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Fate of carbon, nitrogen and phosphorus removal in a post-anoxic system treating low strength wastewater

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ABSTRACT

A lab-scale anaerobic–aerobic–anoxic sequencing batch reactor was operated for 135 days with using acetate as sole carbon source to explore the contribution of denitrifying phosphate accumulating organisms to nitrogen and phosphorus removal in a post-anoxic system. The system was operated at an aerobic sludge age of 2.5 days and DO level greater than 2 mg l^{-1}under variable carbon to nitrogen (C/N) and carbon to phosphorus (C/P) ratios. More than 80% of influent nitrogen and phosphorus were removed simultaneously under aerobic conditions. When aerobic denitrification became limited due to the increase of average dissolved oxygen, overall nitrogen removal continued with the same efficiency, but with a larger contribution from anoxic denitrification. On the other hand, enhanced biological phosphorus removal activity decreased significantly as a result of free nitrous acid (FNA) inhibition. Fluorescence in situ hybridization analysis showed that the relative abundance of *Actinobacter* spp. was decreased by FNA inhibition while the relative abundance of *Accumulibacter* spp. remained unchanged. Conversely, the relative abundance of glycogen accumulating organisms (GAOs) increased from 7.1% to 23% as a result.

1. Introduction

Biological nutrient removal systems have been applied in most of the wastewater treatment plants to prevent eutrophication problems in water sources. Nitrogen is removed in two steps through nitrification and denitrification. In nitrification process, ammonium nitrogen is oxidized to nitrate by autotrophic bacteria under aerobic conditions. In the denitrification step, oxidized nitrogen is reduced to nitrogen gas by heterotrophic bacteria under anoxic conditions. Enhanced biological phosphorus removal (EBPR) process is applied to remove phosphorus. EBPR is achieved by submitting activated sludge to alternating anaerobic and aerobic/anoxic conditions. Under anaerobic conditions, polyphosphate accumulating organisms (PAOs) take up external carbon sources mainly in the form of volatile fatty acids (VFAs) and store them intracellularly as carbon polymers, namely poly-β-hydroxyalkanoates (PHAs). The energy required for the biotransformation is obtained partly from glycogen utilization, but mostly from hydrolysis of stored polyphosphate (poly-P), which is released into the bulk liquid as orthophosphate. In the following aerobic or anoxic phase, PAOs use their stored PHAs as the energy source for biomass growth, glycogen replenishment, phosphorus uptake, and poly-P storage. Net phosphorus removal is achieved by wasting the excess sludge from the activated sludge system when the biomass contains a high level of poly-P (Smolders et al., 1995).

Both nitrogen and phosphorus removal processes require influent carbon which is usually limited and does not satisfy the combined demand of heterotrophic denitrification and of enhanced biological phosphorus removal (Xu et al., 2011). Hence, the importance and overall benefit of denitrifying phosphorus removal has been widely recognized in recent years (Liu et al., 2014; Lopez-Vazquez et al., 2008; Peng et al., 2011; Zeng et al., 2011). These organisms, which can use nitrate/nitrite instead of oxygen as an electron acceptor to remove phosphate, can use the same carbon to simultaneously remove nitrogen and phosphorus with 20–30% less sludge production. In particular, this process is useful to treat wastewaters with a low carbon to nitrogen (C/N) ratio (Kuba et al., 1993; Ostgaard et al., 1997). The most critical point in the application of denitrifying phosphorus removal is oxidation of PHA with nitrite and/or nitrate as the electron acceptors. In two sludge

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systems, nitrifiers and PAOs grow and function at different units (e.g. nitrification in a biofilm reactor or in a SBR) (Kuba et al., 1996). Afterwards, PAOs sludge and nitrified effluent are mixed in an anoxic tank. In the anoxic tank, PHA oxidation with oxidized nitrogen provides phosphorus uptake and denitrification simultaneously. On the other hand, in single sludge systems, this could be achieved using an anaerobic–anoxic–oxic (A2O) configuration (Lopez-Vazquez et al., 2008; Zhang et al., 2011). In this configuration, nitrates produced in the aerobic tank are recycled into the anoxic tank with a high mixed liquor recycle (MLR) rate. The removal of oxidized nitrogen and released phosphorus is ultimately limited by the MLR rate. Although the A2O configuration has the advantage of using intracellular PHAs for nitrogen and phosphorus removal, there are several disadvantages related to MLR pumping. These disadvantages include higher energy costs, the entry of dissolved oxygen (DO) from the aerobic (oxic) zone into the anoxic zone, and the dilution of organic carbon (Winkler et al., 2011). Conversely, there is no need for internal recirculation pumping in the anaerobic–oxic–anoxic (AOA) configuration and the above-mentioned disadvantages can be eliminated. Furthermore, anoxic phosphate uptake can be stimulated in an AOA system, which makes it a promising method for simultaneous nitrogen and phosphorus removal. In this case phosphorus is removed under anoxic conditions with reduced sludge production, aeration cost, and carbon requirement. Contrary to these advantages, the AOA configuration has the disadvantage of depleting intracellular PHAs in the aerobic tank before they are used in the anoxic tank for both nitrogen and phosphorus removals. Under these conditions DNPAOs cannot be enriched in the post-anoxic systems (Lopez-Vazquez et al., 2008). However, enrichment of DNPAOs in the AOA configuration can be achieved if sufficient nitrite or nitrate (as the electron acceptor) and PHAs (as the electron donor) are concurrently available. This requires efficient nitrification accompanied by limited polyhydroxybutyrate (PHB) oxidation under aerobic conditions and can be achieved through simultaneous nitrification, denitrification, and phosphorus removal in the aerobic phase or by partial nitrification followed by anoxic phosphorus removal by the nitrite pathway.

Nitrogen and phosphorus removals in post-anoxic systems have been the focus of a number of recent studies (Coats et al., 2011; Liu et al., 2013, 2014; Winkler et al., 2011). However studies about the simultaneous nitrification, denitrification and phosphorus removal with DNPAOS in the AOA-SBR reactor are very limited (Yilmaz et al., 2008; Zeng et al., 2003a, 2011). In the present study, nitrogen and phosphorus removal mechanisms in an AOA-SBR system were evaluated based under variable influent wastewater characteristics and aerobic and anoxic retention times. Aerobic sludge age of the system and retention time of the aerobic phase were kept at 2.5 days and 1.2 h to limit nitrite and PHB oxidation under aerobic conditions to increase the availability of PHB and nitrite to DNPAOs under anoxic conditions. Besides, the effects of pH, DO, NO_2–N and NO_3–N on nutrient removal dynamics were also assessed together with microbial community changes.

2. Materials and methods

2.1. Reactor

A plexiglass sequencing batch reactor (SBR) with a 3 l active volume was used. The SBR was operated under sequencing AOA conditions for 135 days. The reactor was seeded with sludge obtained from a biological nutrient removal plant located in Istanbul. A plexiglass cover was used to prevent oxygen entrance to the system during the anaerobic period. The system was fed with synthetic wastewater as follows: CH_3COOH (800–1000 mg l^{-1}as COD basis), $(NH_4)_2SO_4$ (40–100 mg l^{-1}as NH_4–N basis), KH_2PO_4 (5–20 mg l^{-1} as PO_4–P basis), and K_2HPO_4 (5–20 mg l^{-1}as PO_4–P basis). Alkalinity for nitrification was provided by the addition of $NaHCO_3$. The trace element solution contained the following (per liter): 0.30 mg H_3BO_3, 0.06 mg $CuSO_4.5H_2O$, 0.24 mg $MnSO_4 \cdot H_2O$, 0.24 mg $ZnSO_4 \cdot 7H_2O$, 0.30 mg $CoCl_2$, 3 mg $FeSO_4 \cdot 7H_2O$, 0.12 mg $Na_2MoO_4 \cdot 2H_2O$, 180 mg $MgSO_4 \cdot 7H_2O$, and 28.5 mg $CaCl_2 \cdot 2H_2O$. Sludge retention time was kept at 10 days by wasting 100 ml of sludge in each cycle. There were three cycles in a day, each 8 h long. During each cycle, 750 ml of synthetic wastewater was fed to the reactor with peristaltic pumps (Watson Marlow 323 S/D, USA). Hydraulic retention time (HRT) was 16 h. Temperature was maintained at 22–23 °C in an air conditioned room. A schematic diagram of the SBR is shown in Fig. 1. During the feeding period, nitrogen gas was introduced into the reactor head space to prevent oxygen entry. Reactor was subjected to anaerobic, oxic, and anoxic (post-denitrification) cycles during the treatment period. The reactor content was mixed with a mechanical stirrer at 200 ± 5 rpm to prevent settling.

2.2. Experimental procedure

The experimental period consisted of three operational phases (Table 1). In each operational phase, influent nitrogen and phosphorus concentrations as well as retention time of the anaerobic–aerobic–anoxic phases were changed. Nitrogen and phosphorus concentrations were increased gradually based on the nitrogen and phosphorus removal performances obtained in aerobic and anoxic phases. Influent COD concentration was not changed since the C/P and C/N ratios of the system were higher than the recommended values for an efficient nutrient removal system. After nitrogen and phosphorus concentrations were increased in operational phases II and III, the retention time of the aerobic phase was increased to provide sufficient time for nitrification and phosphate uptake. Throughout the whole experimental period, the retention time of the aerobic phase was maintained below 2 h to obtain an aerobic sludge age less than 2.5 days to decrease the activity of nitrite oxidizing bacteria (NOB) and limit the PHB oxidation in the aerobic phase. DO was above 2 mg l^{-1} in whole operational period.

2.3. Monitoring reactor performance

Daily samples were taken from the feed tank and effluent collection tank (composite sample) to monitor the nitrogen and phosphorus removal efficiencies of the system. Cycle measurements were performed once a week to calculate nitrogen balance and process rates in anaerobic, aerobic, and anoxic phases. Mixed liquor samples for mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) measurements were taken during the anaerobic phase before feeding the reactor. Temperature, DO, and pH were measured with online measurement devices (HACH Lange, UK) to monitor process dynamics during each phase. All data was recorded at 15-min intervals and stored in a computer.

2.4. Determination of the fraction of DNPAOs

Batch experiments were carried out to determine DNPAO fractions as previously described (Kuba et al., 1997). During the normal SBR cycle, 1 l of mixed liquor was taken from the anaerobic reactor at the end of the anaerobic phase. It was then divided equally into aerobic and anoxic batch reactors. The anoxic reactor was spiked with excess nitrate (30 mg l^{-1}) while the aerobic reactor was aerated only. Both reactor contents were stirred with a magnetic stirrer. Specific phosphorus uptake rates were determined for