Toxicity Effects of Organic Substances on Nitrification Efficiency

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Abstract. Ammonia content in wastewater causes eutrophication in water bodies due to the biological ammonium oxidation process that is by the nitrification process. In this study, the aim was to determine the concentration and maximum efficiency of biodegradation of ammonium in the nitrification process using the help of *Nitrosomonas* and *Nitrobacter* bacteria with carbon sources of autotrophs and heterotrophs. The results of this study indicate that the highest value of nitrification efficiency was achieved by heterotrophic carbon sources with the addition of a catalyst namely H1 with a concentration of 60 mg/L at 30.49%, followed by H1 with a concentration of 80 mg/L at 27.32% and the third largest was 26.12% with the addition of H3 at a concentration of 20 mg/L. However, it should be kept in mind that heterotrophic nitrifying bacteria have a much lower activity level than autotrophic bacteria. This shows that the source of heterotrophs without catalysts had the least efficiency at 0.85% with H3 concentrations of 80 mg/L compared to carbon sources of autotrophs with the smallest efficiency of 3.32% in H2 concentration of 20 mg/L.

Keywords: Ammonium, Autotrophs, Heterotrophs, Catalysts, Nitrification

1. Introduction

In general, it is believed that biological ammonia degradation occurs through the nitrification process which has two stages of reaction, namely oxidation of ammonium (NH₄⁺) to nitrite (NO₂⁻) by *Nitrosomonas* bacteria and oxidation of nitrite to nitrate (NO₃⁻) by *Nitrobacter* bacteria. Both of these bacteria are known as chemoautotrophic bacteria which use inorganic nitrogen compounds as energy sources and live in aquatic and soil environments that have sufficient inorganic nitrogen (Wielgosz et al., 2010). In the process of nitrification, it is actually not only carried out by chemoautotrophic bacteria, but also various other microorganisms, such as heterotrophic bacteria which have the ability to oxidize various components of nitrogen (Agustiyani, 2010). Besides, the ammonia oxidation process that requires large amounts of oxygen can cause dissolved oxygen concentrations in the waters to be low, and a condition like this is very dangerous for aquatic organisms (Hibban, 2016; Mangkoedihardjo, 2007, 2010; Samudro and Mangkoedihardjo, 2012). Therefore, the degradation process of nitrogen compounds, mainly ammonia, is very important in a liquid waste treatment system.

In surface water, ammonium levels are less than 10 mg/L while in wastewater ammonium levels reach 30 mg/L or more (Titiresmi and Sopiah 2006). Ammonium accumulation in water will have a negative impact on the environment, causing ecological problems and health of living things around it, such as eutrophication. According to the Government Regulation of the Republic of Indonesia No. 82
of 2001 concerning Management of Water Quality and Water Pollution Control, the first standard quality of ammonium for water is 0.5 mg/L NH₃-N. According to Widayat (2010), at a concentration of 1 mg/L NH₃, some types of fish will suffocate because ammonium can reduce oxygen concentration in water. Efforts to reduce ammonium concentration in water can be done by nitrification process. However, autotrophic nitrifying bacteria are very sensitive to environmental factors and grow very slowly so that their populations in activated sludge are often competed by heterotrophic microorganisms.

In ecotoxicology it is known that toxic substances in the form of organic chemicals can be toxic or cause adverse effects or negative effects on the aquatic environment. The negative effects of organic chemicals on aquatic organisms are influenced by many factors, such as the concentration of chemical compounds, the physical-chemical quality of water, the type and condition of aquatic organisms and the length of time the organisms are exposed to these chemicals (Aryani et al., 2004). Nitrifying bacteria will negatively affect the nitrification process and become sensitive of toxicity, pH, temperature, oxygen, substrate concentration and the presence of organic material (Wiesmann, 1994). So, this study examines the effect of ammonium addition which has varying concentrations and carbon sources which show the effectiveness of the nitrification process in the reactor. With this research, it is expected to determine the efficiency of nitrification and carbon sources that are effective in the nitrification process.

2. Materials and Methods

2.1 Seeding
The bacteria used in this study were bred first. Seeding was done using Nutrient Broth (NB) liquid media which was then incubated by using a shaker for 48 hours at a temperature of 28°C with a speed of 115 rpm. Nitrifying bacteria require oxygen and food to live and build colonies in the media so that nitrifying bacteria’s replication is lengthy compared to other bacteria’s. In freshwater ponds, bacteria need 8 hours to replicate, while in sea water it takes even longer, around 24 hours. (Budiyanto, 2011)

2.2 Artificial Waste
The selection of this variation is based on the literature which states that the ratio C:N:P must be in the range between 100:10:1 and 100:5:1 (Alexander, 1994). Therefore, the variation used for this study was the ratio of 100:5:1. The composition of nutrient for reactors which were heterotrophic was a mixture of 18 grams of Glucose Monohydrate (pro-analyst) with 0.2675 gram of NH₄Cl and 0.136 grams of KH₂PO₄. While in the mixture for autotrophs was 0.2675 grams of NH₄Cl and 0.136 grams of KH₂PO₄. Three grams of active catalyst Fe(NO₃)₃ 9 H₂O (Sigma-Aldrich) was also added. The mixture was dissolved and diluted with tap water.

The addition of nitrifying bacteria, as needed, was at 4% of the reactor, which was filled with 3 L, so the bacteria needed was 120 mL per reactor. Each reactor also contained 60 mL of Nitrosomonas bacteria and 60 mL of Nitrobacter. In addition, there were several reactors which were given the addition of an iron solution catalyst. The catalyst content added was 1% of the reactor, which was 30 mL per reactor.

2.3 Reactor Assembly
The nitrification reactor used was an aerobic batch reactor. 22 reactors were used, each made of a plastic container with a size of 5L but were only filled with 3L.

The nitrification reactor was made open with the presence of an aerator to supply oxygen to the nitrifying microbes continuously. Aerators used in aerobic reactors were Air pumps type AT410 with the following specifications: voltage: 220 V, frequency: 50 Hz, power: 5W and capacity: 2 X 4 L/minute. Oxygen supply would be supplied with an ¼ inch plastic hose.
2.4 Nitrification Efficiency

Data processing was done by calculating the efficiency percentage of ammonium removal. The efficiency percentage for the provision of these compounds was calculated by the following formula:

\[
\text{Nitrification Efficiency (\%)} = \left( \frac{(NH_4)^{in} - (NH_4)^{eff}}{(NH_4)^{in}} \right) \times 100\%
\]  

Where:

- \((NH_4)^{in}\) = influent (initial incubation / 0 hours)
- \((NH_4)^{eff}\) = effluent (end of incubation / t hours)

3. Results and Discussion

3.1 Bacterial Growth Rate

The bacterial growth rate is the ability of certain bacteria to grow in a reactor. At this stage, Optical Density (OD) was analyzed using a Spectrometer to determine the OD of bacterial culture that had been planted in liquid NB media and in a shaker for 48 hours. The wavelength used was 600 \(\lambda\). The results of Optical Density for Nitrifying bacteria are shown in Table 1 below.

| No. | Name of Bacteria | Optical Density Result |
|-----|------------------|------------------------|
| 1   | *Nitrosomonas*    | 1.848                  |
| 2   | *Nitrobacter*     | 1.559                  |

Based on Table 1 above, the test results show that *Nitrosomonas* bacteria experienced the highest growth, indicated by high turbidity and the ability to reduce ammonia which was also quite high. This is according to Respati (2017), that the results obtained at the time of the initial Optical Density (OD) if the optimal absorbance value has shown value 1.

3.2 Results of Ammonium Removal Efficiency

Calculations of efficiency percentage were carried out on ammonium which had different concentration variations. The efficiency calculation was done once every two days for six consecutive days so that there were three efficiency results, namely H1 for the efficiency of the first and second days, H2 for the efficiency of the third and fourth days and H3 for the efficiency of the fifth and sixth days.

The highest efficiency results were in the Heterotroph carbon source with the addition of Catalysts in H1 with a concentration of 60 mg/L at 30.49%. This was followed by H1 with a concentration of 80 mg/L at 27.32% and the third largest was 26.12% in H3 at a concentration of 20 mg/L. In accordance with Agustiyani (2004) research that ammonia oxidation activity results in higher nitrite in media containing acetate organic carbon or heterotrophic because bacteria are able to utilize organic compounds as carbon sources. In line with the opinion of Agustiyani (2004), Jenie and Rahayu (1993) stated that in addition to autotrophic nitrifying bacteria, there are also heterotrophic bacteria which are capable of using organic compounds, also able to utilize inorganic nitrogen, such as ammonia, as electron donors and energy sources.

However, it should be kept in mind that heterotrophic nitrifying bacteria have a much lower activity than autotrophic bacteria (Ambarsari, 1999). This shows that heterotroph without catalyst carbon sources has the least efficiency efficiency of 0.85% in H3 concentrations of 80 mg/L compared to carbon sources of autotrophs with the smallest efficiency of 3.32% in H2 concentrations of 20 mg/L. Ammonium removal efficiency can be seen in Figure 3 to 6 as follows.
Figure 1. Autotrophic Nitrification Process Efficiency

Figure 2. Heterotrophic Nitrification Process Efficiency

Figure 3. Autotrophic Nitrification Process Efficiency with Catalysts
In Figures 1 to 4 it can be seen that the average value nitrification process efficiency of the carbon source autotrophs without catalysts was 12.72%. Whereas for heterotrophs without catalysts, the efficiency was 9.79%. This shows that the development of autotroph bacteria was better than that of heterotrophic bacteria, in line with Ambarsari (1999) which states that heterotrophic nitrifying bacteria have much lower activity than autotrophic bacteria. A condition like this can also be related to the degree of acidity (pH) which is one of the environmental factors that influence the growth and activity of ammonia oxidizing bacteria (Esoy et al., 1998).

From the results of the pH parameter test, it can be seen that the autotrophic carbon source had a pH ranging from 7.0 to 8.5, whereas heterotrophic carbon sources had a pH ranging from 3.0 to 7.5. Heterotrophic bacteria are more tolerant to acidic environment and grow faster with higher yields in conditions with low DO concentrations (Zhao et al., 1999).

The efficiency of ammonium concentration decrease is in line with increase in concentrations of nitrite and nitrate. It can be seen that the number of bacterial cells increased with increasing pH in H2 and H3 and the increase started on the 4th day of the study. According Agustiyani (2004), this could be because the number of bacterial cells had reached an optimal value at pH 7-8. The results of measurements of total DO showed a range of DO 3.1-5.2 mg/L. Total DO was maintained so that it was always more than 1 mg/L in order for the nitrification process to run well (Revelation 2010).

At pH 8, bacterial cell growth for heterotrophs was very good. Like most nitrifying bacteria, ammonia oxidizing bacteria prefer alkaline environments with optimal pH levels for growth ranging from 7.5 to 8.5 (Imas et al., 1989 and Ambarsari, 1999).

While at pH 5 and below, the growth and oxidation activity of ammonium by heterotrophic bacteria decreased, and this indicated an inhibition. This occurred at pH in H2, on Days 3 and 4. At low pH, cell membranes would become saturated by hydrogen ions thus limiting membrane transport. Poisoning that would occur at low pH could be because some acidic substances that did not decompose would soak into the cell, so that ionization occurred and the pH of the cell changed. This change would cause the process of sending amino acids from RNA to be inhibited so that it would inhibit growth and could even kill microbes. (Agustiyani, 2004).

From this study it was found that oxidizing ammonia showed much better growth and activity in media containing acetate. This fact shows that nitrifying bacteria is an ammonia oxidizer which will be more efficient if it is heterotrophic and with the addition of a catalyst which obtains the highest efficiency value.
4. Conclusion
This research on the toxicity effects of organic substances on nitrification efficiency revealed that the carbon source of heterotrophic with the catalyst in H1 scored the highest concentration in the can at 60 mg/L with an efficiency of 30.49%. This shows that the oxidation activity of ammonia into nitrite was higher in media containing acetate organic carbon or heterotrophic because the bacteria were able to utilize organic compounds as their carbon source especially with the addition of catalysts. However, the efficiency was not high due to the lack of hourly analysis of the rate of bacterial development. Such an analysis opens a possibility for further research.

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