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Effect of nitrite concentration on the distribution and competition of nitrite-oxidizing bacteria in nitratation reactor systems and their kinetic characteristics

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ABSTRACT

Genus *Nitrospira* and *Nitrobacter* species are the key nitrite-oxidizing bacteria (NOB) in nitrifying wastewater treatment plants. It has been hypothesized that genus *Nitrospira* are K-strategists and can exploit low amounts of nitrite more efficiently than *Nitrobacter.* In contrast, *Nitrobacter* species are r-strategists that can grow faster than *Nitrospira*. To elucidate the K/r hypothesis and to analyze the effect of substrate (nitrite) concentration on the competition and distribution of the two NOB, two different reactor types were employed for nitrite oxidation (nitratation) and NOB growth. The continuous biofilm airlift reactor (CBAR) maintained low nitrite concentration due to the complete oxidation of nitrite in continuous operation while the sequencing batch reactor (SBR) was kept in a relatively high nitrite concentration environment due to a cyclic substrate concentration profile. Fluorescence in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM) showed that both *Nitrobacter* species and genus *Nitrospira* were present in the CBAR and the SBR. Quantitative FISH analyses of the CBAR showed that *Nitrospira* occupied 59% of the total bacteria while *Nitrobacter* occupied only 5%. On the other hand, *Nitrobacter*, occupying 64%, was the dominant NOB in the SBR, and only 3% of total bacteria belonged to genus *Nitrospira*. Nitrite oxidation kinetics and quantitative FISH analyses revealed that the specific nitrite oxidation activities of *Nitrobacter* and *Nitrospira* are 93.8 and 10.5 mg/g NOB h, respectively, and the specific activity of *Nitrobacter* is about 9 times higher than that of *Nitrospira*. In conclusion, the results confirm the K/r hypothesis and the distribution of *Nitrobacter* and *Nitrospira* is likely to depend mainly on nitrite concentration. It seems that nitrite load and starvation conditions do not give a direct effect on the distribution of NOB.

1. Introduction

Recent interest in novel nitrogen removal technologies via nitrite pathway, such as SHARON and Anammox process, attract researchers' interest because of their economic advantages in saving costs of aeration and organic carbons (Schmidt et al., 2003). The above processes rely on the competition, elimination or inhibition of nitrite-oxidizing bacteria (NOB) so that the oxidation of nitrite to nitrate is blocked. Nitrite concentration in the conventional nitrogen removal wastewater treatment remains very low by the simultaneous transformation of nitrite to nitrate. Several mechanisms are suggested for the inhibition of nitrite oxidation (Anthonisen et al., 1976; Hellinga et al., 1998; Kuai and Verstraete, 1998; Philips et al., 2002), but they are not always equally effective and it is believed that the nitrite

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oxidation rate and degree of inhibition are different for each NOB species.

Former studies on NOB identification in wastewater treatment plants showed complicated and ambiguous results. *Nitrobacter* had been known as the key NOB in wastewater treatment plants (Grady and Lim, 1980). However, the opinion was dramatically challenged when no *Nitrobacter*-related organisms were detected in nitrifying activated sludge samples by fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes specific for the genus *Nitrobacter* (Wagner et al., 1996). Further investigations with 16S rDNA sequencing and in situ hybridization using oligonucleotide probe revealed that genus *Nitrospira* was the responsible NOB and *Nitrobacter* species were rarely found in nitrification systems (Burrell et al., 1998; Juretschko et al., 1998; Schramm et al., 1998, 1999) indicating that *Nitrospira* species, not *Nitrobacter*, were numerically dominant and carried out nitrite oxidation in the nitrifying bioreactors. In sewage treatment plants *Nitrospira* occurs predominantly embedded in cell aggregates such as flocs or biofilms (Juretschko et al., 1998; Koops and Pommerening-Roser, 2001). Unfortunately, all attempts to isolate *Nitrospira* from activated sludge and to grow them in pure culture have been unsuccessful and, consequently, the knowledge of the microbiology of *Nitrospira* species is still limited.

On the other hand, Daims et al. (2001a) reported that *Nitrobacter* and *Nitrospira*-like bacteria co-existed in a nitrifying sequencing batch reactor (SBR) where nitrite concentration varies significantly during an operation cycle. They suggested that *Nitrobacter* could take advantage of the temporarily elevated nitrite concentration and compete successfully with *Nitrospira* in the SBR. The co-existence of *Nitrospira* and *Nitrobacter* was also observed in a full scale activated sludge plant (Coskuner and Curtis, 2002) and a leachate treatment plant where nitrite concentration was highly maintained (Kim et al., 2006). Recently, Kelly et al. (2005) also reported that membrane hybridization format and terminal restriction fragment length polymorphism (T-RFLP) fingerprinting revealed that both *Nitrospira* and *Nitrobacter* were contributing populations in a full-scale activated sludge treatment plant.

Physiological study on NOB showed that under aerobic conditions *Nitrospira* species only took up inorganic carbon and pyruvate but not acetate, butyrate, and propionate while many species of *Nitrobacter* were able to grow mixotrophically with the above organic compounds (Prosser, 1989). In addition, no uptake by *Nitrospira* of any of the carbon sources was observed under anoxic or anaerobic conditions (Daims et al., 2001a) while *Nitrobacter* utilized pyruvate in an anoxic condition (Bock et al., 1988). Therefore, *Nitrobacter* species might have competitiveness over *Nitrospira* because they can utilize organic components in an aerobic or anoxic condition. The above result also supports the possibility of the co-existence of *Nitrobacter* and *Nitrospira* in a nitrogen removal activated sludge plant by nitrification and denitrification.

With respect to kinetic characteristics of NOB, Schramm et al. (1999) suggested that *Nitrospira* represent K strategists adapted to low nitrite and oxygen concentrations, while *Nitrobacter*, as an r strategist, thrives if nitrite and oxygen are present in higher concentrations. The K/r hypothesis was also supported by the fact that *Nitrospira* can be enriched from activated sludge to a certain degree when nitrite concentration in the growth medium is low, while higher nitrite concentrations select for *Nitrobacter* (Bartosch et al., 2002). Nevertheless, no quantitative data from continuous reactor systems have been reported to confirm the hypothesis in engineered systems except Wagner et al. (2002) who compared the dominance of NOB after addition of *Nitrobacter* to the chemostats. Some pure culture experiments with *Nitrobacter* species showed that the specific nitrite-oxidizing activities were 5.1–42 fmol/cell h (Prosser, 1989). Ehrich et al. (1995) reported the specific nitrite-oxidizing activity of *Nitrospira moscoviensis*, which is isolated from a corroded iron pipe of a heating system in Moscow, to be 6.3 fmol/cell h. It is hard to tell that *Nitrobacter* is an r-strategist from the above specific activities, moreover, considering the potential inaccuracy of the most probable number (MPN) technique used for enumerating NOB.

In situ analysis with microelectrodes estimated the nitrite-oxidizing activity of *Nitrospira* as 0.02 fmol/cell h in a nitrifying biofilm system where ammonia-oxidizing bacteria (AOB) compete with NOB for oxygen (Schramm et al., 1999). The AOB might have out-competed NOB for oxygen because AOB are thought to possess lower K_o values for oxygen than NOB (Focht and Verstraete, 1977; Prosser, 1989). Nitrite-oxidizing activity may also be limited by the activity of AOB which provide nitrite to NOB. For the above reasons, the estimated in situ activity may be different from the physiological potential of NOB. No in situ activities of *Nitrobacter* in wastewater treatment systems are available yet. Therefore, it is meaningful to investigate the distribution and specific activities of *Nitrospira* and *Nitrobacter* in mixed culture systems.

The objectives of this study were to investigate the effects of nitrite concentration, nitrite load, and starvation conditions as a selection pressure on the distribution of *Nitrobacter* and *Nitrospira* in two different nitratation reactor systems, and possibly to measure specific nitrite-oxidizing activities of *Nitrobacter* and *Nitrospira,* and finally to examine the validity of the K/r hypothesis. For the purpose, a continuous biofilm airlift reactor (CBAR) running under nitrite limiting conditions and an SBR with high nitrite conditions were operated for 90 days. Nitrite was supplied to the reactors as the only energy source in order to eliminate the growth of ammonia oxidizers, and subsequently to provide more realistic nitrite-oxidation activity.

The distribution of NOB in the biofilm and sludge flocs was determined by FISH with confocal laser scanning microscopy (CLSM). The specific nitrite oxidation activities of each NOB were also estimated from the results of nitrite oxidation kinetics and NOB concentration measurements in the biofilm samples by quantitative FISH image analyses.

2. Materials and methods

2.1. *Bioreactors for nitrite oxidation*

Both CBAR and SBR used the same size of airlift-type reactors which have a concentric tube inside equipped with a