Population Dynamics of Nitrifying Bacteria in an Aquatic Ecosystem

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Abstract In a space environment such as Space Shuttle or Space Station, animal experiments with aquatic species in a closed system pose a crucial problem in maintaining their water quality for a long term. In nature, ammonia as an animal wastes is converted by nitrifying bacteria to nitrite or nitrate compounds, which usually become nitrogen sources for plants. Thus an application of the biological reactor with such bacteria attached on some filters has been suggested and experimentally studied for efficient waste managements of ammonia. Although some successful results were reported (Kozu et al. 1995, Nagaoka et al. 1998, Nakamura et al. 1997, 1998) in the space applications, purely empirical approaches have so far been taken to develop a biological filter having a stable nitrifying activity. In this study, we constructed a mathematical model to deal with the dynamics of the ammonia nitrifying processes in a biological reactor. The model describes population dynamics of the ammonia-oxidizing bacteria and the nitrite-oxidizing bacteria cultivated on the same filter. We estimated parameters involved in the model using the experimental data. The result shows that these estimated parameters could be applied to general cases and that the two bacteria are in a symbiotic relationship; they can better perform when both coexist, as has been empirically recognized. Based on the model analysis, we discuss how to prepare a high performance biological filter.

Key words: Dynamics of nitrifying bacteria, Bacterial ecosystem, Mathematical model, Biological filter

Introduction Since the first space experiment in Japan, 1992, aquatic animals, especially small freshwater fishes and amphibians, were launched into the space as model animals to investigate the effects of zero gravity on the biological and physiological systems. The goals of these experiments are to elucidate the biological mechanisms responding and adapting to gravity as well as the extended human activity in space.

To maintain these animals in a closed water environment for a long time, we must solve a critical problem of how to process nitrogenous wastes excreted from animals. Ammonia NH₃ is the most critical compound for animal because of its toxicity. Therefore, establishment of a method to remove or convert ammonia into non-toxic substances is a key issue to be resolved. Several methods have been proposed for this purpose: 1) physicochemical adsorption method such as using zeolyte or ion exchanger, and 2) conversion of ammonia by nitrifying bacteria to least toxic compound, nitrate. The former method is not suitable for a prolonged use because a capacity of the filter adsorption is rather limited. On the other hand, the latter method is suitable, because aquatic ammonia excreted is effectively converted by nitrifying bacteria as seen in nature. Two species of bacteria are involved in the process of the ammonia conversion: ammonia oxidizing bacteria which oxidizes ammonia into nitrite, and nitrite oxidizing bacteria which oxidizes nitrite into nitrate, as indicated by the following schemes:

\[
\text{NH}_4^+ + \frac{3}{2} \text{O}_2 \rightarrow \text{NO}_2^- + 2 \text{H}^+ + \text{H}_2\text{O}^- \\
\text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^- 
\]

The nitrate can be ultimately converted to nitrogen N₂ and oxygen O₂ by denitrifying bacteria, thus becomes complete ammonia conversion. However, we will not consider this final process, because the denitrifying process is not well established in laboratories and also nitrate is less harmful than ammonia or nitrite in a practical point of view.

Nitrifying bacteria (ammonia and nitrite oxidizing bacteria) are known to be slow growth bacteria compared with other ordinary species such as yeast and B. subtilis. For example, it takes only 30 ~ 60 min for these bacteria to reproduce, while the doubling time of nitrifying bacteria is much longer; 29 hours for ammonia oxidizing bacteria and 21 hours for nitrite oxidizing bacteria (Yoshioka, 1982; Toray Research Center, 1994). Therefore, it is necessary to establish an efficient method to culture these bacteria.

For the space experiment project, IML-2, an Aquatic Animal Experimental Unit (AAEU) was used for embryology and physiology investigations maintaining freshwater fishes, Medaka and goldfish in a closed environment. The apparatus includes a water tank called fish package to hold several fishes with a biological filter.
where nitrifying bacteria are colonized and serve to convert the ammonia and nitrite. It is known that there exist many strains of nitrifying bacteria. At the beginning of the space experiment project, some nitrifying bacteria strains isolated from active sludge were tested for a space aquatic life support. These strains, however, turned out to be difficult to maintain their nitrifying activities. Subsequently, other kinds of strains isolated from a freshwater fish, gold fish, were found to be more stable and controllable. Thus a biological filter with these bacterial strains was selected for the biological filter of AAEU in the IML-2 experiment. However, the efficiency of the biological filter depends both on the bacterial strains and the fish to be maintained, so that how to optimize its efficiency remains open to a question.

Shimura et al. (1996) studied several properties of the biological filter attached to the AAEU and reported details about the dynamics of the nitrifying process of ammonia. They focused on 1) the process of establishment of the nitrifying bacteria derived from gold fish on a bacteria-free filter and measured the activity development of the filter to nitrify ammonia, and 2) the effect of exogenous addition of ammonium chloride to the water on the bacterial growth.

In this paper, we develop a mathematical model to describe the processes of the bacterial growth based on these experimental data, and its relation to ammonium and nitrite oxidization activities in terms of the bacterial population dynamics. Based on the model analysis, we discuss the conditions to make a biological filter that achieves high efficiency of ammonia conversion. First, we give a brief introduction of the experiments carried out by Shimura et al. (1996), and then go on to the construction and analysis of the model.

**Experiment of Shimura et al.**

The biological filter installed in the AAEU used by Shimura et al. (1996) is composed of porous glass beads of diameter 2~3 mm, (Siporax, Schott Japan, Inc.), on which ammonia and nitrite oxidizing bacteria derived from gold fish were colonized. The water is cycled by an electric pump through the biological filter at a constant rate. Using this apparatus, they carried out the following two experiments to study the detailed dynamics of the nitrifying process. Here we summarize their experiments.

### Table 1 Conditions for Experiments 1 and 2 (from Shimura et al., 1996)

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of gold fishes</td>
<td>(Ammonia excreted) 5 (4 mg NH4+-N / day)</td>
<td>8 (6.4 mg NH4+-N / day)</td>
</tr>
<tr>
<td>Water capacity</td>
<td>5 liter (pH 7.5)</td>
<td>8 liter (pH 7.5)</td>
</tr>
<tr>
<td>Volume of Siporax</td>
<td>230 ml (Bacteria free)</td>
<td>1000 ml (100 ml balanced + 900 ml bacteria free)</td>
</tr>
<tr>
<td>Temperature</td>
<td>23 °C</td>
<td>23 °C</td>
</tr>
<tr>
<td>Light, condition</td>
<td>Light, 14 hours Dark, 10 hours</td>
<td>Light, 14 hours Dark, 10 hours</td>
</tr>
<tr>
<td>Ammonia added</td>
<td>None</td>
<td>5 mg/L at every 2 days</td>
</tr>
<tr>
<td>Water replacement</td>
<td>Replace 1/3 with fresh water when NH4-N&gt;10 mg/L or NO2-N&gt;15 mg/L, respectively</td>
<td>Replace half with freshwater per week</td>
</tr>
</tbody>
</table>

**Experiment 1**

Experiment 1 was aimed to newly establish the nitrifying bacteria on a bacteria-free Siporax. Five gold fishes were reared in a water tank of 5 liter (23 °C, pH 7.5) attached with a filter containing 230 ml of Siporax. Gold fishes produced approximately 4 mg / day of NH4+-N per day, which was processed by the nitrifying bacteria. Appropriate amounts of exogenous ammonium chloride were added so as to facilitate the bacterial growth. However, the accumulation of ammonia and nitrite could have negative impacts on gold fish, so that one third of the tank water was replaced with new freshwater when the ammonia or nitrite concentration increased above 10 mg/L or 15 mg/L, respectively. The experimental conditions are listed in Table 1. The temporal changes in the concentrations of NH4+-N and NO2--N are shown by closed and open squares in Fig. 1, respectively. The NH4+-N concentration increased monotonically in the initial stage until it exceeded 10 mg/L after around 13 days. It then began to decline after 20 days, becoming undetectable after around 25 days. On the other hand, the concentration of nitrite began to rapidly accumulate from 20 days, and it abruptly decreases to zero at around 50 days. Thus it took about 50 days for the nitrifying bacteria to be effectively established on the bacteria-free Siporax, upon which the water quality was stabilized. In this study, Shimura et al. defined the activity of the ammonium and the nitrite oxidizing bacteria as follows.

**Activity of the ammonia-oxidizing bacteria:** The amount of ammonia oxidized per day by 100 ml Siporax in 1 liter of water with 5 mg/L NH4+-N initially added (mg NH4+-N / 100 ml Siporax / day)

**Relative activity of the nitrite-oxidizing bacteria:** (1 – the ratio of NO2--N produced to NH4+-N oxidized per day by 100 ml Siporax in 1 liter of water with 5 mg/L NH4+-N initially added) ∕ 100 (%).

According to these definitions, the activities of the ammonia-oxidizing bacteria and the nitrite-oxidizing bacteria after 25 days were measured to be 18.6 mg and 8.6 %, respectively. When NO2--N became undetectable after 50 days, these activities were 18.3 mg and 73.7%, respectively. Because the NH4+-N oxidization activity remained almost the same (18.6 mg and 18.3 mg) during...
the last half of the experiment, this level of activity seems to represent the maximum functional capacity of the ammonia-oxidizing bacteria established on the Siporax used in the AAEU. Therefore, we adopt this maximum activity of the ammonia-oxidizing bacteria (18.6 mg NH₄⁺-N / 100 ml Siporax / day) as a standard index in the following analysis.

Experiment 2

Experiment 2 was aimed to investigate how the AAEU system could be scaled up using the pre-established Siporax as a seed that had been obtained in Experiment 1. Eight gold fishes were reared in a 8-liter tank attached with a biological filter composed of 100 ml pre-established Siporax as a seed and 900 ml of bacteria-free Siporax. The nitrifying bacteria were cultivated only with ammonia excreted from gold fish for the first 10 days to adapt the bacteria to the new environment and then 5 mg/L ammonia was artificially added every two days to facilitate the bacterial reproduction on the new Siporax. Half of the water was replaced with fresh water every week (Table 1).

The temporal changes in the concentrations of NH₄⁺-N and NO₂⁻-N are shown in Fig. 2 (closed and open squares). The endogenous ammonia and nitrite were almost completely decomposed for the first 10 days. Exogenous ammonium chloride was added in very two days from 10 days. On each addition, ammonium was rapidly transformed into nitrite, which in turn was changed to nitrate with a slight delay, so that the concentrations of both ammonium and nitrite dropped to almost null before the next addition of ammonia. The temporal changes of the nitrifying activities are shown in Fig. 2. The ammonia oxidizing activity increased monotonically and reached a saturated level of 18 mg NH₄⁺-N/100 ml Siporax / day after 30 days. Then, the ammonia oxidizing activity remained at the same level for about one and a half months. On the other hand, the nitrite oxidizing activity fluctuated between 50~75% and then began to increase after about 2 months and reached 97.2% after two and a half months. This shows that the nitrifying bacteria was effectively established on the new Siporax using a piece of pre-established Siporax as a seed. In addition, the balance between the ammonia and nitrite oxidizing activity was autonomously restored even if it was temporarily perturbed. In the next section, we will build a mathematical model to describe these dynamical processes based on the data observed by Shimura et al. (1996).

The Model

The nitrifying process is driven according to scheme (1). The ammonia produced from fishes or added artificially enters the experimental system and its concentration is denoted as \( A(t) \) as a function of time \( t \) (the unit is mg/L / day). The ammonium and nitrite concentrations in the tank water is denoted as \( I \) and \( u \) (the unit is mg/L), and the density of the ammonium oxidizing and nitrite oxidizing bacteria as \( x \) and \( y \), respectively (per 1 ml Siporax), all of which are functions of time. The unit of time is day.

We assume that the oxidizing rate by the ammonium oxidizing bacteria depends on the concentration of ammonia, \( I \), and that a unit density of the bacteria oxidizes \( f(I) \) ammonia (NH₄⁺-N mg / day oxidized by a unit density of bacteria) as a function of \( I \). In the same way, we assume that the oxidizing rate of the nitrite oxidizing bacteria is a function of \( u \), given as \( g(u) \) (NO₂⁻-N mg / day oxidized by a unit density of bacteria). It would be natural to assume
inter- and intra-specific competitions between bacteria because they grow on the surface of Siporax and the total surface available is limited. So we assume that both kinds of bacteria have their own carrying capacities and that they exert mutual influence on the other’s reproduction. Then we obtain the following differential equations that describe the temporal changes of $I$, $u$, $x$, and $y$:

\[
\frac{dI}{dt} = A(t) - \frac{V}{V'} f(I)x, \quad (2-1)
\]

\[
\frac{du}{dt} = \frac{V}{V'} f(I)x - \frac{V}{V'} g(u)y, \quad (2-2)
\]

\[
\frac{dy}{dt} = \sigma g(u) \left(1 - \frac{y}{k_y} - \frac{x}{k_x}\right), \quad (2-3)
\]

\[
\frac{dx}{dt} = \alpha f(I) \left(1 - \frac{x}{k_x} - \frac{y}{k_y}\right), \quad (2-4)
\]

where $V$ is the total volume of water in the tank (liter), $V_s$ is the capacity of Siporax biological filter (cc), $\alpha$ is the growth yield of the ammonia oxidizing bacteria due to the ammonia oxidation, $\sigma$ is the growth yield of the nitrite-oxidizing bacteria, $k_x$ and $k_y$ are the carrying capacities of the ammonia-oxidizing and nitrite-oxidizing bacteria per 1 ml Siporax, respectively. $\gamma$ represents the coefficient of interspecific competition of the ammonia-oxidizing bacteria against the nitrite-oxidizing bacteria and $\gamma$, that of the nitrite-oxidizing bacteria against the ammonia-oxidizing bacteria.

Equation (2-1) indicates that the rate of the temporal change in the ammonia concentration is given as the rate of exogenous addition of the ammonia subtracted by that of ammonia oxidized. The second term of the right hand side represents the concentration of oxidized ammonia within Siporax converted to the concentration in the tank. Equation (2-2) describes that the reproduction of the ammonia-oxidizing bacteria follows the Lotka-Volterra competition: when the densities of both bacteria are low enough, the ammonia-oxidizing bacteria increases exponentially at a rate, $\alpha f(I)$, but the growth rate is slowed down, as the densities increase. If $\gamma > 0$, the nitrite-oxidizing bacteria has a negative impact on the growth of the ammonia-oxidizing bacteria, but if $\gamma < 0$, it facilitates the other’s reproduction. Equation (2-3) indicates that the rate of the temporal change of nitrite is given as the addition by the ammonia oxidization minus the decline by the nitrite oxidization. Likewise, equation (2-4) describes the reproduction of the nitrite oxidizing bacteria. In general, the rate of oxidization of a substrate increases linearly with increases in the substrate concentration at first, but it reaches a plateau when the substrate concentration becomes large enough. Therefore, we assume in this model that the functional forms of $f(I)$ and $g(u)$ are given by Michaelis-Menten functions as follows:

\[
f(I) = \frac{\mu I}{g_a + I}, \quad (3-1)
\]

\[
g(u) = \frac{\mu u}{g_a + u}, \quad (3-2)
\]

where $\mu_a$ and $\mu_u$ are the maximum ammonium and nitrite oxidation rates and $g_a$ and $g_u$ are the half saturation constants, respectively.

Now we have obtained a complete set of the differential equations that describes the temporal changes in the
cultivation experiments as concentrations are saturating, have been obtained from when their densities are low and the substrate is estimated. Among them, the growth rate of the bacteria ammonia-oxidizing and nitrite-oxidizing bacteria. Parameter estimation

The model equations (2) contain 10 parameters to be estimated. Among them, the growth rate of the bacteria when their densities are low and the substrate concentrations are saturating, have been obtained from cultivation experiments as $\mu_u = 0.58$ and $\mu_v = 0.81$ (Yoshioka, 1982; Toray Research Center, 1994). We estimate the other parameters by fitting the solution of equation (2) to the data shown in both Fig. 1 (a), (b) and Fig. 2 (a), (b) at the same time. The parameter values are thus estimated as: $\mu_u = 0.58$, $\mu_v = 0.81$, $\gamma_1 = 0$, $\gamma_2 = -8.33$, $g_v = 0.2$, $g_u = 0.8$, $\mu_v \kappa_v = 0.24$, $\mu_u \kappa_u = 0.1$. Although $\mu_u$, $\mu_v$, $\mu_v \kappa_v$ and $\mu_u \kappa_u$ are not decoupled, it is not necessary to obtain the independent values of constituent parameters for the purpose of the subsequent analysis. The fitted curves for these estimated values are shown by the solid and dashed curves in Figs 1 and 2.

In Fig. 1 (a), the modeled dynamics is well fitted to the observed result except that the simulated nitrite concentration is higher than the observed concentration at around day 40. The activities of the ammonia and nitrite oxidizing bacteria are shown in Fig. 1 (b). Although the activities were measured only at two time points, the simulated values fit well to the observed values except for the activity of ammonia oxidation at around day 25. The temporal changes in the densities of ammonia-oxidizing and nitrite-oxidizing bacteria that are measured in $1/\mu_u$ and $1/\mu_v$ as units are shown in Fig. 1 (c). Note here that this is the first prediction of the bacterial densities in a mixed culture system, for which no experimental data can be available. The ammonia oxidizing bacteria begins to increase rapidly after 20 days with concomitant disappearance of ammonia, and several days later, the growth rate suddenly declines. The nitrite-oxidizing bacteria also begins to increase after 40 days with a reciprocal decline in the nitrite concentration. The densities of both ammonia-oxidizing and nitrite-oxidizing bacteria approach some constant levels after 50 days, suggesting that both bacteria have been established to be stabilized on the Siporax biological filter.

The simulated dynamics corresponding to Experiment 2 is shown in Fig. 2. As before, the fit between the simulations and the experiments is reasonably good. The activities of the ammonia and the nitrite oxidation also fit well to the observed dynamics except for the relative nitrite oxidation activity after day 50 (Fig. 2 (b)). The ammonia oxidation activity increases in parallel with the density of the ammonia oxidizing bacteria. The relative nitrite oxidation activity reaches a plateau of around 70% at a very early stage. This means that the relative activity of the nitrite oxidizing bacteria to that of the ammonia oxidizing bacteria are maintained at high, constant levels. In fact, the densities of both bacteria monotonically increase and reach saturation levels after 40 days. The bacterial densities converge to the same levels as the simulated values corresponding to Experiment 1 (Fig. 2 (c)).

Overall, we conclude that this model along with the estimated parameters can faithfully simulate the experiments by Shimura et al. (1996) and hence this model could be used to make “virtual experiments”, which might be helpful to understand the dynamics of the nitrifying process of ammonia.

Discussion

Validity of parameter estimation

We have modeled the dynamics of the concentrations of ammonia, nitrite, and the densities of the ammonia-oxidizing and nitrite-oxidizing bacteria using differential equations. Based on the experiments by Shimura et al. (1996), we estimated the parameter values involved in the model. These estimated parameters give a remarkably good fit to the observed dynamics by Shimura et al. To further test the validity of the present model, we examine whether these estimations could be applied to the data obtained in other experiments. Kondo and colleagues measured the oxidation activities of the two bacteria established on Siporax (Kondo at Toray Research Center, Personal communication). They investigated on the temporal changes in the activities of ammonia- and nitrite-oxidization using Siporax filters on which the corresponding bacteria were separately cultivated, respectively. Conditions used are listed in Table 2.

In Experiment (a) of Fig. 3 (a), the ammonia concentration decreased linearly with time, while nitrite accumulated in a reciprocal manner because no nitrite oxidizing bacteria was present. In the same way, they

Table 2 Conditions for Experiments (a) and (b) (from Toray Research Center)

<table>
<thead>
<tr>
<th></th>
<th>Experiment (a)</th>
<th>Experiment (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial concentration of ammonia</td>
<td>100 mg/L</td>
<td>0 mg/L</td>
</tr>
<tr>
<td>Initial concentration of nitrite</td>
<td>0 mg/L</td>
<td>100 mg/L</td>
</tr>
<tr>
<td>Water volume</td>
<td>0.85 liter</td>
<td>0.85 liter</td>
</tr>
<tr>
<td>Volume of Siporax</td>
<td>32 ml</td>
<td>32 ml</td>
</tr>
<tr>
<td>(Bacteria established on Siporax)</td>
<td>(Ammonia-oxidizing bacteria)</td>
<td>(Nitrite-oxidizing bacteria)</td>
</tr>
</tbody>
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investigated the process of nitrite oxidation in experiment (b) as shown in Fig. 3 (b).

We simulated these experiments using the same parameter values as estimated above from Shimura et al. (1996). In both cases, the simulations fit very well to the observations as shown by the solid and dashed lines in Fig. 3.

Both bacteria are in symbiotic relationship

We estimated the coefficients of interspecific competitions between the two bacteria as $\gamma_x = 0$, $\gamma_y = -8.33$. This means that the growth of the nitrite oxidizing bacteria is not affected by the ammonia oxidizing bacteria, but that of the ammonia-oxidizing bacteria is positively affected by the nitrite oxidizing bacteria ($\gamma_y < 0$). It has been known empirically that the nitrifying activity of bacteria system shows a best performance when both bacteria are present on the same location. Furthermore there is the suggestion that both bacteria are in a symbiotic relationship. The parameter estimation for the present model supports this, which in turn imply the robustness of our model.

How to make an effective biological filter

In this section, we discuss how to make an effective biological filter to convert ammonia by using the model as a virtual experiment. The nitrifying bacteria used in the experiments by Shimura et al. were derived from freshwater fishes and they were allowed to establish on the Siporax filter by using natural source of ammonia from fishes as a substrate. By artificially adding ammonia, we could enhance the bacterial reproduction, which might shorten the time needed for these bacteria to be established on the biological filter. The ammonia, however, could be harmful to fishes when its concentration is too high. Thus, how best to add ammonia is a crucial problem.

We performed a virtual experiment in which 10 gold fishes are reared in a water tank of 10 liter. As the biological filter

![Fig. 3 Results from Experiments (a) and (b) (Toray Research Center, 1994) and the theoretical results calculated from the model. (a) The temporal changes in the ammonia and nitrite concentrations. (b) The temporal change in nitrite concentrations. Solid and dashed lines are theoretical curves and closed and open squares are the observed data for the concentrations of ammonia and nitrite, respectively.](image)

![Fig. 4 The dynamics of ammonia and nitrite concentrations and bacterial densities when ammonia is added continuously with time (1mg/L/day). (a) The dynamics of the concentrations of ammonia $R(t)$ and nitrite $u(t)$ shown in solid and dashed lines, respectively. (b) The dynamics of the bacterial densities, $x(t)$ and $y(t)$, shown in solid and dashed lines, respectively.](image)
filter, we use 1000 ml Siporax, of which a 100-ml portion has been pre-established as a seed and the remainder is new and bacteria-free. We add some amount of ammonia into this virtual model in the following two ways: the ammonia is added either continuously with time or intermittently at a constant interval. The total amount of the ammonia added is kept the same in both cases. Figures 4 and 5 illustrate the results of simulations for the continuous and intermittent modes of ammonia addition (1 mg/L / day), respectively. In Fig. 4, both the ammonia- and nitrite-concentrations show gradual increases and come to saturate at constant levels. On the other hand, in Fig. 5, the peak of the ammonia concentration is quite high because we added the ammonia intermittently. However, in both cases, the bacterial reproduction is not affected greatly in either way of ammonia addition at the dose of 1 mg/L / day. Nevertheless, the continuous addition should be better to achieve and build a biological filter with a higher efficiency, because the peak concentration of ammonia in the intermittent addition might have negative impacts on live fishes when its daily dose exceeds the suggested safety limit, 10 mg/L (see Fig. 6).

**Perspectives**
In this study, we have only focused on the dynamics of the ammonium and nitrite oxidizing processes, which leaves nitrate as the final product. However, nitrate could still have negative influence on biological organisms at high concentrations. Therefore we should ultimately consider denitrifying all inorganic nitrogen to gas form of nitrogen and oxygen. This process is not fully explored yet both experimentally and theoretically. Thus, the present model should be extended to a more complete ammonia disposal system incorporating the denitrifying process.

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