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Molecular detection of microbial community in a nitrifying–denitrifying activated sludge system

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ABSTRACT

Presence of different forms of ammonia in the wastewater treatment plants are toxic to aquatic life and promote eutrophication in receiving water bodies. It becomes imperative to lower the ammonia concentration in the effluents for which characterization of microbial diversity is foremost objective. Study was planned to identify the diversity of nitrifiers and denitrifiers from activated sludge using PCR technique. Cultural techniques revealed high bacterial diversity was found in the aeration tank where majority of the isolates belongs to class γ proteobacteria followed by β proteobacteria suggesting the adaptability of these groups. Presence of *Nitrosomonas europaea* revealed better ammonium oxidation condition at longer SRT of 6 and 7 d with DO concentration of 2.5 mg/L. As a part of NOB, *Nitrospira* sp. found to be more dominant than *Nitrobacter winogradskyi* as its detection was achieved in all samples of 6 and 7 d SRT, while later was detected only on 7 d SRT. Use of cultural techniques in combination with PCR analysis with 16S rRNA approach revealed *Pseudomonas aeruginosa* as important denitrifier. Highest removal efficiencies of COD, BOD, TN and NH_4^+–N were found to be 81, 78, 70 and 69% respectively at optimized SRT of 7 d.

1. Introduction

The Biological wastewater treatment using activated sludge process (ASP) for the transformation of organic and inorganic pollutants to CO_2 and water has contributed greatly to the improvement of the aquatic environments worldwide (Moura et al., 2009). However, efficiency of the ASP system is greatly influenced by the design and operation of the treatment system. Appropriate design and optimized condition of ASP can affectively attain organic carbon removal including nitrogen and phosphorus (Vaiopoulu et al., 2007). Biological nitrogen and phosphorus have however, limited removal efficiencies because of the different optimum operating conditions. Nitrogen appears in wastewater in several free and ionized forms like ammonia (NH_3 and NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and as organic compounds, collectively as total nitrogen (Rodriguez et al., 2011).

Nitrogen, being essential nutrient for biological growth, is one of the main constituents in all living organism. However, an excessive presence of nitrogen in the effluents should be avoided for several reasons; (i) nitrogen in the reduced forms will exert oxygen demand in the receiving water (Poller et al., 1987); (ii) NH_3 and NO_2^- are toxic to fish and requires large amounts of chlorine for disinfection during treatment of water to potable standards (Dapena et al., 2006); (iii) NO_2^- and NO_3^- can cause eutrophication of lakes and landlocked water bodies resulting in the uncontrolled growth of algae and other aquatic plants (Lim et al., 2007). Hence, it is imperative to remove nitrogen form the wastewater using biological nitrification–denitrification naturally occurring as a part of biogeochemical cycle (Dionisi et al., 2002). For the efficient nitrogen removal, the understanding of relationship between microbial community dynamics and their functional consistency (Wang et al., 2011) to recognize and assure linear working of treatment systems is a valued element for the improvement in the design of ASP (Moura et al., 2009).

Nitrification is an aerobic process which oxidizes NH_4^+ to NO_2^- with the help of ammonia oxidizing bacteria (AOB) followed by the conversion of nitrite to nitrate by nitrite oxidizing bacteria (NOB) (Siripong and Rittmann, 2007). AOB and NOB are termed as nitrifying bacteria (chemolitho autotrophs). *Nitrosomonas*, *Nitrosococcus* and *Nitrosospira* are mostly reported to oxidize NH_4^+ while *Nitrobacter*, *Nitroeystis* and *Nitrospira* species are reported for NO_2^- oxidation (Bothe et al., 2000). Different nitrifiers performing the same task imply functional cease, which can allow microbial groups

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to compete when condition changes. High level of nitrifier variety is considered to affect performance stability (Kowalchuk et al., 2000).

Denitrification is generally considered as a special case of dissimilation where NO_3^- is acted upon by denitrifying bacteria (facultative heterotrophs) to be converted to gaseous NO, N_2O and N_2 (Geets et al., 2007). *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Thauera* sp. and *Flavobacterium* are generally reported to cause denitrification (Lim et al., 2007) while *Pseudomonas* is considered to be one of the most important bacteria that cause denitrification (Khan et al., 2013).

Advent of the molecular techniques permitted to overcome the culture dependent problems which result in an underestimation of the real diversity of microbes (Otawa et al., 2006). As nitrifying bacteria are very slow growing, they are mostly enumerated using molecular techniques such as polymerase chain reaction (PCR), with denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) etc. (Gilbride et al., 2006; Kyunghwa et al., 2010). However, the efforts to assess the microbial diversity of a full scale wastewater treatment plant have limited attention.

The present study was designed for performance evaluation and microbial characterization of full scale sewage treatment plant (STP) which was established in 2007 at sector I-9, Islamabad employing ASP for municipal wastewater treatment. The plant is capable of treating 17 MG of sewage per day. Study have been conducted to optimize performance of STP (Fatima and Khan, 2011), however a study that compares performance and bacterial diversity of the treatment plant has not been evaluated yet. In this context, bacterial consortia responsible for treatment performance (nitrification-denitrification) at various SRTs was investigated to enhance the efficiency of STP.

2. Materials and methods

2.1. Sewage treatment plant and sampling

The phase IV of full scale plant in this research was operated having a treatment capacity and inflow rate of 37,854 and 16,000–20,000 m^3/d respectively. During the study period, HRT, SRT, DO and MLSS concentration of aeration tank (having volume of 3600 m^3) were maintained at 5–6 h, 5–7 d, 2–2.5 mg/L and 3000–3500 mg/L respectively. The temperature during the study period was found to be 16–30 °C. The study was carried out over a period of 160 days starting from mid of October 2011 to March 2012. Activated sludge samples were evaluated for microbial diversity with special focus on nitrifying and denitrifying bacterial composition. Composite samples of wastewater were collected from four locations i.e. influent, primary sedimentation tank, aeration tank and effluent of STP as shown in Fig. 1 (Fatima and Khan, 2011). Activated sludge was sampled from the aeration tank at different SRTs. Temperature of the wastewater and the sludge samples were measured on site and stored at 4 and −20 °C respectively, in the laboratory for microbial and analytical analysis.

2.2. Isolation and identification of microbial species

To isolate dominant microbial consortia serial dilution of samples were prepared upto 10^{-7}. 0.1 ml of each dilution was plated on nutrient agar (Oxoid, UK) and petri plates were incubated at 37 °C for 24 h. Morphological distinct colonies were isolated and confirmed through Analytical Profile Index (API) 20E (Biomeurix France).

Wastewater samples were plated on selective media i.e. cetrimide agar (Oxoid, UK) using the spread plate technique and yellow green colonies were picked and purified. Further confirmation of the yellow green isolate (*Pseudomonas aeruginosa*) was done using an API 20E kit. A pure culture of *P. aeruginosa* ATCC 27853 was also obtained for verification and handled as per manufacturer's instructions.

2.3. DNA extraction and purification

Genomic DNA was isolated for detection of major nitrifiers present in activated sludge using soil DNA spin kit (Norgen, Canada) following the manufacturer's instructions. While a PrepEase kit (Affymetrix, Canada) was used for genomic DNA extraction of *P. aeruginosa*. The purified genomic DNA was stored at −20 °C.

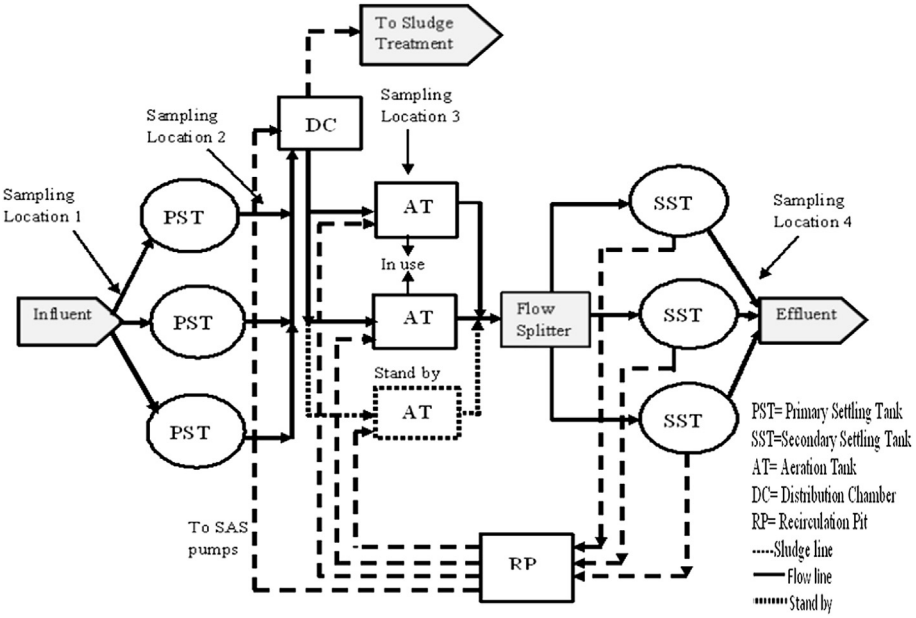

Fig. 1. Detailed schematic of sewage treatment plant, Islamabad (Fatima and Khan, 2011).