Effects of Organic Substance on Denitrification Efficiency

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Abstract. Toxicity can refer to the impact on all organisms, such as animals, bacteria, or plants. Other effects can occur in the substructure of an organism, such as cells or organs. This shows that toxicity can cause problems for the environment. In this study artificial sample, denitrifier and several reagents were used to analyze the main parameters, namely Dissolved Oxygen (DO), pH, Nitrate (NO$_3^-$-N). The variables used were variations in nitrate concentration, process variation, and administration of selenium as a catalyst. Variations in nitrate concentration used were 20 mg/L, 40 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. The process variations used were the autotroph and heterotroph processes. The reactor used was a batch system denitrification reactor. The microorganism used was \textit{Thiobacillus denitrificans}. Research data were taken every day for Dissolved Oxygen (DO), pH, Nitrate (NO$_3^-$-N). This study aims to determine the efficiency of the denitrification process by autotrophs and heterotrophs using both catalysts and without catalysts. Of the two types of processes, autotrophic denitrification was efficient for processing with low nitrate concentrations. The highest efficiency value reached was 89%. Denitrification of heterotrophs was better able to process nitrates with high concentrations. The value of efficiency using this process reached 92%.

Keywords: Denitrification, Efficiency, Organic Substance, Toxicity

1. Introduction

Toxicity in general is the level of damage to a substance if exposed to organisms. Toxic substances that can cause toxic effects are called toxicants. Toxicity can refer to the impact on all organisms, such as animals, bacteria, or plants. Other effects can occur in the substructure of organisms, such as cells or organs such as the liver. Toxicity shows an ecological response that depends on the situation. The presence of toxicity will affect the denitrification process. In this study we examined the toxicity effects of organic matter on the effectiveness of denitrification. The toxicant used was derived from a solution of KNO$_3$. KNO$_3$ is used as a nitrogen source (Nugroho, 2003). The excess accumulation of nitrate (NO$_3^-$) in drinking water is harmful to human health because of its contribution to methemoglobinemia, gastric cancer, and non-Hodgkin lymphoma (Sunger and Bose in Wang et al., 2012).

The variables used in this study were variations in nitrate concentration, process variation, and use of catalysts. The process variations used were autotroph and heterotroph, while the catalyst used was selenium. The process of denitrification with the help of microbial autotrophs was enough to use inorganic materials and carbon sources from CO$_2$. One type of microbe is \textit{Thiobacillus denitrificans},
which can reduce nitrate to nitrogen gas. The process uses heterotrophic microbes, namely by adding organic matter as a hydrogen donor into waste water (Nugroho, 2003).

2. Methods

2.1 Microbial Preparation
The microorganism used in this study was *Thiobacillus denitrificans*. The microbe was in a culture solution that was ready for use. The volume of microbes prepared was 150mL. The microbes used in this study were 5% of the reactor volume.

2.2 Artificial Preparation
Waste used was artificial waste derived from chemicals made from pro analyst (pa) or pure. This study did not use case studies of domestic waste in purpose because by using artificial waste we could make the desired variation of concentration. Ingredients to make this artificial waste included glucose, KNO₃, and KH₂PO₄. Variations in concentration in each reactor were 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. A C:N:P ratio of 250:5:1 was used to maintain nutrient requirements of bacteria.

In the autotroph denitrification reactor, the material used was only KNO₃ as a source of N and KH₂PO₄ as a source of P. While in heterotrophic reactors, the materials used were glucose, KNO₃, and KH₂PO₄. The waste made for each reactor was 1.8 liters, which consisted of one liter of the composition of nitrate concentration and 800 mL of nutrient composition.

2.3 Reactor Construction
The denitrification reactor was made of 2 L plastic jars with 22 pieces. The reactor circuit included 2 control reactors for autotrophs and heterotrophs respectively. Five autotroph process reactors without the addition of a catalyst, five heterotrophic reactors without the addition of a catalyst, five autotroph reactors with the addition of a catalyst, and five heterotrophic reactors with the addition of a catalyst. The catalyst used was selenium with a ratio of 1% reactor volume. Anaerobic reactors were made closed, maintained no oxygen and covered with rubber caps (Putri et al., 2013). The reactor was connected to a 1000 mL measuring cup using a ¼-inch hose. In a reactor that required a catalyst, 10 mL of catalyst was added. An illustration of the reactor used can be seen in Figure 1.

![Figure 1. Illustration of the reactor used in the research](image)

2.4 Denitrification Efficiency
Denitrification efficiency was calculated using the following formula:

\[
\text{Efficiency (\%)} = \frac{\text{NO}_3\text{(in)} - \text{NO}_3\text{(out)}}{\text{NO}_3\text{(in)}} \times 100\% \tag{1}
\]
Where:
\[ \text{NO}_3 \text{(in)} = \text{NO}_3 \text{ influent} \]
\[ \text{NO}_3 \text{(out)} = \text{NO}_3 \text{ effluent} \]

3. Results and Discussions

3.1 pH Measurement

The degree of acidity (pH) is one of the environmental factors that influence bacterial growth and activity (Esoy, et al., 1998). Denitrification is most effective at pH between 7.0-8.5 and optimal at around pH 7.0. Alkalinity and pH rise during denitrification (Herlambang & Marsidi, 2003). In this study, the measured pH ranged from 4.2 to 8.92. At the autotroph reactor the pH was measured between 7.1-8.9. According to Ratledge (1994), the optimum acidity (pH) for the growth of autotrophic ammonia oxidizing bacteria ranged between 7.5 and 8.5. Whereas in heterotrophic reactors the measured pH ranged from 4.2-6. This shows that heterotrophic bacteria are more tolerant to the acidic environment, and grow faster with higher yields in conditions with low dissolved oxygen concentrations (Zhao, et al., 1999).

3.2 Dissolved Oxygen (DO) Measurement

DO measurement aims to find out whether the process that runs in the reactor is at low DO. The denitrification process occurs anaerobically. The anaerobic process occurs in low DO. Therefore, the process of denitrification requires a closed bioreactor that does not come into contact with outside air. In this study, the measured DO values ranged from 0.7 to 4.6. Dissolved Oxygen (DO) level which was measured quite high would be due to air contact during sample measurement. The dissolved oxygen level fluctuations resulted in fluctuations of the efficiency of the denitrification process being studied.

3.3 Efficiency of Autotrophic Denitrification Process without Addition of Catalysts

The graph of efficiency can be seen in Figure 2. The 100 mg/L reactor showed the lowest efficiency. According to Scott and Crunkilton (2000), toxicity of nitrates increases with increasing nitrate concentration and exposure time. This shows that nitrate at a concentration of 100 mg/L caused toxic properties in the microorganisms. Toxicity can refer to the impact on all organisms, such as animals, bacteria, or plants. Other effects can occur in the substructure of organisms, such as cells or organs such as the liver. Toxicity shows an ecological response depending on the situation. Pollutant toxic effects can be identified through microbial expression adapted to the microbial test. In general, microbial expression can be chosen between death, cell growth disorders, disruption of cell activity, respiratory disorders and also fermentation disorders (Razif et al., 2006; Mangkoedihardjo, 2006, 2014). The highest efficiency in the reactor was 60 mg/L on day 4, or 89%.

![Figure 2. Efficiency of Autotrophic Denitrification Process without Addition of Catalyst](image-url)
3.4 Efficiency of Heterotrophic Denitrification Process without Addition of Catalyst

The highest efficiency of heterotrophic denitrification process was achieved at 20 mg/L reactor on the seventh day which was equal to 92%. On the first and second days in all reactors there were no nitrate removal so that efficiency was very low. This condition shows that one day retention time was too short for processing nitrate waste (Herlambang and Marsidi, 2003). In the 60 mg/L, 80 mg/L, and 100 mg/L reactors, the efficiency produced on the sixth day was the highest. High efficiency indicated effective nitrate removal. The efficiency chart can be seen in Figure 3.

![Figure 3. Efficiency of heterotrophic denitrification process without addition of catalyst](image)

3.5 Efficiency of Autotrophic Denitrification Process with Addition of Catalyst

In the 20 mg/L reactor, efficiency increased on the third day compared to the first and second days. However, on the fourth day there was a decline. Another increase in efficiency occurred again on the fifth day. After the fifth to seventh day, efficiency tended to be stable. This fluctuation might have occurred because the conditions in the reactor were less homogeneous. In the 40 mg/L and 60 mg/L reactors, the highest efficiency was shown on the seventh day. Graph of efficiency calculation results can be seen in Figure 4.

![Figure 4. Denitrification process Efficiency by Autotrophs with addition Catalyst](image)
3.6 Efficiency of Heterotrophic Denitrification Process with Addition of Catalyst

In this process, all reactors showed high efficiency on the seventh day, indicating that the heterotrophic denitrification process was able to run well at high nitrate concentrations. According to the results in the autotroph process, the first and second days of processing were not efficient. This condition shows that one day retention time would be too short for processing nitrate waste (Herlambang and Marsidi, 2003). Graph of efficiency calculation results can be seen in Figure 5.

![Graph of efficiency calculation results](image)

**Figure 5.** Efficiency of Heterotrophic Denitrification Process with Addition of Catalyst

4. Conclusions

Autotrophic process resulted in efficient denitrification for processing with low nitrate concentrations, with the highest efficiency reaching 89%. Denitrification of heterotrophs was better able to process nitrates with high concentrations. With the addition of a catalyst, the autotroph process produced the highest efficiency of 96% in the 20mg/L reactor on the seventh day, while the heterotrophic process produced the highest efficiency of 75% in the 60 mg/L reactor on the seventh day.

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