



Research article

Dynamics of microbial community structure and nutrient removal from an innovative side-stream enhanced biological phosphorus removal process



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ABSTRACT

Biological phosphorus (P) and nitrogen (N) removal from municipal wastewater was studied using an innovative anoxic-aerobic-anaerobic side-stream treatment system. The impact of influent water quality including chemical oxygen demand (COD), ammonium and orthophosphate concentrations on the reactor performance was evaluated. The results showed the system was very effective at removing both COD (>88%) and $\text{NH}_4^+\text{-N}$ (>96%) despite varying influent concentrations of COD, $\text{NH}_4^+\text{-N}$, and total $\text{PO}_4^{3-}\text{-P}$. In contrast, it was found that the removal of P was sensitive to influent $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ concentrations. The maximum $\text{PO}_4^{3-}\text{-P}$ removal of 79% was achieved with the lowest influent $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ concentration. Quantitative PCR (qPCR) assays showed a high abundance and diversity of phosphate accumulating organisms (PAOs), nitrifiers and denitrifiers. The MiSeq microbial community structure analysis showed that the *Proteobacteria* (especially β -*Proteobacteria*, and γ -*Proteobacteria*) were the dominant in all reactors. Further analysis of the bacteria indicated the presence of diverse PAO genera including *Candidatus Accumulibacter phosphatis*, *Tetrasphaera*, and *Rhodocyclus*, and the denitrifying PAO (DPAO) genus *Dechloromonas*. Interestingly, no glycogen accumulating organisms (GAOs) were detected in any of the reactors, suggesting the advantage of proposed process in term of PAO selection for enhanced P removal compared with conventional main-stream processes.

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1. Introduction

Discharge of nitrogen (N) and phosphorus (P) via wastewater effluents into receiving water bodies causes poor water quality that may lead to eutrophication threatening both human and environmental health (Wang et al., 2015a). Numerous biological treatment approaches have been applied in municipal wastewater treatment

plants (WWTPs) for the removal of N and P (Wang et al., 2015a). Generally, N removal is performed by exposing bacterial populations to an aerobic followed by an anoxic environment (Wang et al., 2015b; Wu et al., 2014). For P removal, enhanced biological phosphorus removal (EBPR) processes are utilized by exploiting the ability of polyphosphate accumulating organisms (PAOs) to accumulate P, store it as intracellular polyphosphate (poly-P) under aerobic conditions and release under anaerobic conditions (Bowman et al., 2007; Seviour et al., 2003). However, glycogen accumulating organisms (GAOs) can also become abundant in EBPR systems that compete with PAOs and negatively impact the EBPR process removal performance (Oehmen et al., 2007; Ong et al., 2014). A more advantageous biological wastewater treatment approach would include the simultaneous removal of N and P from wastewater by exploiting various bacterial populations based on oxygen availability.

The removal of P has been successful in WWTPs using

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alternating anaerobic-anoxic-aerobic (A^2O) processes (Oehmen et al., 2007; Wang et al., 2015a) with main-stream (e.g., Phoredox, University of Capetown, Bardenpho, Johannesburg) (Metcalf et al., 2003) or side-stream (e.g., Phostrip) (Kaschka and Weyer, 1999) anaerobic reactor arrangements. Conventionally, the main stream A^2O processes have been widely utilized where the feed wastewater is introduced into the anaerobic reactor (Qiu et al., 2010; Zeng et al., 2010). In the conventional process, the entire influent wastewater stream passes through the anaerobic reactor making it vulnerable to fluctuations in raw wastewater characteristics. By comparison, a better process control can be maintained through the use of side stream processes (Kaschka and Weyer, 1999). The solids retention time (SRT) in the anaerobic reactor of a typical side-stream process is 3–5 times longer than a similar volume main stream process reactor (Kaschka and Weyer, 1999; Metcalf et al., 2003). This longer SRT leads to improved P release from sludge in the anaerobic zone, especially in the presence of nitrate. In addition, the reduced P of the returning sludge allows for more efficient uptake of P in the influent wastewater by the PAOs in the aerobic reactor. Moreover, the increased P release in the anaerobic reactor creates a smaller, more highly-concentrated, P wastewater stream from the supernatant zone allowing for recovery of P which can be valuable for uses such as incorporation into fertilizer. Additionally, recent studies have demonstrated that P removal can be enhanced by denitrifying PAOs (DPAOs) found in anoxic reactors (Wang et al., 2015b). These DPAOs allow for the simultaneous removal of N and P, however, reactor configurations to optimize these removals have not been fully developed.

In the current study we demonstrate an innovative anoxic-aerobic-anaerobic side-stream process with the feed wastewater entering into an anoxic reactor followed by an aerobic reactor (prenoxic configuration) and final side-stream anaerobic reactor. In this process, the P-rich supernatant is separated in the anaerobic reactor from the sludge recycled into the aerobic reactor, instead of the anoxic reactor, to promote P uptake. In addition to the benefit of P recovery from the supernatant of the anaerobic reactor, the sludge in the anaerobic reactor will be very voracious to make P uptake after returning to aerobic reactor. This anaerobic tank also provides additional readily biodegradable COD (rbCOD) from cell lysis for the synthesis of polyhydroxyalkanoates (PHAs) that, when returned to the aerobic tank, helps the PAOs to uptake P more efficiently by providing a carbon and energy source. The internal recycle between aerobic reactor and anoxic reactor could minimize the amount of nitrate entering the anaerobic reactor. Overall, the process could enhance P removal by both DPAOs in the anoxic reactor and PAOs in the aerobic reactor. The main objectives of this study were to evaluate the simultaneous N and P removal and to investigate the microbial community compositions (including PAOs, DPAOs and GAOs) in the various treatment reactors. To meet these objectives, the N and P loadings were varied to determine their impacts on their removals and community compositions. With the aid of qPCR and high-throughput sequencing, the microbial community dynamics were studied with close scrutiny for the abundance of major PAOs, DPAOs and GAOs in each reactor.

2. Materials and methods

2.1. Raw wastewater collection and mixed wastewater preparation

The raw WWTP influent wastewater was collected in two separate sampling events (March 08, 2015 and May 12, 2015) in 200 L HPDE barrels from the Gold Bar Wastewater Treatment Plant (GBWWTP; Edmonton, AB, CA) which uses the Johannesburg

Process (main stream process) followed by an oxidation ditch for biological nutrient removal. This raw wastewater was stored in 4 °C prior to use for Stage 1 experiments. To evaluate the system performance at various N and P concentrations, mixed wastewaters (raw and treated) were prepared for Stage 2 and 3 experiments (see [Supplementary Materials](#) for the details). Before starting experiments, the raw and mixed wastewater were characterized for typical wastewater parameters after filtration using nylon membrane filters with pore size of 0.2 μ m (Fisher Scientific Company, ON, CA) as shown in [Table S1 \(Supplementary Materials\)](#).

2.2. Experimental schematic and operation

An innovative side-stream EBPR process was designed for the treatment of GBWWTP wastewater to remove N and P. This system consisted of an anoxic reactor, followed by an aerobic reactor and anaerobic digester as shown in [Fig. 1](#). Peristaltic pumps (Cole-Parmer, QC, CA) with Masterflex tubing (Cole-Parmer, QC, CA) were used for all process flows. As the feed (line 1) was introduced into the anoxic reactor at 4.0 mL/min, NaOAc (0.4 mL/min at 4000 mg/L) (line 10) was added to the anaerobic reactor (final concentration of ~400 mg COD/L) as a carbon source to help increase the reactor P uptake. The internal recycle (line 3) and sludge recycle (line 8) ratios of 3 and 0.35, respectively, were taken from the literature based on previous anoxic treatment processes (Metcalf et al., 2003). The uniqueness of the process was: (a) an internal recycle (line 3) from aerobic to anoxic reactor was used to allow the anoxic uptake of P by DPAOs/PAOs instead of recycling the clarified activated sludge as used in the extended Phostrip process; (b) the anaerobic digested sludge was recycled to the aerobic reactor instead of sending to the anoxic reactor to enhance the P uptake; (c) a single sludge recycle stream was used instead of multiple recycle streams as used in the extended Phostrip process, that reduces the complexity of the side stream process. The bioreactors had a total working volume of 8.0 L (anoxic reactor: 2.4 L, aerobic reactor: 3.0 L, final settling tank: 0.8 L, anaerobic reactor: 1.8 L). Based on the flow rate, the hydraulic retention times were 2.5, 3, 2.5 and 14 h, respectively. An air pump with fine bubble air diffusers was used in the aerobic reactor to maintain a dissolved oxygen (DO) concentration of 3–4 mg/L. The reactors were operated continuously at room temperature (21 ± 1 °C).

A mixed liquor suspended solids (MLSS) sample from the GBWWTP aerobic reactor was taken during the first sampling event and immediately used to seed the aerobic/anaerobic reactor. After seeding, the treatment process was run continuously for 4 months under various loading conditions to evaluate the impact of N (as NH_4^+-N) and P (as $PO_4^{3-}-P$) on the process performance. There were three major Stages (1, 2 and 3) using variable P influent doses of ~7, ~4 and ~21 mg/L in the feed wastewater, respectively ([Table 1](#)). The Stage 1 Phase I $PO_4^{3-}-P$ concentration was the natural concentration of the GBWWTP influent on the first sampling date and Phase II concentration was from the second sampling date. Each Stage was operated until reaching steady-state based on $PO_4^{3-}-P$ removal rates (at least 3 weeks) using identical operating conditions outlined above. The overall process and individual aerobic and anaerobic reactor performances were evaluated using various chemical and physical parameters of the raw and treated wastewaters including the COD, NH_4^+-N , $PO_4^{3-}-P$ (reactive and total), nitrite (NO_2^-), nitrate (NO_3^-), the redox potential, alkalinity and pH. The biological characterization of sludge samples from the phosphorous uptake zone (aerobic reactor), the phosphorous release zone (anaerobic reactor), the N removal zone (anoxic reactors) and feed wastewaters were analyzed for microbial community structures by using qPCR and MiSeq analysis at various Stages (Section 2.3).

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