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Differences in nitrite-oxidizing communities and kinetics in a brackish environment after enrichment at low and high nitrite concentrations

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ABSTRACT

Nitrite accumulation in shrimp ponds can pose serious adverse effects to shrimp production and the environment. This study aims to develop an effective process for the enrichment of ready-to-use nitrite-oxidizing bacteria (NOB) inocula that would be appropriate for nitrite removal in brackish shrimp ponds. To achieve this objective, the effects of nitrite concentrations on NOB communities and nitrite oxidation kinetics in a brackish environment were investigated. Moving-bed biofilm sequencing batch reactors and continuous moving-bed biofilm reactors were used for the enrichment of NOB at various nitrite concentrations, using sediment from brackish shrimp ponds as seed inoculum. The results from NOB population analysis with quantitative polymerase chain reaction (qPCR) show that only *Nitrospira* were detected in the sediment from the shrimp ponds. After the enrichment, both *Nitrospira* and *Nitrobacter* coexisted in the reactors controlling effluent nitrite at 0.1 and 0.5 mg-NO_2^--N/L. On the other hand, in the reactors controlling effluent nitrite at 3, 20, and 100 mg-NO_2^--N/L, *Nitrobacter* outcompeted *Nitrospira* in many orders of magnitude. The half saturation coefficients (K_s) for nitrite oxidation of the enrichments at low nitrite concentrations (0.1 and 0.5 mg-NO_2^--N/L) were in the range of 0.71–0.98 mg-NO_2^--N/L. In contrast, the K_s values of NOB enriched at high nitrite concentrations (3, 20, and 100 mg-NO_2^--N/L) were much higher (8.36–12.20 mg-NO_2^--N/L). The results suggest that the selection of nitrite concentrations for the enrichment of NOB inocula can significantly influence NOB populations and kinetics, which could affect the effectiveness of their applications in brackish shrimp ponds.

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Introduction

Nitrite accumulation is usually a concern in shrimp ponds that lack sediment, as sediment is the major source of nitrifying microorganisms, including nitrite-oxidizing bacteria (NOB). The accumulation of nitrite greater than 1 mg/L-N in shrimp ponds can cause serious adverse effects on aquaculture (Hart and O'Sullivan, 1993). Therefore, the development of techniques for controlling nitrite concentrations in shrimp ponds is essential. Bioaugmentation with NOB inocula is considered an attractive approach for resolving intermittent nitrite accumulation in shrimp ponds. Bioaugmentation for nitrification has previously been demonstrated in activated sludge systems (Yu et al., 2012; Leu and Stenstrom, 2010; Parker and Wanner, 2007). The approach has great potential to be further developed and applied in aquaculture.

NOB are groups of microorganisms that are capable of converting nitrite into nitrate, which can be applied to resolve nitrite accumulation in shrimp ponds. *Nitrobacter*, *Nitrospira*, and *Nitrotoga* are the three main genera of NOB commonly observed in nitrification systems. These groups of NOB usually thrive under different environmental conditions (Daims et al., 2001). Studies have shown that physicochemical and operational parameters, such as nitrite concentrations, dissolved oxygen (DO) concentrations, temperature, and salinity affected NOB populations (Daims et al., 2001; Huang et al., 2010; Moussa et al., 2006). In the past, *Nitrobacter* was believed to be the key NOB in wastewater treatment plants (Grady and Lim, 1988). However, *Nitrobacter*-related organisms were not detected in nitrifying activated sludge samples by fluorescence *in situ* hybridization (FISH) (Wagner et al., 1996). In addition, NOB communities in a nitrifying bioreactor were investigated using FISH, and the results revealed that *Nitrospira* spp. were the responsible NOB in nitrification systems (Schramm et al., 1999). Moreover, a recent study found that *Nitrotoga*-like bacteria were key nitrite oxidizers in full-scale wastewater treatment plants (Lücker et al., 2015). Nevertheless, until now, the occurrence and importance of *Nitrotoga* in brackish systems have not yet been reported. Therefore, *Nitrobacter* and *Nitrospira* are still considered to be the two main genera of NOB commonly observed in brackish environments.

With the kinetic characteristics of NOB, it has been suggested that *Nitrospira* are K-strategists that can adapt to low nitrite concentrations, while *Nitrobacter* are r-strategists that thrive when nitrite is at high concentrations (Daims et al., 2001; Schramm et al., 1999). *Nitrospira* generally have higher nitrite affinities (lower K_S values) compared to *Nitrobacter* (Nowka et al., 2015). Many studies have also supported the K/r hypothesis. For examples, Kim and Kim (2006) reported that the distribution of *Nitrobacter* and *Nitrospira* depended on nitrite concentrations. Nogueira and Melo (2006) studied the competition between *Nitrospira* and *Nitrobacter* in nitrite-oxidizing bioreactors. The dominance of *Nitrobacter* over *Nitrospira* appeared to be caused by the elevated nitrite concentrations in bioreactors, which confirmed the K/r hypothesis (Nogueira and Melo, 2006). These previous findings suggest that nitrite concentrations can significantly influence NOB communities. Therefore, the selection of nitrite concentrations to enrich NOB inocula is crucial since it can lead to different NOB communities and kinetics, which could affect their applications in brackish shrimp ponds.

In general, nitrite concentrations in shrimp ponds are in the range of 0.02–0.17 mg-NO_2^--N/L, in which only *Nitrospira* spp. were observed (Srithep et al., 2015). However, higher nitrite concentrations are usually used for the enrichment of NOB inocula for applications in shrimp ponds. The differences in nitrite concentrations used for the enrichment could result in different nitrite-oxidizing bacterial communities and kinetics. The effects of nitrite concentrations on NOB communities and kinetics remain unclear, especially in brackish environments. Such information would be useful for the development of NOB inocula appropriate for nitrite removal in aquaculture ponds. The objective of this study is to investigate the effects of nitrite concentrations on microbial communities and the kinetics of NOB enrichments. The approach for NOB enrichment in this study can also be further applied to develop NOB inocula suitable for aquaculture ponds.

1. Material and methods

1.1. Moving-bed biofilm sequencing batch reactors

Sediment from two outdoor brackish shrimp ponds in Chachengsao, Thailand, was collected and mixed to use as seed inoculum for the enrichment of NOB on biofilm carriers (2H-BCN 012 KLL, Kunststoff GmbH, Germany) in two moving-bed biofilm sequencing batch reactors (50 L). The biofilm carriers (Appendix A Fig. S1) had specific surface area of 859 m^2/m^3, a protected area of 704 m^2/m^3, and a weight of 150 ca. kg/m^3. The first (Reactor A) and second (Reactor B) moving-bed biofilm sequencing batch reactors were fed intermittently with nitrite at a low concentration (1 mg-NO_2^--N/L) and at a high concentration (50 mg-NO_2^--N/L), respectively. The NOB enrichment in these moving-bed biofilm sequencing batch reactors was aimed to increase the amount of NOB populations at low and high nitrite concentrations for further NOB enrichment in continuous-flow moving-bed biofilm reactors.

The synthetic wastewater used in this experiment consisted of $NaNO_2$ (1 or 50 mg/L NO_2^--N), 0.2 g/L of KH_2PO_4, 0.4 g/L of $MgSO_4 \cdot 7H_2O$, 0.1 g/L of KBr, 200 g/L of $NaHCO_3$, 35.8 g/L of Marinium™ reef sea salt (Mariscience, USA), 1 mL/L of nonchelated trace element mixture, 1 mL/L of vitamin mixture, 1 mL/L of vitamin B12 solution, 1 mL/L of vitamin B1 solution, 1 mL/L of selenite-tungstate solution (modified from Könneke et al. (2005)). The nonchelated trace element mixture, vitamin mixture, vitamin B12 solution, vitamin B1 solution, and selenite-tungstate solution were prepared according to Widdel and Bak (1992). The reactors were operated for 90 days at room temperature (28 ± 3°C). Both reactors were operated within a pH range of 7.5–8.5 and an alkalinity range of 120–150 mg-$CaCO_3$/L. DO was controlled to be greater than 4 mg-O_2/L throughout the operation. The salinity of the medium was 15 ppt. The nitrite and nitrate concentrations in both reactors were monitored.

1.2. Continuous-flow moving-bed biofilm reactors

The biofilm carriers that were enriched in Reactor A were then transferred to 2 aerobic continuous-flow moving-bed biofilm