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Research paper

Inhibition of High Substrate Concentration of Ammonia-Nitrogen in Bio-Filter

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Abstract

Nitrification process is very important to oxidize ammonia-nitrogen to nitrite-nitrogen and nitrate-nitrogen via ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). However, the high concentration of ammonia-nitrogen would inhibit the nitrification process. The presence of excessive ammonia-nitrogen would retard the ammonia oxidation by nitrifying bacteria. Hence, the aim of this research is to evaluate the biological process of AOB in different concentration of high concentration of ammonia-nitrogen (200 mg/L to 500 mg/L) on batch culture system using bio-filter. The result showed that the percentage removal rate at concentrations higher than 400 mg/L ammonia-nitrogen would reduce the ammonia oxidation of AOB. The decay rate, k, for the concentration of 500 mg/L ammonia-nitrogen was calculated as -0.055. This value was the highest decay rate compared to the other decay rates obtained from different concentrations of ammonia-nitrogen that were analyzed. It can be concluded that the AOB in bio-filter attached growth system can tolerate the ammonia-nitrogen up to 400 mg/L.

Keywords: *ammonia-nitrogen; ammonia oxidizing bacteria; decay rate; inhibition; percentage removal.*

1. Introduction

Ammonia and nitrite oxidation in nature are carried out mainly by autotrophs of the types *Nitrosomonas sp.* and *Nitrobacter sp.* [1]. This bacteria would perform the nitrification process thus are capable of oxidizing ammonia-nitrogen and nitrite-nitrogen. This microorganism of AOB is the first and rate-limiting step for the removal of nitrogen in wastewater treatment plant [2].
The most environmental parameters influencing nitrification are the dissolved oxygen (DO) concentration, temperature, pH and free ammonia (FA) and free nitrous acid (FNA) concentration [3]. It has been reported that free ammonia (FA) had inhibition on nitrifying bacteria under aerobic and anaerobic condition [4-5]. High concentration of FA can promote nitrite accumulation in the process of nitration by inhibiting the NOB activity [4].
Parameters affecting nitrifying bacteria or their activity (nitrification) as observed by various researchers are summarized and the circumstance of observation must be considered carefully to achieve the optimal values [6]. It has been found that, both *Nitrosomonas sp.* and *Nitrobacter sp.* are sensitive to their own substrate and more so to the substrate of the other [7]. According to Anthonisen [8], the degree of inhibition depends upon the ammonia-ammonium and the nitrite-nitrous acid equilibrium. Other researchers [7, 9] support the suggestion that inhibition is due to free ammonia and un-dissociated nitrous acid; concentrations of these species have significance in the inhibition of nitrification. Jubany [9] also reported that the start-up of biological nitrifying systems to treat high-strength ammonium wastewater must be done carefully to avoid ammonium or nitrite build-up and subsequently system destabilization due to the inhibition of both substances.
Wide ranges of ammonia and nitrite ion concentrations could be oxidized by the nitrifying bacteria [10]. Differences in conditions can account for the apparent discrepancies. Normal ammonia and nitrite ion concentrations in domestic wastewaters are not in the inhibiting ranges [7]. However, substrate and product inhibition are of significance in treatment of industrial, poultry and agricultural wastes [10].
This study focused on the high concentration of ammonia-nitrogen form 200 mg/L to 500 mg/L ammonia-nitrogen in batch culture experiment. This research would demonstrate the reduction of ammonia-nitrogen concentration by AOB and NOB in bio-filter attached growth system.

2. Methodology

An experiment was carried out to investigate ammonia oxidation with high concentrations of ammonia-nitrogen, from 200 mg/L up to 500 mg/L. The analysis was carried out using the same one litre culture bottles as described in [11] with the attached growth of the batch culture on the bio-filter using K2 AnoxKaldnes packing material.
Medium A was prepared with formulation from Table 1 [11]. A sterile stock solution of $(NH_4)_2SO_4$ was prepared separately and autoclaved for 15 min. This stock solution was added aseptically to give the final concentration from 200 mg/L to 500 mg/L ammonia-nitrogen in four different culture bottles (reactor) together with autoclaved Medium A. Hence, an amount of 100 mL of fish effluent enrichment culture from the serial batch [11] was also added in the reactor. The temperature was controlled at 30°C and the pH was set to 8 [11]. The aeration was maintained above 6 mg/L, and the values were constantly checked with a dissolved oxygen probe [11].

Table 1: Nitrification Medium A [11]

Formulation of Medium A	
Formulation	Weight (g)
Na_2HPO_4	13.5
KH_2PO_4	0.7
$NaHCO_3$	0.5
$MgSO_4.7H_2O$	0.1
$FeCl_3.6H_2O$	0.014
$CaCl_2.2H_2O$	0.18

The cultured was sampled on a daily basis and the samples were analyzed for ammonia-nitrogen, nitrate-nitrogen and nitrite-nitrogen [12]. The incubation period for the high concentrations of ammonia-nitrogen was 22 days.

3. Results and Discussion

The results of the experiment are shown in Fig. 1, which shows the concentration of ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen in batch reactors with nitrifying bacteria on the bio-filter of K2 AnoxKaldnes packing material.

As shown in Fig.1, A) for the ammonia-nitrogen concentration of 200 mg/L, the value for nitrate-nitrogen increased steadily when the ammonia-nitrogen value decreased towards zero and nitrite-nitrogen was consumed by the NOB. In Fig. 1, B) for the ammonia-nitrogen concentration of 300 mg/L, nitrate-nitrogen slowly built up and the ammonia oxidation process began to slow down toward the end of the incubation period.

In Fig. 2, A) for the ammonia-nitrogen concentration of 400 mg/L and B) for the ammonia-nitrogen concentration of 500 mg/L, the ammonia oxidation process was retarded, possibly due to the high concentration of ammonia-nitrogen present in the batch reactor with the bio-filter of K2 AnoxKaldnes packing material.

According to the experimental analysis, the nitrification process was significantly affected by a high concentration of ammonia-nitrogen in the batch reactor. The ammonium ions were converted to nitrite ions at a faster rate than nitrite ions were converted to nitrate ions. Therefore, excessive ammonium ion discharge or deamination of organic-nitrogen compounds may inhibit nitrification [4]. Based on these results, the nitrification process of the nitrifying bacteria began to slow down when a high concentration of ammonia-nitrogen was introduced to the reactor system. These high concentrations of ammonia-nitrogen produced a certain amount of free ammonia (FA) that could not be tolerated by the nitrifying bacteria. Following from the results shown in Fig. 2, a concentration above 400 mg/L ammonia-nitrogen contributed to excessive ammonia discharge or FA present in the batch culture system. This inhibition decreased the microbial activity in the batch reactor with the bio-filter of K2 AnoxKaldnes packing material. In response to this situation, a longer period of time was needed by the nitrifying bacteria to fully oxidize ammonia-nitrogen.

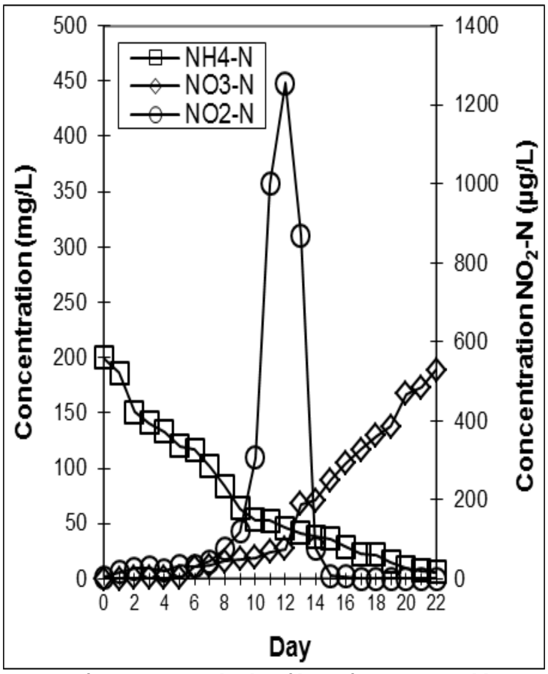

Fig. 1A: Nitrification using the bio-filter of K2 AnoxKaldnes packing material in batch reactor experiments with high concentrations of ammonium sulfate, 200 mg/L ammonia –nitrogen.

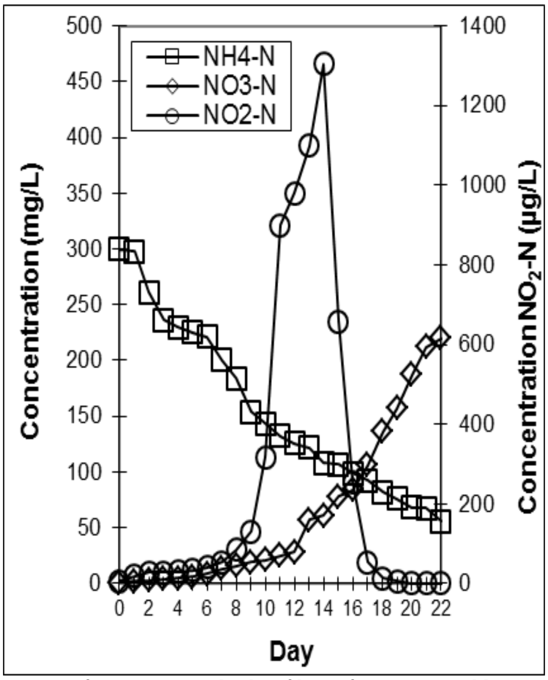

Fig. 1B: Nitrification using the bio-filter of K2 AnoxKaldnes packing material in batch reactor experiments with high concentrations of ammonium sulphate, 300 mg/L ammonia-nitrogen.

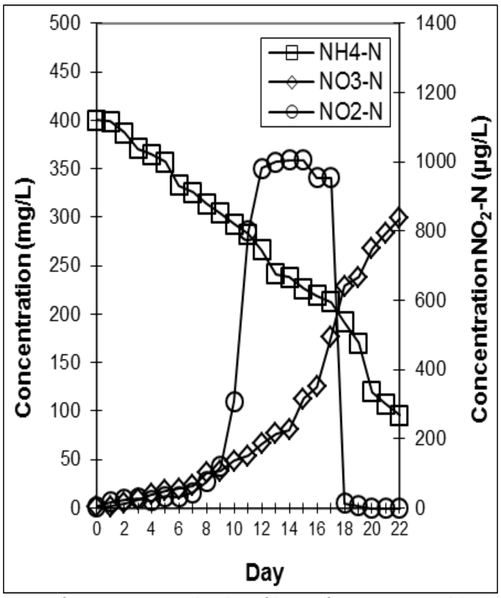

Fig. 2A: Nitrification using the bio-filter of K2 AnoxKaldnes packing material in batch reactor experiments with high concentrations of ammonium sulphate, 400 mg/L ammonia –nitrogen.

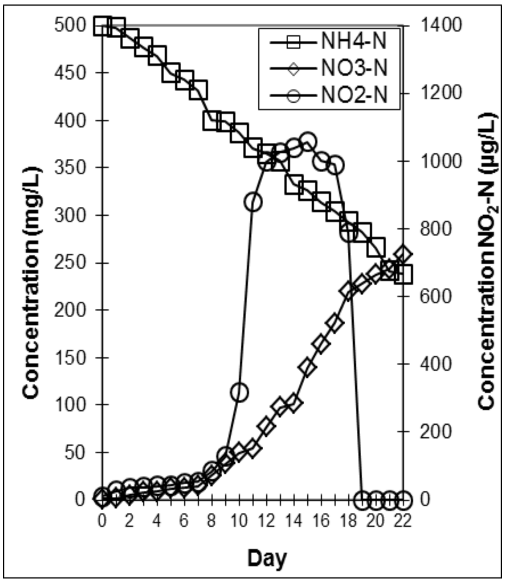

Fig. 2B: Nitrification using the bio-filter of K2 AnoxKaldnes packing material in batch reactor experiments with high concentrations of ammonium sulphate, 500 mg/L ammonia –nitrogen.

Several studies have shown that the inhibitory effect of ammonia on nitrification is due not to the ammonia itself, but rather due to FA, which can form with high concentrations of total ammonia [4,8]. Furthermore, the deterioration in nitrogen removal efficiency was mainly due to ammonia accumulation in the reactor system. A possible reason for this depreciation in nitrogen removal performance could be due to the increase in FA and free nitrous acid (FNA). FA is inhibitory to AOB and NOB. However, FNA, rather than ammonium ions (NH_4^+) and nitrite ions (NO_2^-), is inhibitory only to NOB [4].

From this point of view, a long retention time is required for the AOB and NOB to adapt to changes in the environmental conditions when a high level of ammonia-nitrogen is found in the batch reactor system. A novel approach to operating the immobilized system with the bio-filter of K2 AnoxKaldnes packing material resulted to complete nitrogen removal. The suspended carriers in the batch reactor provided intermediate support for the nitrifying bacteria. New colonies of nitrifying bacteria could be developed and attached to the support carrier. High nitrogen transformation could be achieved through the action of nitrifying bacteria on the ammonia-nitrogen accumulated by the filters. This activity plays a role in producing an oxygen diffusion gradient that will create conditions favourable for the nitrification process [13].

Fig. 3 shows the reduced nitrification values at five different concentrations of ammonia-nitrogen with the bio-filter of K2 AnoxKaldnes packing material in the batch reactor. The concentrations of 100 mg/L ammonia-nitrogen [11], 200 mg/L ammonia-nitrogen, 300 mg/L ammonia-nitrogen, 400 mg/L ammonia-nitrogen and 500 mg/L ammonia-nitrogen were assessed. Fig. 3 shows decreased amounts of ammonia-nitrogen after 22 days of incubation, with the highest decline in ammonia-nitrogen at the concentration of 100 mg/L ammonia-nitrogen [11].

The formula for exponential decay [14] was used to describe the limitation of nitrifying bacteria in the presence of different high concentrations of ammonia-nitrogen shown in Fig. 3 for the experimental study using the batch reactor immobilized system with the bio-filter of K2 AnoxKaldnes packing material.

$$N = N_o e^{kt}, \ k \leq 0 \quad (1)$$

Where;

N = concentration of ammonia-nitrogen remain in the system after a certain incubation period.
N_o= initial concentration of ammonia-nitrogen
t = time
k = decay rate

The decay rate, k, for the concentration of 500 mg/L ammonia-nitrogen was calculated as -0.055. This value was the highest decay rate compared to the other decay rates obtained from different concentrations of ammonia-nitrogen that were analyzed. The values for the decay rate of nitrifying bacteria at 400 mg/L, 300 mg/L, and 200 mg/L ammonia-nitrogen were -0.124, -0.133 and -0.177, respectively.

The decay rate in this study was found to be relatively similar to the decay rate obtained for nitrifying bacteria in activated sludge, with a value in the range of 0.2 to 0.06 in similar conditions [15]. For all activated sludge models, a default decay rate with a value of 0.15 at 20°C was used under all conditions [16]. However, the decay rate of nitrifying bacteria is an uncertain parameter [15]. Based on the study by [17], the decay rate measured by anoxic conditions was 85% lower than under aerobic conditions; in spite of 92% decay rate reduction was obtained under anaerobic condition.

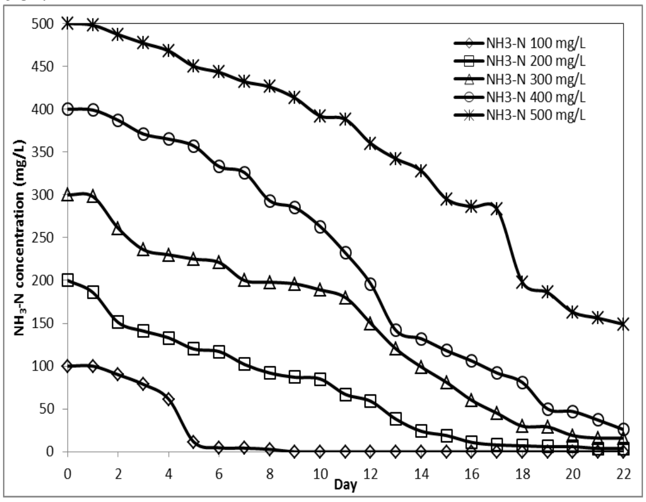

Fig. 3: The effect of high concentrations of ammonia-nitrogen in the batch culture with the bio-filter of K2 AnoxKaldnes packing material.

The decay process is in general, a complex process modelled in a relatively simple mathematical manner [17]. The decay process comprises maintenance energy requirements, the real decay of cells, protozoa grazing and other important factors. The rate of decay can be expected therefore to vary strongly depending on the conditions in a system. Maintaining the optimum incubation conditions for a certain period of time was also an essential aspect to prevent water evaporation and the loss of biomass. In practice, good pH control and maintaining incubation conditions during the experimental analysis has proven to be a crucial factor to ensure good results [18].

This experiment determined the influence of high ammonia-nitrogen concentrations on the growth and/or decay of nitrifying bacteria in the batch culture reactor. The procedure in the batch culture system performed under the optimum conditions was designed to assess the reduction in activity of both AOB and NOB correlated with different concentrations of ammonia-nitrogen.

Here, from the calculation of the decay rate, the reduction in endogenous respiration activity within the AOB and NOB were correlated with high ammonia-nitrogen concentrations. When the ammonia-nitrogen level was high in the reactor system, the nitrifying bacteria were exposed to extreme conditions [5], whereas the slow growth of nitrifying bacteria under high concentrations of free ammonia reduced the activity of nitrifying bacteria. A prolonged incubation time was needed for the nitrifying bacteria to readjust to the surrounding conditions to achieve higher ammonia oxidation for a complete nitrification process.

Nitrifying bacteria use ammonia as a source of energy and electrons. The nitrification reaction can be inhibited by ammonia itself at high concentrations [4]. Figure 4 shows the percentage of ammonia-nitrogen removal rate at low concentrations to high concentrations of ammonia-nitrogen according to [11]. The results for the amounts of ammonia-nitrogen are obtained from [11] and [19]. The ammonia-nitrogen removal rate increased with an increase in the ammonia-nitrogen concentration up to 400 mg/L and then apparently declined with higher amounts of ammonia-nitrogen. This result indicates that an ammonia-nitrogen concentration below 400 mg/L ammonia-nitrogen does not inhibit the microbial activity of nitrifying bacteria in the batch reactor with the bio-filter of K2 AnoxKaldnes packing material.

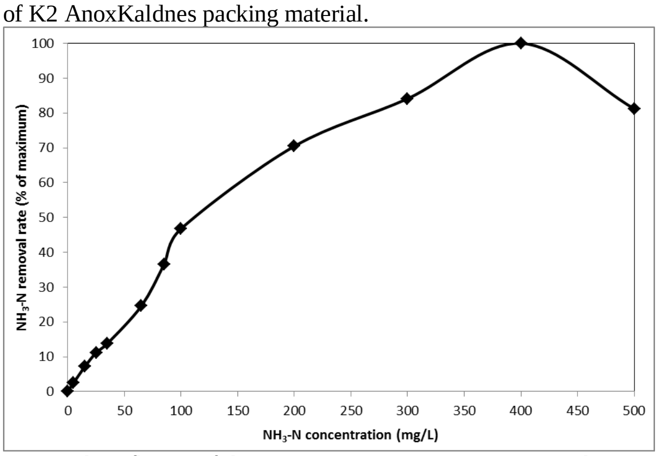

Fig. 4: The influence of the ammonia-nitrogen concentration on the ammonia-nitrogen removal rate in batch culture using the K2 AnoxKaldnes packing material.

As the ammonia-nitrogen concentration increased, FA also increased. The inhibitory effect of ammonia on nitrification was due to FA in the system, which can be formed at high concentrations of total ammonia [4, 8]. NOB such as *Nitrobacter* will become inhibited [4]. FA opposed the nitrification process and caused a reduction in ammonia oxidation and a failure of nitrite-nitrogen build-up. From the results shown in Figure 4, ammonia-nitrogen concentrations above 400 mg/L contributed to the inhibition of FA present at these high concentrations of ammonia-nitrogen in the batch reactor. This inhibition decreased the microbial activity of nitrifying bacteria in the batch reactor with the bio-filter of K2 AnoxKaldnes packing material.

4. Conclusion

The reduction of the percentage ammonia-nitrogen removal rate at concentrations higher than 400 mg/L suggested that nitrifying bacteria activity was being obstructed in the presence of high ammonia-nitrogen concentrations. Nitrifying bacteria are sensitive to the changing environment as they are slow-growing bacteria. Implementing the bio-filter of K2 AnoxKaldnes packing material in the batch reactor system allowed the nitrifying bacteria to be cultivated more easily in the attached system. A high population of nitrifying bacteria could be achieved in a certain period of time to carry out the ammonia oxidation process.

Furthermore, the transport mechanisms in immobilized systems with suspended carriers might even enhance nitrite-nitrogen and nitrate-nitrogen accumulation. Dissolved oxygen is normally consumed only in the first 50-100 μm of biofilms due to deficient oxygen transfer [20]. Therefore, biofilm reactors using immobilized suspended carriers such as the K2 AnoxKaldnes packing material are designed to move in the batch reactor system to achieve high mass transfer coefficients at the biofilm/liquid interphase, which may be advantageous for the complete nitrification process.

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