rate(μ_m) for nitrification bacteria is 0.62 to 0.92day^{-1}. The $\mu_m$value was smaller and indicated that the maximum growth rate of bacteriawas slow. This can be caused oxidize ammonia nitrogen required a long time. According Bitton (1998), the value of $\mu_m$is influenced by the type of bacteria and environmental conditions of growth such as pH. In addition, the value of Kswas 0.189mg/l. Ks value showed an affinity of bacteria to substrate. According to Bejarano(2005), the Ks value for nitrification range between0.15 to 2mg/l. The results showed that the value of Kswas in the range0.15 to 2mg/l.

CONCLUSION

Ammonia nitrogen removal only occured during four dayswiththe greatest removal efficiency in R2(2 mgNH_3-N/l) was25.74%. This was due to the decreased of pH value below pH 6 on the 3rd day so that the oxidation of ammonia nitrogen to nitrite can be stopped. In addition, nitrite accumulation did not occurduring reactor operation. Nitrification occured within the reactor involves heterotrophic bacteria. Furthermore, the value of the maximum specific growth rate (μ_m) was 0.038day^{-1}and a half saturation constant (Ks) was 0.189mg/l. This indicated that bacteria can not utilize the substrate available because unfavorable environmental conditions(pH) so that the maximum growth rate of bacteria was slow.

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