Enhanced Biological Phosphorus Removal During Simultaneous Nitrification and Denitrification in an Oxidation Ditch

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Point and nonpoint source nutrient discharges to surface waters can lead to eutrophication and endanger aquatic life. After years of eutrophication, the Tampa Bay Estuary Program (TBEP) was successful in restoring Tampa Bay water quality by controlling the discharge of point sources, such as wastewater effluent (Morrison et al., 2011). According to the National Pollutant Discharge Elimination System (NPDES), treated effluent at Hillsborough County wastewater plants, including the Falkenburg Advanced Wastewater Treatment Plant (FAWTP), must contain no more than an annual average of 3 mg/L of nitrogen (as N) and 1 mg/L of phosphorus (as P) when it's discharged to the surface water.

Biological and/or chemical processes can be used for removal of nitrogen and phosphorus from wastewater. Biological nitrogen removal uses ammonia-oxidizing microorganisms (AOM) and nitrite-oxidizing bacteria (NOB) to oxidize ammonia to nitrate under aerobic conditions. Subsequently, denitrifying microorganisms transform nitrate to nitrogen gas (N_2) under anoxic conditions. Enhanced biological phosphorus removal (EBPR) uses polyphosphate-accumulating organisms (PAOs) to release phosphorus from bacterial cells under anaerobic conditions, and then take up phosphorus under anoxic or aerobic conditions, yielding a net removal of phosphorus from the aqueous solution.

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Chemical techniques for phosphorus removal are based principally on the addition of coagulants, such as aluminum sulfate (alum), ferric chloride, or lime, all of which can result in the precipitation and subsequent sedimentation of a solid compound, such as $AlPO_4$ (Tchobanoglous et al., 2014).

Biological treatment is usually preferred in Florida, where warm temperatures favor EBPR; however, biological treatment does not always meet the requirement for phosphorus removal, so it's frequently accompanied by chemical addition to remove the remaining phosphorus.

Different reactor configurations are used for implementing biological removal of nutrients. In many of these configurations, such as A^2O or Bardenpho, separate basins or zones are required with different oxidation-reduction conditions and carbon sources in each basin to induce the required conditions. In contrast, an oxidation ditch (OD) is a configuration in which only one reactor is used instead of separate aerobic and anoxic reactors for biological nitrogen removal. The OD can be preceded by an anaerobic stage to initiate EBPR. Many wastewater treatment plants across the United

Figure 1. Aerial photograph of Falkenburg Advanced Wastewater Treatment Plant. *(courtesy of Hazen & Sawyer)*

States, including FAWTP, employ ODs. The OD is an economical and efficient design in Florida where land costs are:

- Relatively low in many regions
- Effluent carbon, nitrogen, and phosphorus limits are strict
- Large numbers of utilities have practical experience with this technology

Previous research has shown that simultaneous nitrification and denitrification (SND) occurs in ODs (Rittmann & Langeland, 1985; Daigger & Littleton, 2000; Sager, 2016). The SND is believed to occur by any of three possible mechanisms (Daigger and Littleton, 2000):

- *Bioreactor macroenvironment.* A single bioreactor, such as an OD, can contain both aerobic and anoxic microenvironments, supporting nitrification and denitrification, respectively.
- *Floc microenvironment.* A biological floc can contain a gradient of oxygen concentration, such that nitrification occurs near the aerated surface of the floc and denitrification occurs in the anoxic interior of the floc.
- *Novel microorganisms.* Microorganisms that use denitrification by PAOs in a "previously unrecognized pathway" are able to remove nutrients from wastewater (e.g., denitrification by PAOs).

A number of prior studies have been carried out to assess nitrogen removal during SND (Rittmann & Langeland, 1985; Daigger & Littleton, 2000; Hao et al., 1997; Liu et al., 2010; Sager, 2016); however, the fate of phosphorus during SND is still not well understood. A few studies investigated phosphorus removal in SND systems at the bench scale (Zeng, 2003; Rout et al., 2007; Datta & Goel, 2010; Filipe & Daigger, 1999), pilot scale (Peng et al., 2007), and full scale (Littleton et al., 2003; Datta & Goel, 2010).

Some researchers have reported the occurrence of EBPR during SND (Datta & Goel, 2010; Peng et al., 2007; Littleton et al., 2003; Zeng, 2003), but the removal mechanisms and expected removal efficiency are still not well understood. Further research is needed on the fate of phosphorus during SND to efficiently and reliably meet permit limits by employing an OD, and to minimize the cost of additional reagents for chemical precipitation.

The overall purpose of this study was to determine the fate of phosphorus during SND at FAWTP. More specifically, this article will:

- Assess treatment performance and SND occurrence at FAWTP
- Analyze phosphorus behavior and fate at FAWTP
- Compare the results of this study to previously published results

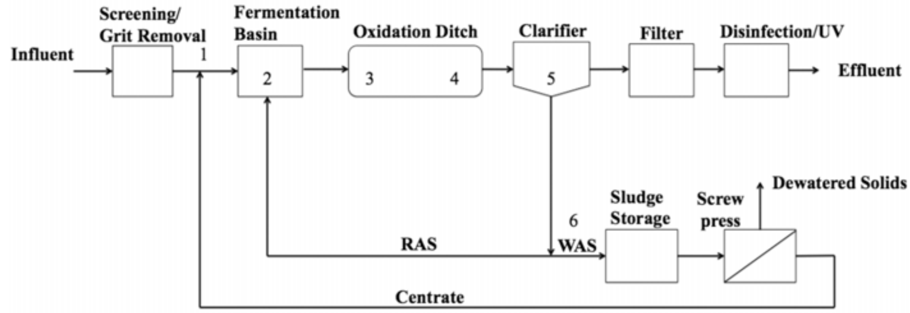

Figure 2. Layout of the Falkenburg Advanced Wastewater Treatment Plant and sampling locations. Numbers indicate sampling points. Location 1 represents plant influent and centrate combined; location 2 indicates fermentation liquid combined with return activated sludge.

Table 1. Volume and hydraulic retention time of fermentation basin, OD, and clarifier at the Falkenburg Advanced Wastewater Treatment Plant.

	Fermentation Basin	Oxidation Ditch	Clarifier
Number of tanks	4	4	5
Total volume (gal)	1,215,800	7,130,000	4,112,300
Hydraulic retention time (hours)	3	18.5	11

Methods and Materials

Site Description

The FAWTP is an advanced wastewater treatment facility in Hillsborough County that incorporates nutrient removal in its treatment system. It has a daily average influent flow rate of 9.27 mil gal per day (mgd), with a permitted annual average daily flow rate of 12 mgd. An aerial photograph of FATWP is shown in Figure 1 and the treatment process train at FAWTP is shown in Figure 2. Stages involved in wastewater treatment at FAWTP are as follows:

- Screening and grit removal
- Fermentation for phosphorus release and EBPR promotion
- Carrousel® OD with two 200-horsepower mechanical aerators located at both ends for removal of biochemical oxygen demand (BOD), SND (nitrogen removal), and biological phosphorus uptake
- Sedimentation in circular secondary clarifiers for liquid and solids separation
- Filtration of clarifier supernatant
- Disinfection with ultraviolet (UV) radiation
- Discharge to the Palm River, Hillsborough River Bypass Canal, or reuse for irrigation

During sedimentation, alum ($Al_2[SO_4]_3$) is added in the clarifier to chemically remove phosphorus not taken up by EBPR. A portion of the settled solids from the clarifier is returned to the fermentation basin, while the rest is wasted. The volume and hydraulic retention time (HRT) of the fermentation basin, OD, and clarifier are shown in Table 1. The average mean cell residence time (MCRT) and mixed liquor suspended solids (MLSS) between Sept. 1, 2010, and August 31, 2013, as well as mixed liquor volatile suspended solids (MLVSS) between Sept. 1, 2010, and August 1, 2011, at FAWTP were reported by Knapp (2014). The MCRT (7-d moving average), MLSS, and MLVSS varied between 15 and 51 days, 4000-7000 mg/L, and 3800-5200 mg/L, respectively, for all four ODs at FAWTP.

Sampling Campaigns

Three sampling campaigns were carried out at FAWTP on Oct. 13, Nov. 3, and Dec. 30, 2015. Six locations were sampled:

- Influent to the fermentation basin (mixture of plant influent and filtrate)
- Fermentation basin combined with return activated sludge (RAS)
- Toward the beginning of the OD #1
- Toward the end of the OD #2
- Secondary clarifier
- Waste activated sludge (WAS)

Samples were collected in 1-liter acid-washed containers and transported on ice to the environmental engineering laboratory at the University of South Florida (USF) within two hours of collection and analyzed in duplicate for total phosphorus (TP), ammonium, nitrite, nitrate, phosphate, and alkalinity immediately upon arrival at the laboratory. The pH was measured in the laboratory and onsite. Additional details can be found in Sager (2016).

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Analytical Methods

Samples were tested for TP via Standard Method 4500-P-E (Rice et al., 2012), employing Hach phosphorus (reactive and total) TNT plus test kits. Method detection limits are 0.5 mg/L when using low-range kits, 1.5 mg/L when using high-range kits, and 6 mg/L when using ultra-high-range kits. Samples tested for TP were not filtered, representing phosphorus concentration in both solid and liquid wastewater fractions. For the phosphate test, pretreatment was necessary by centrifuging (at 8.5 revolutions per minute for 10 minutes) and filtering (using 0.45µm HA filter paper) samples. After pretreatment, samples were analyzed for phosphate via ion chromatography (IC) with chemical suppression of eluent conductivities using a Metrohm 850, Professional IC (MDL [mg/L]: PO_4^{3-}, 0.02).

Ammonium, nitrate, and nitrite were measured via IC with chemical suppression of eluent conductivities (Dionex 2001), employing a Metrohm 850, Professional IC. Method detection limits are 0.2 mg/L for NH_4-N^+, 0.01 mg/L for NO_3^--N, and 0.04 mg/L for NO_2^--N.

Alkalinity was tested with a Metrohm Dosimat Plus multipurpose dispensing unit according to Standard Method 2320 B (Rice et al., 2012). In the laboratory, dissolved oxygen (DO) and pH were measured respectively with a Hach SC1000 Controller (range 0-90 mg/L) and an Orian 5 Star Meter Probe. A YSI 556 Handheld Multiparameter Instrument (range 0-14) was used to measure pH onsite. The pH was measured according to Standard Method 4500-H+B (Rice et al., 2012).

Results and Discussion

Plant Performance

In order to understand phosphorus removal in ODs, it's essential to assess plant performance. The pH and alkalinity across FAWTP processes are presented in Figure 3. The pH ranged between 7.1 and 7.4 across the six sampling locations. The drop in pH in the fermentation basin can be an indication of organic acids production by anaerobic organisms. Alkalinity varied between 150 and 260 mg/L as calcium carbonate ($CaCO_3$) across the plant. A noticeable decrease in alkalinity was observed in the clarifier, which can be explained by the addition of alum.

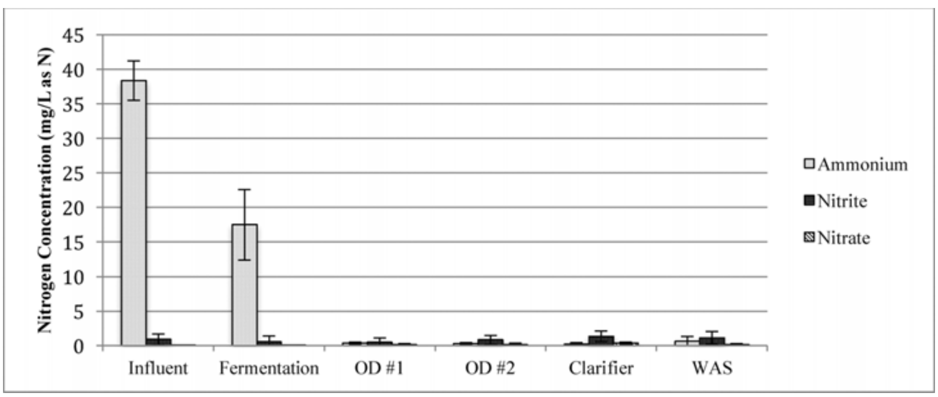

Figure 3. Average alkalinity and pH in the influent, fermentation basin, oxidation ditch #1, oxidation ditch #2, clarifier, and waste activated sludge at the Falkenburg Advanced Wastewater Treatment Plant.

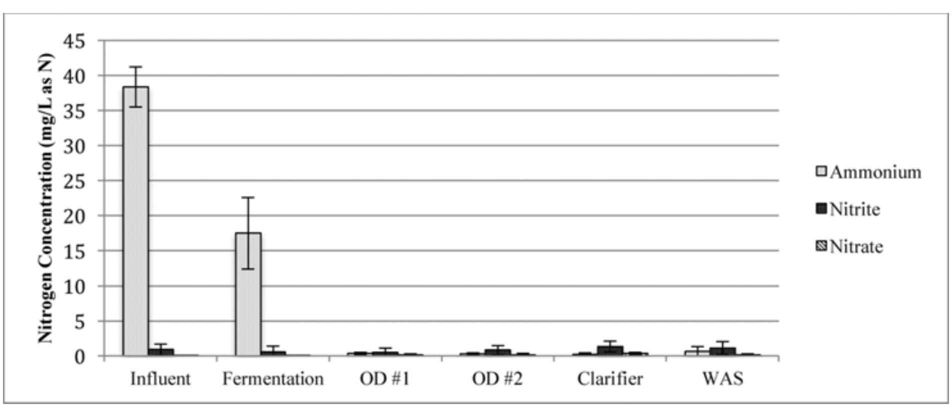

Figure 4. Average concentrations of nitrogen species (ammonium, nitrite, and nitrate) in the influent, fermentation basin, oxidation ditch #1, oxidation ditch #2, clarifier, and waste activated sludge at the Falkenburg Advanced Wastewater Treatment Plant.

Simultaneous Nitrification and Denitrification

Successful nitrogen removal in the OD at FAWTP is shown in Figure 4. Ammonium removal efficiency in the plant was 99.5 percent, reducing the concentration from 38.4 to 0.2 mg/L, as N. Approximately half of the removal (54 percent) was observed in the fermentation basin, indicating possible volatilization or occurrence of anaerobic ammonium oxidation. Further research should be carried out to explore the mechanisms responsible for the removal of ammonium in the fermentation basin at FAWTP. Nitrite and nitrate concentrations were low and similar across the six sampling locations. The near-complete removal of ammonium without accumulation of nitrite or nitrate is evidence of SND occurring in the OD.

Since nitrification and denitrification occur under aerobic and anoxic conditions respectively, SND at FAWTP can be attributed to different aeration zones within the OD. Sager (2016) investigated SND mechanisms in the MLSS from FAWTP by running a controlled bench-scale experiment and reported that SND did not occur unless DO was cycled between 0.5 and 3 mg/L. The DO impact on SND is critical because denitrifying organisms are facultative aerobes, so they start to use oxygen as an electron acceptor instead of nitrate at high DO, which interrupts denitrification (Rittmann & Langeland, 1985). Also, very low DO can inhibit nitrifying organisms, leading to partial nitrification and nitrous oxide (N_2O) emission. Nitrite formation was insignificant at FAWTP, which can indicate the occurrence of complete nitrification.

Besides the macroenvironment, other mechanisms could be responsible for SND within the OD. Anoxic microenvironment theory suggests the presence of oxygen gradient within the aerated floc, creating anoxic conditions in the inner layer, which supports denitrification (Schramm et al., 1999). Satoh et al. (2003) studied the impact of the DO level in the reactor on the oxygen gradient within the floc and reported that SND can be achieved when DO in the reactor is between 0.3 and 1.1 mg/L. Other suggested mechanisms that can facilitate SND are denitrification by autotrophic ammonia oxidizers (e.g., anaerobic ammonia oxidation [anammox] organisms) and aerobic denitrification (Littleton et al., 2003).

Anammox organisms can oxidize ammonium to nitrogen gas (N_2) using nitrite as oxidant, in the absence of oxygen (Jetten et al., 1999). In case of partial nitrification in the OD, nitrite production can serve as electron acceptor for anammox organisms in unaerated zones and result in SND. Some heterotrophs, such as *Thiosphaera* *pantotropha*, are able to denitrify nitrate under aerobic conditions (Robertson et al., 1988).

Biological Phosphorus Removal in the Oxidation Ditch

The average TP concentration in the influent wastewater was 12 mg/L as P, which slightly exceeded the typical influent upper range of 11 mg/L as P expected in municipal wastewater (Tchobanoglous et al., 2014). The phosphate concentration fraction in the influent was approximately 50 percent of TP, falling in the range of the expected inorganic phosphorus concentration in municipal wastewater, which is typically 3-8 mg/L as P (Tchobanoglous et al., 2014).

Average phosphate concentrations in the influent, fermentation basin, at two points in the OD #1, OD #2, and the clarifier, are shown in Figure 5. Phosphorus release by PAOs can be observed in the fermentation basin, where phosphate concentration went from 6.3 mg/L as P in the influent to 28 mg/L as P in the fermentation basin. Also, phosphorus uptake by PAOs in the OD can be observed. Phosphate concentrations decreased to 0.6 mg/L as P, indicating the ability of EBPR to occur in the OD. The efficiency of EBPR at FAWTP can be calculated to be 90 percent based on the decrease of phosphate from 6.3 mg/L in influent to 0.6 mg/L in OD, before alum addition. Knapp (2014) observed a similar release and uptake of phosphate at FAWTP. The remaining phosphorus was precipitated by alum addition in the clarifier, where the concentration of phosphate was below detection limits. These observations demonstrate the ability for EBPR to occur in an OD during SND; however, the mechanism driving the biological phosphorus removal is not yet identified.

Observed phosphorus removal can be attributed to different mechanisms:

- Phosphorus uptake in the aerated zones of the OD can be achieved by PAOs using poly-ß-hydroxyalkanoate (PHA) stored during the previous fermentation stage as carbon source (Tchobanoglous et al., 2014; Peng et al., 2007).
- Phosphorus uptake in anoxic zones of the OD can be carried out by denitrifying PAOs (DPAOs), such as the *Bacillus cereus* GS-5 strain, that have shown to denitrify nitrate/nitrite and take up phosphorus under anoxic conditions using an external carbon source other than PHA (Rout et al., 2017). Rout et al. (2017) explored the ability of the *Bacillus cereus* GS-5 strain to remove nitrogen and phosphorus from domestic wastewater by running a controlled experiment. The authors reported 96, 95, 84, and 81 percent removal of ammonium, nitrate, nitrite, and phosphate, respectively. Further research should be conducted to investigate the presence of DPAOs at FAWTP.
- Phosphorus uptake can be carried out across flocs. Anaerobic conditions may be present in the inner layer of the floc, enabling the release of phosphorus that accompanies the release in the fermentation basin. Phosphorus uptake occurs in the outer layers of the floc where aerobic conditions are available. In order to evaluate this mechanism, Datta & Goel (2010) conducted a bench-scale experiment where they assessed phosphorus release and uptake in mixed liquor with flocculated and nonflocculated biomass for comparison; however, results showed that intra floc micro zones did not add efficiency to EBPR. Further research should be conducted to assess the importance of this mechanism.

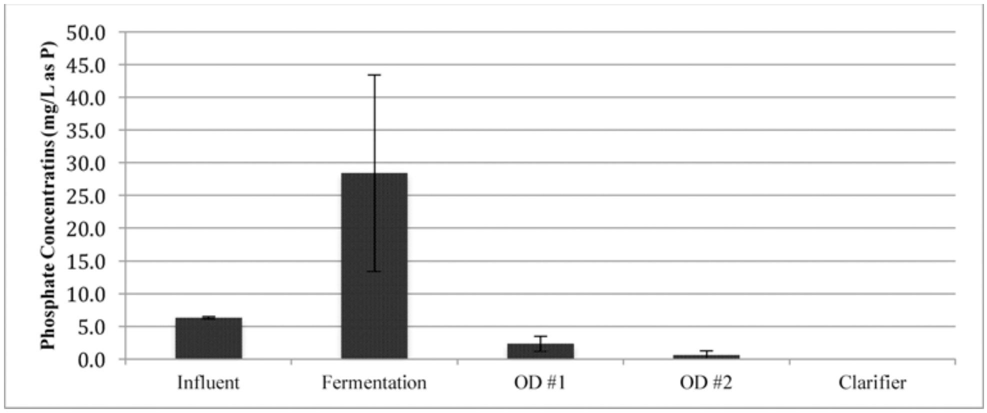

Figure 5. Average phosphate concentrations in the influent, fermentation basin, oxidation ditch #1, oxidation ditch #2, and clarifier at the Falkenburg Advanced Wastewater Treatment Plant (average based on sampling campaigns 2 and 3 only).

Comparison of the Falkenburg Advanced Wastewater Treatment Plant With Other Published Studies

Biological phosphorus removal during SND has been reported in previously published papers (Datta & Goel, 2010; Peng et al., 2007; Filipe & Daigger, 1999). Peng (2007) looked into P removal in a pilot-scale anaerobic-anoxic OD system consisting of anaerobic, anoxic, and aerobic zones. Results showed that the OD achieved efficient biological phosphorus removal of 85 percent, which is similar to the removal accomplished by FAWTP (90 percent).

Datta & Goel (2010) monitored phosphorus removal in full-scale oxidation ditches at four wastewater treatment plants. None of these ODs were designed to remove phosphorus; nevertheless, it was found that phosphorus release and uptake occurred. The release rate, however, was higher than the uptake rate at the four full-scale ODs.

Conclusions

The SND and EBPR were shown to occur in an OD at FAWTP. Biological ammonium and phosphorus removal efficiency at FAWTP were 99.5 and 90 percent, respectively. The net production of nitrate and nitrite was insignificant. The SND in the OD can be attributed to different aerobic and anoxic zones in the OD, intra floc microanoxic zones and/or aerobic denitrification (e.g., *Thiosphaera pantotropha*). Typical EBPR behavior was observed, where phosphate was released in the fermentation basin and taken up in the OD. Phosphorus uptake can be carried out in aerobic zones by PAO and/or in anoxic zones by DPAO (e.g., *Bacillus cereus* GS-5 strain) in the OD. Another not-well-understood mechanism of phosphorus uptake could occur on a microenvironment level. Further research should be conducted at FAWTP to investigate the presence of DPAOs. Comparing these results to other published studies showed similar behavior of phosphorus removal at different wastewater treatment plants employing ODs for biological treatment.

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