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INORGANIC NITROGEN COMPOUND REMOVAL EFFICIENCY OF AN ADDED INITIAL BACTERIUM IN THE BIOLOGICAL FILTRATION SYSTEM

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ABSTRACT

The efficiency of inorganic nitrogen compound removal using *Nitrosomonas* sp. strain P-1 was investigated in a filtering system. Bacterial cell densities and concentrations of inorganic nitrogen compounds including ammonia, nitrite, and nitrate were determined every 4 days. Ammonia decreased immediately after treatment for 4 days and was reduced from an initial concentration of 65.75 to 17.57 mg/l during the 28-day experiment. Nitrite and nitrate increased from 0 to 0.239 mg/l and 4.292 mg/l, respectively, over this same period. Total amount of inorganic nitrogen compound removed from the filtering system was 46.91 mg/l. Result also showed that the number of ammonia oxidizing bacterium, *Nitrosomonas* sp. strain P-1, increased from 101 to 627 cfu/ml over the 28 days. Comparison to a control where no bacteria were added, showed a highly significant difference ($P<0.01$) in the efficiency of inorganic nitrogen compound removal.

Keywords : Biological filtration system; *Nitrosomonas* sp. strain P-1; ammonia-oxidizing bacteria.

INTRODUCTION

Aquaculture is being used increasingly for food production. Mass production levels are necessary to meet consumer demand. Preferably, the culture water should be reused or recirculated to reduce the effect on the environment. An alternative to an open-system of recirculation is a closed-system in which 95 to 100% of the culture water is recirculated (Liao and Mayo, 1972). One of the major problems with a closed-system is the removal of metabolic wastes when animal density and feeding rates are high. Bacteria in the system cannot grow and cannot eradicate nitrogen quantity as above- mentioned. Thus essential microorganisms grow slowly, resulting

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in the accumulation of inorganic nitrogen quantity in the system (Mahne et al., 1996; Pakasam and Loesr, 1972; and Yang et al., 1995). The culture water becomes rotten and is toxic to aquatic animals. Ammonia and urea are toxic to fish if allowed to accumulate in the culture medium (Jones, 1964). Ammonia removal is usually accomplished by nitrifying bacteria, which are readily enriched on aerated surfaces in a "biological filter" (Liao and Mayo, 1974). However, nitrification results in a slow but gradual increase (depending on the density of the fish) in nitrate-nitrogen; also toxic to fish at levels of 181 mg of NO_3-N/l (Jones, 1964). An alternative to this problem is to add essential microorganisms into the filtration unit or waste-water treatment unit of an aquaculture system. Therefore, we have undertaken a study on a biological nitrification and denitrification filtration system using *Nitrosomonas* sp. strain P-1 to remove nitrogen.

Previously we isolated an ammonia-oxidizing bacterium, *Nitrosomonas* sp. strain P-1, from the filtration system at the Institute of Marine Science, Burapha University, Bangsaen, Chonburi, Thailand. In the present study, we report for the first time, some properties of this isolated bacterium, in regards to the oxidation of ammonium to nitrite.

MATERIALS AND METHODS

Screening and isolation of ammonia-oxidizing strains

Samples were collected from water and sediment in a closed recirculating system at the Institute of Marine Science, Burapha University, Thailand. One milliliter of sample suspension was spread on Drews' mineral salt agar plates (Drews, 1974). The plates were incubated at 28°C in the dark for 2-4 weeks. The strain that was designated as ammonia-oxidizing bacteria, appeared as red colonies on agar plates, and oxidized ammonia to nitrite (Suzuki et al., 1974).

Characterization and identification of isolate

Morphological properties and taxonomic characteristics of the isolate were studied according to the methods described in Bergey's Manual of Systematic Bacteriology (Staley et al., 1989).

Media and culture conditions

The bacteria were grown in 100 ml Drews' mineral salt medium for 30 days or the color of the indicator turned from orange to yellow. Bacterial cultures were incubated at 28°C in the dark and bacterial cells were harvested for mass culture in fresh medium or added into the filtration system at 100 cfu/ml density.

The filtration system and condition

Supporting materials (5 kg. of gravel, sand, limestone 5 mm in diameter, and charcoal powder) were packed into a 100 liter tank. An aeration lift system recirculated water from the bottom to the water zone on top of the gravel layer.

Ammonia at 65 mg/l in the form of ammonium chloride was added to the filtration system.

Bacterial cell density measurement

Bacterial cell density was determined by counting cells grown on Drews' mineral salt agar at 28°C in the dark for 2 weeks.

Ammonia concentration assay

Ammonia concentration was determined on the supernatant culture filtrates by using the methods of seawater analysis (Grasshoff et al., 1983).

Nitrite and nitrate concentration assay

Nitrite and nitrate concentration were determined on the supernatant culture filtrates using the method of Strichland and Parsons (Strichland and Parsons, 1972).

Total inorganic nitrogen concentration assay

Total inorganic nitrogen concentration was determined by the summation of ammonia, nitrite, and nitrate concentrations.

RESULTS AND DISCUSSION

Characteristics of *Nitrosomonas* isolate

The isolated strain was an aerobic, gram negative, non-motile, non-endospore forming, rod shaped bacterium (0.4-0.6 μm × 1.9-2.9 μm). Cells occurred in short chains. The bacterium produced slime-forming cells. The major source of energy and reducing power was from the oxidation of ammonia to nitrite. Based on these characteristics, the bacterium was identified as a member belonging to the genus *Nitrosomonas* according to Bergey's Manual of Systematic Bacteriology. The *Nitrosomonas* strain grows slowly and may be incubated for several weeks to a month.

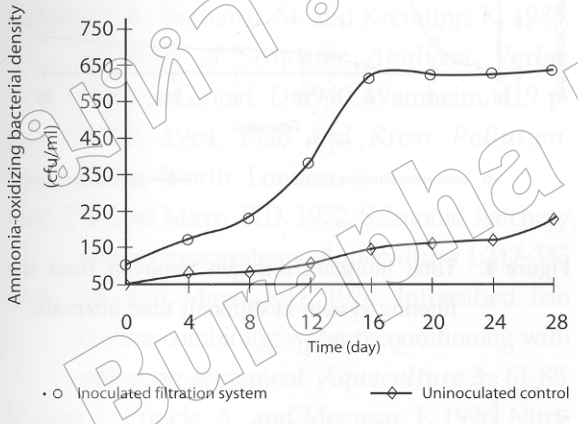

Figure 1. Ammonia-oxidizing bacterial density in a filtering system at different time intervals.

Ammonia-oxidizing bacterial density measurement

After growing *Nitrosomonas* sp. strain P-1 in Drews' mineral salt medium for 30 days, bacterial cells were harvested and added to the filtration system. Ammonia-oxidizing bacterial density increased immediately between 0 and 16 days after which it was stationary. These may be owing to a decrease in ammonia concentration or some elements and product of ammonia-oxidizing pathway accumulation such as nitrite and nitrate. It was related to ammonia, nitrite and nitrate concentration in the filtration system (Figures 2, 3, 5 and 6). During the experimental period, oxidizing ammonia bacterial density increased from 101 to 627 cfu/ml. In comparison to the control, in which no bacteria were added, bacteria increased faster and reached a higher density as shown in Figure 1. The small increase in the ammonia-oxidizing bacteria in the control system was probably due to contamination from supporting materials and seawater.

Inorganic-nitrogen removal efficiency of *Nitrosomonas* isolate

Time course of the removal efficiency of inorganic nitrogen is shown in Figure 2. With the initial bacteria addition, inorganic nitrogen removal was significantly different ($P<0.01$) from that where no bacteria were added. The ammonia concentration at different time intervals is shown in Figure 3. The concentration of ammonia decreased whereas that of nitrite and nitrate increased (Figures 5 and 6). This may be a consequence of bacterial activity (Figure 1). In the initial bacteria addition treatment, the ammonia concentration decreased immediately after treatment for 4 days at which time air bubbles on the surface area of the filtration system tank were more abundant than at the start. Ammonia was reduced from an initial concentration of 65.75 to 17.57 mg/l during the 28-day period of experiment. The air bubbles were probably nitrogen gas, because total inorganic nitrogen of the system also decreased over this time interval. The reduction of ammonia in this study was less than that reported by St-Arnaud et al. (1991). They cultured biofilm of *Nitrosomonas europaea* ATCC 19718 with fecal matter of swine and found

ammonia decreased 270 mg/l/d. However, the biofilm of *N. europaea* may have had a greater cell density than this study and the fecal matter may have provided organic carbon for cell reproduction and metabolism. In this study, organic carbon sources were free. Bacteria could acquire carbon from carbonate in the water or carbon compounds from dead cells, so that ammonia oxidation was less than that reported by St-Arnaud et al. (1991). The total amount of inorganic nitrogen compounds removed from the filtration system was 46.91 mg/l, while in the control it was 7.13 mg/l (Figure 4). Ammonia concentration decreased during experimental period, while the amount of nitrite and nitrate concentrations slowly increased from 0 to 0.239 mg/l and 4.292 mg/l as shown in Figures 5 and 6, respectively. However, the total inorganic nitrogen compounds at different time intervals were less than initial concentration. This indicates that the addition of bacteria promotes inorganic nitrogen removal. Inorganic nitrogen removal efficiency rate increased to maximum value during 12-day experimental period and decreased after that. The inorganic nitrogen loss from the system was not significantly different (P>0.05) between 16 and 28-days of the experiment (Figure 2).

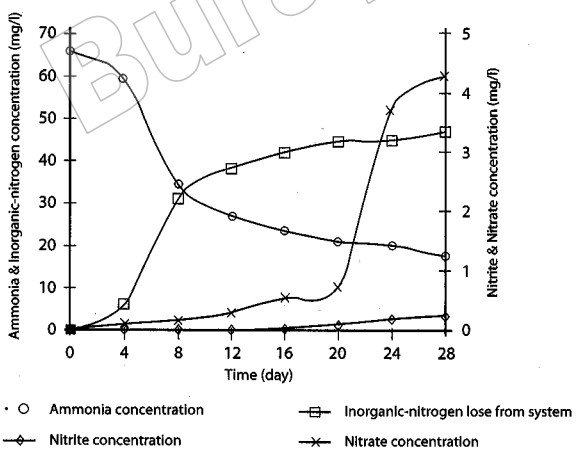

Figure 2. Time courses of inorganic-nitrogen removal efficiency.

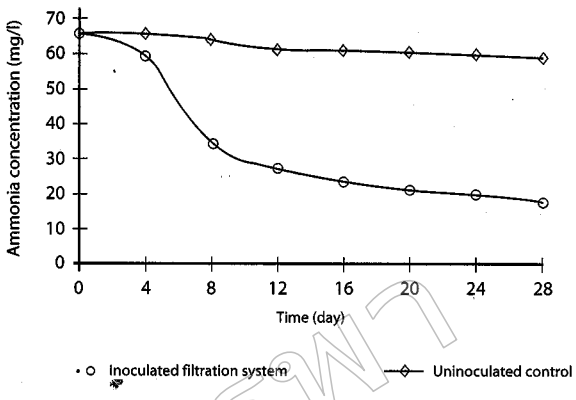

Figure 3. Ammonia concentration in the filtration system at different time intervals.

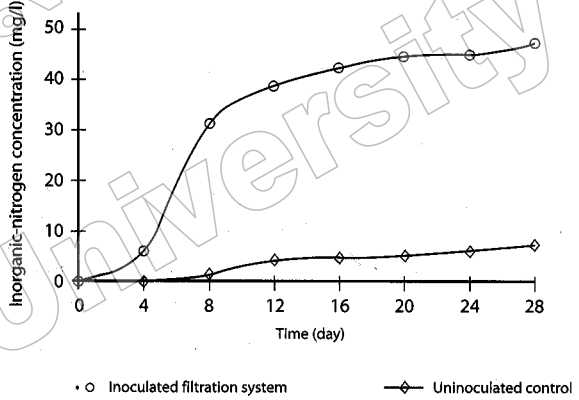

Figure 4. Total inorganic-nitrogen removed from the filtering system at different time intervals.

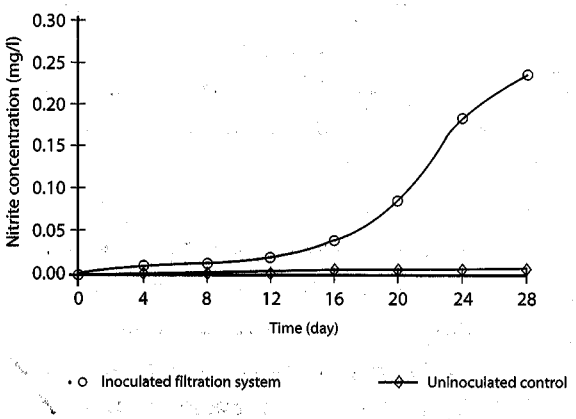

Figure 5. Nitrite concentration in the filtering system at different time intervals.

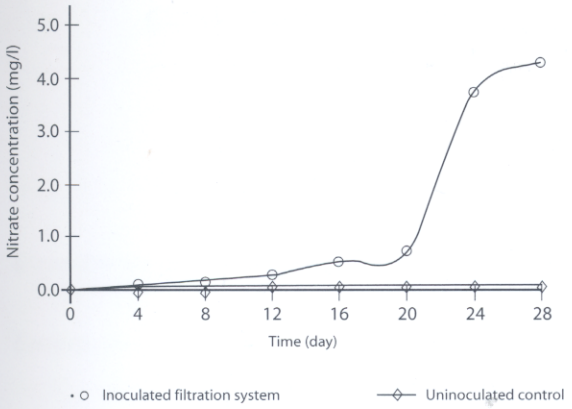

Figure 6. Nitrate concentration in the filtering system at different time intervals.

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