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Nitrifying community structures and nitrification performance of full-scale municipal and swine wastewater treatment plants

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ABSTRACT

This study evaluated nitrification performance and microbial ecology of nitrifying sludge in two full-scale wastewater treatment plants (WWTPs) including a municipal WWTP treating 20 mg N L^{-1} of ammonium and a swine WWTP treating 220 mg N L^{-1} of ammonium. These two plants differed in both wastewater characteristics and operating parameters, such as influent COD, TKN, ammonium, hydraulic retention time, and solids retention time, even though both plants achieve >85% nitrification efficiency. By employing molecular techniques, including terminal restriction fragment length polymorphism, cloning-sequencing and phylogenetic analyses targeting the 16S ribosomal RNA and group specific ammonia-monooxygenase functional gene (*amo*A), microbial community structures of nitrifying sludge and their significance to nitrification performance were evaluated. The results reveal that for the municipal WWTP *Nitrosomonas marina*-like AOB (ammonia-oxidizing bacteria) and *Nitrospira*-like NOB (nitrite-oxidizing bacteria) were the ubiquitously dominant nitrifiers, while *Nitrosomonas europaea*-, *Nitrosomonas oligotropha*-, and *Nitrosospira*-like AOB and *Nitrobacter*- and *Nitrospira*-like NOB were the major nitrifying populations found in the swine WWTP. The observed dissimilar nitrifying populations prevailing in these two plants may be related to niche differentiation concerning ammonium concentrations, system operation, and salinity. Moreover, our results suggest that the swine nitrifying sludge, involving relatively diverse AOB and NOB populations that perform the same task but with distinct growth and survival characters, may allow communities to maintain nitrifying capabilities when conditions change such as sudden increases in ammonium concentrations as examined with nitrification kinetic batch tests.

1. Introduction

Ammonia in water environments can be toxic to aquatic life at sufficiently high levels and creates a large oxygen demand in receiving waters. Nitrogen removal from wastewater is extremely important to protect water resources from pollution discharges, as release of untreated wastewater can result in eutrophication. Nitrification is a series of microbial oxidation processes performed by two different bacterial groups involving ammonia-oxidizing bacteria (AOB) that is responsible for the oxidization of ammonium to nitrite and nitrite-oxidizing bacteria (NOB) that converts nitrite to nitrate. In addition to AOB, a novel bacterium called the ANAMMOX bacterium has also been discovered for its ability of using ammonium as its electron donor and nitrite as its electron acceptor (Strous et al., 1999). Activated sludge is a common biological process for wastewater treatment, but nitrification failure can occur easily, since nitrifiers appear to be inhibited by several environmental and operating factors, including low temperature, extreme pH, low dissolved oxygen (DO) concentration, and a wide variety of chemical inhibitors (Prosser, 1989). Therefore, monitoring of nitrifying populations for early detection of a change in the nitrifying populations should be valuable for securing stable nitrification.

The distribution patterns of distinct AOB/NOB species in the environments reflect the physiological properties of AOB/NOB. Among these, ammonium and nitrite concentrations are considered to be important factors for selection of distinct AOB and NOB species, respectively. According to their physiological properties on ammonium affinity, members of *Nitrosospira spp.* and/or *Nitrosomonas oligotropha* clusters are the prevailing AOB in the environment with low ammonium, whereas *Nitrosomonas europaea* cluster is dominant in the environment that is rich in ammonium (Koops et al., 1991; Koops and Pommerening-Röser, 2001). With respect to NOB, several studies (Both et al., 1992; Bartosch et al., 1999; Schramm et al., 1999; Wagner and Loy, 2002; Gieseke et al., 2003) have indicated that nitrite concentration is the major factor controlling the competition between *Nitrospira* and *Nitrobacter*. Based on models of r- and K-selection in microbial ecology

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(Andrews and Harris, 1986), *Nitrobacter* species represent r-strategists with a low-nitrite affinity, high growth rate and develop large populations when nitrite is present at high concentrations. Conversely, *Nitrospira*-like nitrite oxidizers, as K-strategists, have a high nitrite affinity and a low maximum growth rate adapted to low-nitrite concentrations. In early studies, *Nitrosomonas* and *Nitrobacter* are considered to be the most important nitrifying bacteria responsible for ammonium and nitrite oxidation, respectively. However, recent observations, based on tools and analyses at a molecular level, indicate that *Nitrosospira* also are present along with well-known *Nitrosomonas* (Schramm et al., 1998, 1999; Park et al., 2002), and *Nitrospira* often are the dominant NOB in activated sludge systems (Burrell et al., 1998; Juretschko et al., 1998; Schramm et al., 1998, 1999; Daims et al., 2001; Dionisi et al., 2002; Harms et al., 2003). Although a number of studies have investigated nitrifying bacteria populations in wastewater treatment processes (Daims et al., 2001; Park et al., 2002; Dionisi et al., 2002; Harms et al., 2003; Limpiyakorn et al., 2005; Siripong and Rittmann, 2007), our understanding of linking their presence to nitrification performance at a full-scale level is still limited (Koops et al., 2006). In a study of collecting activated sludge samples from twelve sewage treatment systems, Limpiyakorn et al. (2005) noted the effects of influent characteristics, treatment processes, system operation, and seasonal variation on the AOB communities. By employing molecular tools of denaturing gradient gel electrophoresis (DGGE) and cloning/sequencing of 16S rRNA, they found that the AOB presented in these low-ammonium systems differed and were influenced by distinct environmental factors (Limpiyakorn et al., 2005). However, in another study of surveying seven full-scale municipal wastewater treatment plants (WWTPs) using 16S rRNA-based and *amoA*-based terminal restriction fragment length polymorphism (T-RFLP) methods, Siripong and Rittmann (2007) observed similar AOB and NOB community structures for WWTPs performing stable nitrification, despite significant differences in temperature, solids retention time (SRT), and input of industrial wastewater.

The goal of this study is to provide a better understanding of the linkage between dominant nitrifying populations and their nitrification performance in full-scale wastewater treatment bioreactors by surveying two different WWTPs. One is a municipal WWTP plant treating low ammonium concentrations, while the other is a swine WWTP with high ammonium concentrations. Furthermore, operation with continuous-stir tank reactor (CSTR) mode for the municipal WWTP is also different from that with sequencing batch reactor (SBR) mode for the swine WWTP. In this study, in addition to analysis of wastewater characteristics and nitrification kinetics, molecular techniques, including T-RFLP and cloning-sequencing of 16S ribosomal RNA and functional gene *amo*A, were applied and evaluation of their significance to nitrification performance was discussed in this study.

2. Materials and methods

2.1. Full-scale wastewater treatment plants

Two full-scale WWTPs were studied in this study. The municipal WWTP (Tainan, Taiwan) is a conventional activated sludge process treating 10 000 $m^3\ d^{-1}$ of domestic wastewater. The operation of this plant is characterized by a relative long SRT, generally 20 d, and a hydraulic retention time (HRT) of 10 h. The swine WWTP (Tainan, Taiwan) is an aerobic SBR process with a volume of 2100 m^3 and treats 1000 $m^3\ d^{-1}$ of swine wastewater. The SBR was operated in a 24-h cycle – fill (45 min), aeration (180 min), fill (45 min), aeration (1000 min), settle (60 min), decant (60 min), and idle (50 min). Mixed liquor was withdrawn daily at the end of the second aerobic phase to maintain a SRT of 35 d and a HRT of 2 d. Grab samples of influent and effluent wastewater as well as activated sludge were taken at both plants. After collection, samples were stored in an icy cooler and immediately transported to the laboratory for further analysis. The determination of COD, mixed liquor volatile suspended solids (MLVSS) and nitrogen species followed standard procedures (APHA, 1995).

2.2. Genomic DNA extraction, PCR amplification and TRFLP analysis

The UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA) was used to obtain genomic DNA from the activated sludge samples. Primers *amoA*-1F and *amoA*-2R were used to amplify a 491-bp fragment of the *amoA* gene, according to the protocol described by Park and Noguera (2004). For T-RFLP, *amoA*-1F and *amoA*-2R were labeled with 6-carboxyfluorescein and tetrachlorofluorescein, respectively. PCR (Polymerase Chain Reaction) products were purified using a PCR product purification kit (Qiagen Inc., Valencia, CA, USA) and digested with *TaqI* restriction endonuclease for 3 h at 42 °C. Digested samples were analyzed by capillary electrophoresis in the Nucleic Acid Analysis and Synthesis Core Laboratory at the National Cheng Kung University in Tainan, Taiwan, to determine the size of fragments using an ABI Prism 377 automated sequencer (Perkin-Elmer Corp., Wellesley, MA, USA). Only the T-RFs with abundance greater than 1% of total intensity were used. The observed T-RF lengths were later compared with and identified from the predicted T-RF lengths of the dominant clones obtained in the bacterial *amoA* clone library. For all the analyses, the expected 48 bp terminal fragment could not be unequivocally detected, due to high background noise for fragments smaller than 100 bp.

2.3. Cloning and sequencing

PCR products of unlabeled *amoA* gene fragments or specific amplification products of NOB 16S rRNA for *Nitrobacter* and *Nitrospira* (Regan et al., 2002) were ligated to the pGEM-T Easy Vector System (Promega, Madison, WI, USA) and transformed into *Escherichia coli* DH5α competent cells following the manufacture's protocol (Invitrogen Corp., Carlsbad, CA, USA). Plasmids of clones were extracted by WizardR *Plus* minipreps DNA purification system (Promega, Madison, WI, USA). DNA sequencing reactions were performed using ABI 3100 and 3730 capillary sequencers (Applied Biosystems, Foster City, CA, USA). BioEdit was used to align cloned and published *amoA* sequences in GenBank using the Basic Local Alignment Search Tool program developed by U.S. National Center for Biotechnology Information (Altschul et al., 1997). The sequences determined in this study have been deposited in the GenBank database under accession numbers as follows: *amoA*, EF431852-EF431886; NOB, EF434829-EF434858.

2.4. Batch experiments

In order to evaluate the dependences of nitrification kinetics on ammonium concentrations, a series of batch tests were performed using activated sludge taken from the municipal and swine WWTPs, respectively. For each batch experiment, 800 mL of examined mixed-liquor-suspended solids (MLSS) were centrifuged at 10 000g for 10 min. The supernatant was discarded, and the solids were resuspended in 800 mL of the Bushnëll and Haas medium (Bushnell and Hass, 1941) containing (in g L^{-1}) $MgSO_4\ 7H_2O$: 0.2, K_2HPO_4: 1.0, KH_2PO_4: 1.0, $FeCl_3$: 0.05, $CaCl_2$: 0.02, and $NaHCO_3$: 0.5. NH_4Cl was added to an initial concentration in the range between 0 and 600 mg N L^{-1}. The resuspended solids were placed in a 1 L flask, which was mixed by a magnetic stirrer and aerated to maintain the DO concentration above 3 mg L^{-1}. The pH of the