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Novel Biological Strategies for Phosphorus Recovery from Wastewater

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Abstract

In recent decades the exponential trend of population increase has resulted in ever increasing requirements for resources to satisfy basic human needs. Food as one of the most important basic human needs gains priority over other issues. Food production is entirely instituted in the agricultural industry and constantly evolving agricultural practices are looking for efficient end-product fertilizers that provide a high nutrient availability and elevated micronutrient exposure to optimize crop production. Phosphorus (P), as one of the key micronutrients in the growth cycle of a plant, is thus an increasingly valuable commodity. However, the over-consumption of natural P resources, through mining of phosphate rock for agricultural and industrial purposes, has put the future adequacy of this resource in question. If the current consumption trends continue, future P scarcity and the associated negative consequences will undoubtedly occur.

A big proportion of P that is contained in waste either from industry, household or agricultural usage ends up in municipal wastewater streams and hence, these waste streams are a potential resource for recovery of P. For much of the last century, the focus has been on reduction of P from wastewater to protect the environment from toxic effects of nutrient loading into water bodies, but now a shift in the paradigm from just removal to "removal and recovery" has occurred. Ideally the most applicable strategies are to develop materials that increase the total net value of P recovery.

P recovery from wastewater regardless of the technology must follow three steps, namely P accumulation, P release and P extraction. Among all the available approaches, biological methods generally minimise adverse environmental impacts, energy consumption and acidification of downstream waters or soils. Biological P accumulation and release processes usually occur in the sludge line of an enhanced biological system equipped with anaerobic digesters. However, many treatment plants are still operating with less sophisticated facilities, without advanced EBPR operation. While sludge treatment through dewatering or anaerobic digester units provides a high opportunity for P recovery, many plants do not have these facilities. Hence the objective of this thesis was to address this gap by proposing simple strategies for P recovery that are adoptable and potentially retro-fittable to the majority of conventional municipal wastewater treatment systems.

For this purpose, the potential of some of the alternative available treatment methods to incorporate P recovery was examined. Phostrip, a simple but largely overlooked technology was systematically studied in order to determine the kinetics of P release, and the potential for providing a P recovery stream in non-EBPR plants. It was shown (Chapter 3) that in designing such a process there are many detailed considerations to be accounted for but that it is feasible to practically implement the process to recover a significant amount of waste P which would considerably add to the total value of P recovery. Findings suggested that high concentrations of P (above 100 mg/L p) can be achieved in the returned activated sludge (RAS) of a municipal wastewater treatment plant and that a tank as small as 9 m^3 can facilitate the recovery of the influent incoming P from a municipal wastewater treatment plant with a capacity of 61 ML/d. In Chapter 4 it was demonstrated that the application of granular sludge, due to the high density and bioconversion potential (also known as biotransformation capability of a bacterial community), can make it possible to obtain concentrations of up to 500 mg/L P. Under laboratory condition, using an alternating aerobic/anaerobic sequencing batch reactor (SBR), it was shown that P accumulating granular biomass can increase the concentration of P by 45-fold, by reducing the volume of the recovery stream by 5-fold in the anaerobic P release phase. It was shown that the approach of taking up P from a dilute stream and releasing it into a smaller concentrated stream is not only feasible with granular sludge but is also sustainable. Operation of a reactor for a year in this configuration revealed that this process has the capability to be operated for long periods without being compromised in terms of function (Chapter 5). The anoxic/anaerobic configuration of this study showed that granular sludge technology can be adopted as a post denitrification facility to further reduce nitrogen (N) content of the final effluent of a conventional treatment plant while also facilitating P recovery. It was found that factors such as food to microorganisms (F/M) ratio and the amount of wasted sludge play a key role in the optimum function of this configuration. The use of granular sludge with a well stablished, mainstream process so-called simultaneous nitrification, denitrification, and phosphorus removal (SNDPR) process was investigated in the final chapter. The results showed the granular sludge SNDPR technology can be utilised for P recovery and nutrient removal using the available C in wastewater if operated under controlled conditions (Chapter 6). The feasibility to increase P concentration and generate a P rich liquor (>70 mg/L) suitable for a latter P recovery step was successfully demonstrated. The in-depth

studies revealed the importance of dissolved oxygen (DO) for stablishing a balanced microbial community in a system that shows complete nutrient removal and also provides an opportunity for P recovery.

Summing up, this work was an attempt to provide new visions in the well-recognised area of biological processes for P recovery. The findings confirm the outstanding potential of granular sludge technology through systematic examinations of the capabilities of such technology. The research findings in the following chapters suggest that granular sludge processes employed in novel-engineered configurations can be the basis for new sustainable methods for resource recovery.

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"Nature composes,
some of her loveliest poems,
for the microscope and the telescope."
- Theodore Roszak

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List of Abbreviations

AOB	Ammonia oxidising bacteria
COD	Chemical oxygen demand
CST	Capillary suction time
CUPS	Curtin University Postgraduate Scholarship
DGAO	Denitrifying glycogen accumulating organisms
DPAO	Denitrifying phosphorus accumulating organisms
EBPR	Enhanced biological P removal
EPS	Extracellular polymeric substances
FISH	Fluorescence in situ hybridization
GAO	Glycogen accumulating organisms
HRT	Hydraulic retention time
ISP	Ion Sphere Particles
MLSS	Mixed liquor suspended solids
NOB	Nitrite oxidizing bacteria
OTU	Operational taxonomic units
PAO	Phosphate accumulating organisms
QIIME	Quantitative Insights Into Microbial Ecology
RAS	Returned activated sludge
RFIC	Reagent-free ion chromatography
RO	Reverse osmosis
SBR	Sequencing batch reactor
SND	Simultaneous nitrification-denitrification

SNDPR	Simultaneous nitrification, denitrification, and phosphorus removal
SRT	Sludge retention time
SST	Secondary sedimentation tank
TSS	Total suspended solid
UPGMA	Unweighted Pair Group Method with Arithmetic
VFA	Volatile fatty acids
WWTP	Waste Water Treatment Plants

1 INTRODUCTION AND SCOPE OF THE RESEARCH

1.1 *THE LOST P*

Phosphorus (P) is a limited resource and a vital nutrient for all forms of life, from complex organisms such as mammals and plants to simpler single celled organisms such as bacteria (Cordell et al., 2009, Yang et al., 2017). Modern agricultural methods rely heavily on utilisation of fertilizers that contain P in large portions, since it is an essential micronutrient for plant development (Rittmann et al., 2011). In addition, the rapidly increasing human population puts pressure on global crop production. To meet this growing demand, fertilizer production and consequently, P consumption, has increased significantly (Withers et al., 2015). The increasingly unsustainable use of P has become a worldwide concern, and consequently, many recent studies have highlighted concerns about the future adequacy of P, and the risk that its scarcity imposes on future food production (Pearce, 2015). Extensive mining of P is leading to rapid depletion of this critical yet finite resource (Chen and Graedel, 2016). Considering that natural deposits of P are not evenly distributed in the world imposes even higher stress on global P stocks, resulting in rising prices (Rittmann et al., 2011). In addition, the vast mining of P rocks has led to massive amounts of P entering water bodies. Even low levels of P in aquatic ecosystems lead to eutrophication (Mehta et al., 2015), which has brought about many negative effects, including degradation of waterways from fish deaths, proliferation of algae and other unwanted plants and production of algal toxins.

1.2 *RETURNING THE LOST P*

Considering the above-mentioned issues, there is an urgent need to find sustainable strategies to secure P reserves for future food production (Mayer et al., 2016). In the absence of large new discoveries of P rock, recycling of P is the logical approach for preservation of global P resources. The first step is therefore to identify the potential sources that provide an opportunity for P recovery. In general sources of P that may be suitable for recovery are present in wastewater, sludge or farm runoff, which ultimately will end up either in the water or soil (Figure 1.1) (Pearce, 2015, Cordell et al., 2011, Withers et al., 2015).

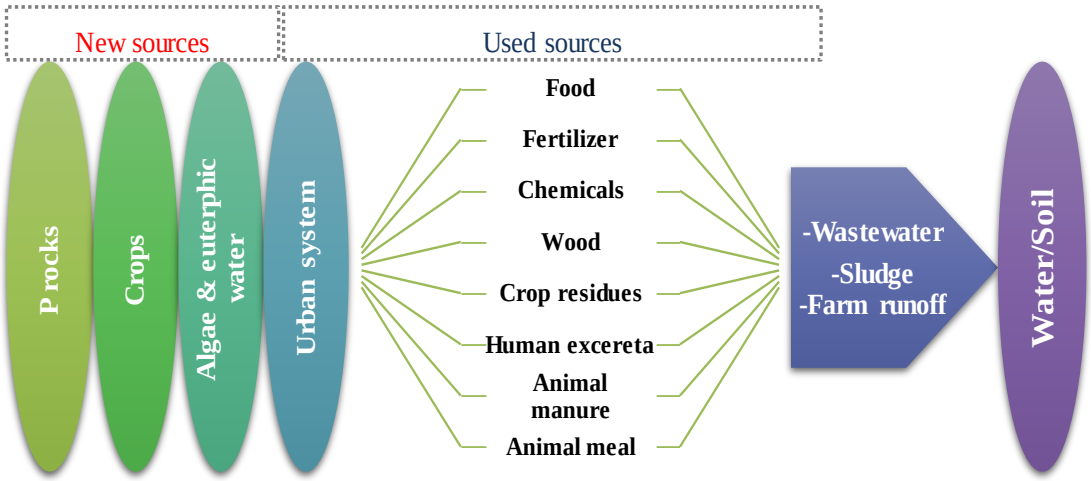

Figure 1.1. Phosphorus resources and phosphorus flow in urban areas. (Pearce, 2015) (Cordell et al., 2011, Withers et al., 2015)

1.3 *WASTEWATER, A PROMISING RESOURCE FOR P RETURN*

As shown in Figure 1.1, wastewater provides one of the biggest opportunities for P recovery since there is a reasonably high and constant P flow into waste streams (Schoumans et al., 2015; Smil, 2000). Historically environmental and regulatory agencies have been largely concerned with the removal of P, rather than its recovery, since the primary goal was to meet discharge limits and to protect surface waters from eutrophication. However, the focus is gradually shifting to P recovery due to increasing trends of P consumption and recognition that stocks of this element are finite (Cieslik and Konieczka, 2017). Wastewater has good capacity for P recovery, since the total theoretical P recovered from wastewater can potentially provide 15–20% of global P demand (Yuan et al., 2012). For this reason, development of technology for P recovery from wastewater has become not only an option but a priority. Some of the many drivers of using waste streams as a potential platform for P recovery are presented in Figure 1.2.

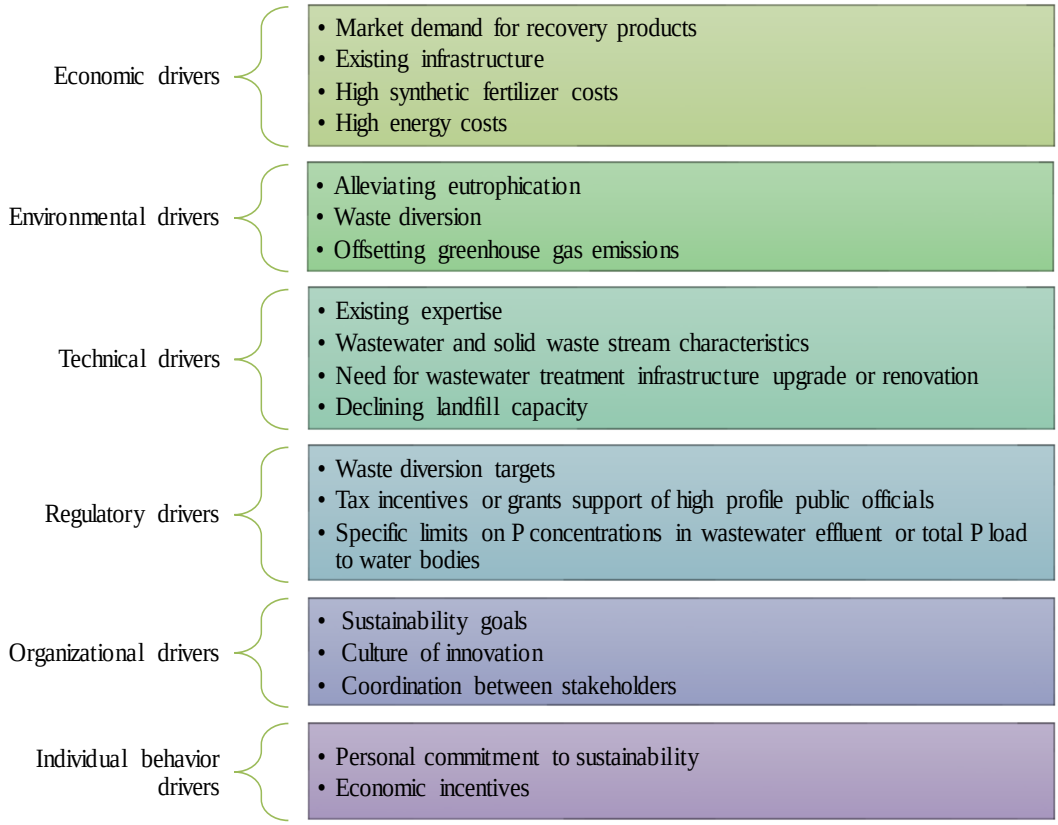

Figure 1.2. Drivers of recovering P from wastewater. (Roy, 2017, Cordell et al., 2011)

1.4 *BARRIERS TO RECOVERY OF P FROM WASTEWATER*

Despite many benefits of recycling of P from wastewater there are some practical limitations (Tarayre et al., 2016). Firstly, more than 90% of the P in the wastewater is contained within the sludge and the direct application of the sludge can be problematic. This is partly because the sludge contains various types of contaminants including pathogens, heavy metals, and toxic organic compounds. In addition the P in the sludge may not be sufficiently bioavailable to promote adequate plant growth due to the formation of strong bonds with trivalent metals such as iron or aluminium (Melia et al., 2017, Ye et al., 2016). Another limitation is the cost and inconvenience of transporting or dewatering the bulky and dense sludge. Based upon these concerns, most of the P recovery methods aim for P precipitation from the aqueous phase to a solid form that can be used as a safe fertiliser (Mehta et al., 2015).

However, P recovery through chemical precipitation requires P to be present at a relatively high concentration, which is a major limitation in terms of municipal

wastewater. For example, the production of struvite ((NH4) MgPO4.6H2O), a chemical compound that has a fertilising property similar to commercial mono calcium phosphates, is an established approach to recover P. One major benefit of using struvite as a fertiliser in agriculture is its low solubility, which prolongs nutrient release minimizing wastage and crop root burns. Recovering P in wastewater as struvite however is only feasible when the phosphate concentration in wastewater is at least 50 mg-P/L although some authors state that, in practice, levels of up to 100-200 mg-P/L are required (Kleemann, 2016, Kumar and Pal, 2015). Since municipal wastewater typically contains about 10 mg mg-P/L of phosphate, if P recovery is to be achieved in the form of struvite, methods are required to concentrate phosphate in wastewater.

Numerous strategies have been proposed for recovery of P from wastewater treatment plants in various forms and these are reviewed in the following section.

1.5 *TECHNOLOGIES FOR RECOVERING P FROM WASTEWATER*

Overall, the methods of recovering P from wastewater can be categorised into three main groups: (a) recovery from liquid; (b) recovery from sludge; and (c) recovery from sludge ashes (Withers et al., 2015). As presented in Figure 1.3, there are a wide variety of technologies developed at various point-sources of the wastewater treatment plant, but much of these commercial methods are targeted to digesters and only very little options are catered for the main treatment train. In addition, none of these technologies have widespread application, and choosing the right method depends on a number of factors such as their adoptability to the existing treatment configuration, being economically justified, having low environmental impacts and their contribution to the total value of P recovery. For making the right decision on the optimum configuration, a good understanding of the processes involved at each stage of various technologies is required (Mayer et al., 2016, Cordell et al., 2011).

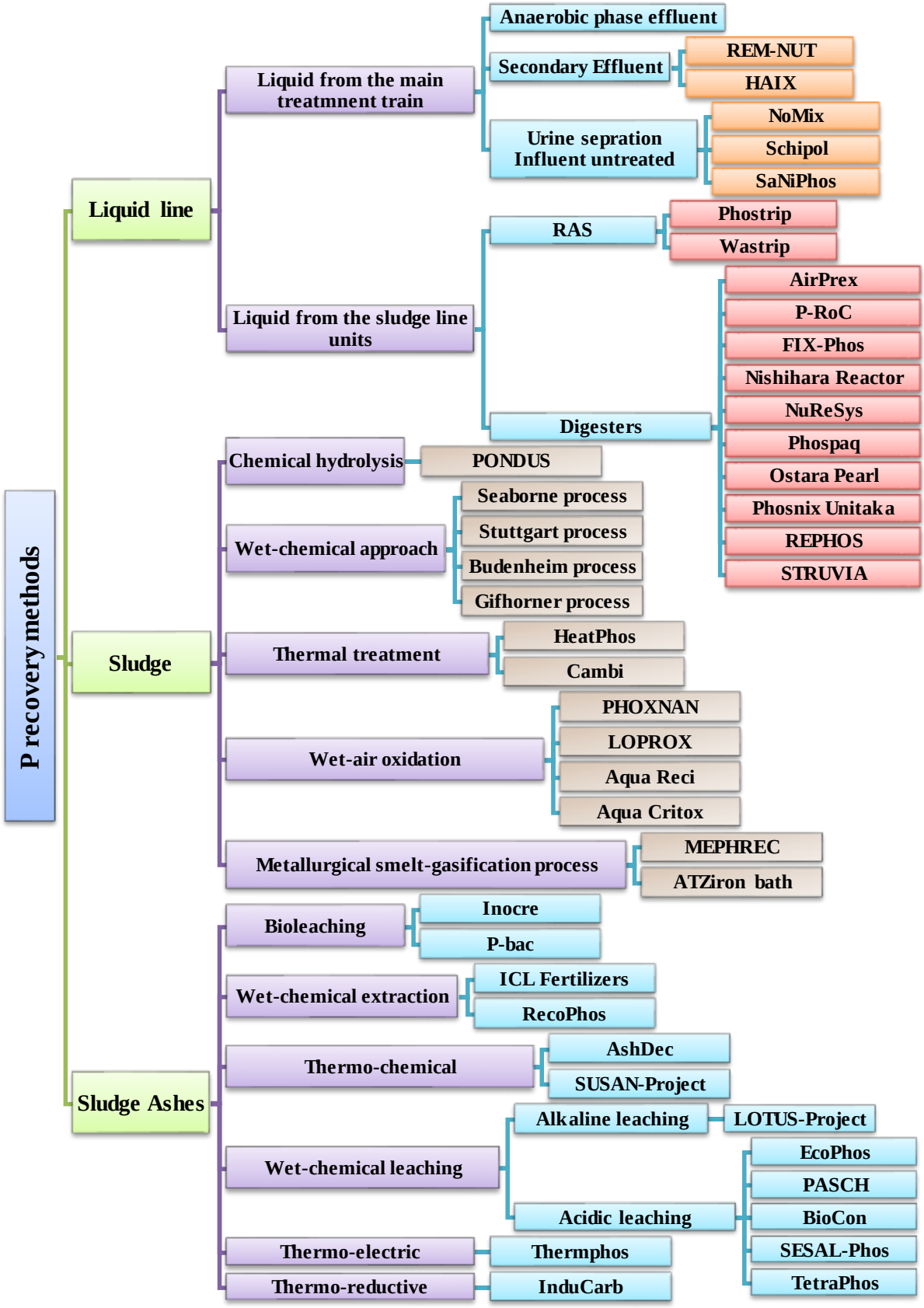

Figure 1.3. An overview of P recovery practices, with example technologies

1.6 *BREAKING DOWN TECHNOLOGIES TO PROCESSES*

The recovery of P in general has three main steps which apply to all the technologies, regardless of their specific approach. These main three steps are nutrient removal/accumulation, nutrient release, and nutrient extraction (Mehta et al., 2015) (Figure 1.4).

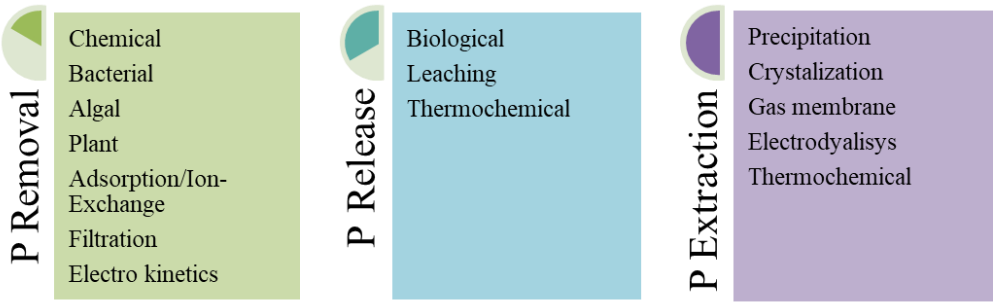

Figure 1.4. Main steps included in P recovery process

In a typical municipal wastewater treatment configuration, bacterial or chemical processes are used for the removal of P. Chemical P removal processes are generally based on coagulation and floculation using metal ions such as Fe, Al or Ca. The precipitated P, or that bound to colloids, can then be separated from the wastewater in a clarifier (Nancharaiah et al., 2016, Mehta et al., 2015). The chemical precipitation approach has some benefits such as the removal of organic matter, pathogens, some contaminants and other undesired elements and it provides flexibility to suit changing operational conditions. However chemical approaches have disadvantages, such as high chemical cost, increasing salinity, upsetting downstream biologial activities and increase in sludge production (Melia et al., 2017). Biological processes to remove P are preferable since the agricultural value of biological sludges (biosolids) is superior compared to chemical sludges, due to the fact that their available phosphate content is higher. Also, removing P via the formation and wastage of biological sludge is more economical and sustainable because the need for chemical addtion is omitted (Egle et al., 2015).

Biological methods are also suitable for recovering P from wastewater. For instance, anaerobic digestion is an established biotechnology that has been widely used to facilitate nutrient recovery from muncipal wastewater plants (Nancharaiah et al.,

2016). It plays a key role in many sewage sludge treatment processes as it provides an opportunity for simultaneously achieving stabilization of sludge, organic solids degradation, pathogen removal, and biomethane production for energy recovery. Another technology that is widely embranced for nutrient removal from wastewater is enhanced biological P removal (EBPR) process. The processis based on the activity of bacteria collectively named as phosphate accumulating organisms (PAO), which can uptake (remove) and release (recover) soluble P depending on the condition of the treatment process. Further detail on the nature of the biochemical reactions that take place in the presence of these organisms is given in the following section.

Among the methods for exracting P, chemical precipitation is widely used, and is capable of removing up to 90% of the P from the bulk water. The separation of precipitants or crystals from the liquid phase is technically easy to achieve, and the end product such as struvite often has good fertilizer characteristics Struvite can be produced in large scale wastewater treatment plants and its production has other advantages in addition to P recovery: the controlled removal of struvite in wastewater treatment plants can eliminate undesired struvite precipitation within plant infrastructure such as in anaerobic digesters, sludge transfer lines and centrifuges (Bhuiyan et al., 2007, Mehta et al., 2015).

1.7 *SHORTCOMINGS OF THE CURRENT METHODS*

As discussed in previous sections, numerous commercial technologies and different approaches are available for recovering P at different stages in a wastewater treatment process (Figure 1.3). Direct chemical precipitation of P is becoming less popular because of the usage of chemicals, and the general lower recovery potential in comparison to the biological methods (Egle et al., 2015). The use of methods such as ion exchange for P enrichment is less favoured due to the complexity of the process, the low selectivity and adsorption of unwanted elements. Recovery from sewage sludge is usually complex and demands significant resources. The creation of another waste stream is another possible drawback of direct chemical precipitation because there is a need for wet chemical processes such as acidic leaching to mobilize a higher level of P (Egle et al., 2015, Tarayre et al., 2016). Moreover, the cost of implementing these systems is a trade-off factor and completing the recovery through chemical approaches can at most be only 40% effective (Tarayre et al., 2016, Egle et al., 2015).

Similarly, the methods available for recovering P from sewage sludge ash are expensive and resource intensive. The need for using large quantities of chemicals, the presence of heavy metals, the high capital operating costs, and the low applicability of the end-product in agriculture are among the drawbacks of these methods (Mayer et al., 2016, Roy, 2017, Cieslik and Konieczka, 2017).

Overall, the search of technologies for P recovery from wastewater should not only consider the immediate outcome of the process, but also the connected downstream impacts, the long-term sustainability, and environmental consequences.

1.8 *RECOVERING FROM THE AQUEOUS PHASE, WHY?*

In a study on environmental impacts of different P recovery methods, Amann et al. (2018) defined three environmental indicators as: cumulative energy demand; global warming potential and acidification potential, based on lifecycle assessment methodology. The authors then based on these three indicators compared the three main paths of P recovery from wastewater (described in section 1.5), including P recovery from (i) the liquid phase, (ii) sewage sludge and (iii) sewage sludge ash. Their results showed that recovery from the liquid phase scored lower in all the three environmental indicators defined by Amann et al. (2018), but with lower P recovery potential in comparison to the other two methods. Despite a lower P recovery potential, the recovery from the aqueous phase is still the most attractive option as it enables the production of a valuable fertilizer product and has more operational, environmental, and economic benefits in comparison to the other methods (Egle et al., 2015, Melia et al., 2017, Mayer et al., 2016). The biologically accumulated P within the sludge has the potential to be dissolved (via a process described in the following section) through a simple set-up and provide an opportunity for P precipitation which in most EBPR plants will also address the problem of unwanted precipitation (Melia et al., 2017). The factors that make the P recovery from aqueous phase attractive are thus as follows: the technology is generally well developed and already in operation; there is the opportunity to obtain a well stabilised end product like struvite; the ability to avoid using chemicals; and the cost-effectiveness and practicality (Kleemann, 2016, Cieslik and Konieczka, 2017, Ye et al., 2016).

1.9 *RECOVERY FROM AQUEOUS PHASE BASED ON EBPR PROCESS*

Extended from

GINIGE, M. P., LASHKAJANI, S. S. & CHENG, K. Y. 2013. Biological recovery of phosphorus from municipal wastewater. *Microbiology Australia,* 34**,** 194-196.

Enhanced biological P removal (EBPR) as a contemporary emerging method adapts a group of microorganisms, phosphate-accumulating organisms (PAOs), to remove P from wastewater. The process of luxury P uptake is the basis of the biochemical pathway of the EBPR process (Barnard, 1983, Ginige et al., 2013). Polyphosphate-accumulating organisms (PAOs) are bacteria that can take up P in excess of their metabolic requirements and excess P is stored intracellularly as polyphosphate (poly-P) under aerobic or anoxic conditions. The dominant PAO typically found in activated sludge has been classified under class *Betaproteobacteria* and is named *Candidatus Accumulibacter Phosphatis* (Figure 1.5). Biological P removal from wastewater is facilitated with the assistance of PAOs and this process is commonly known as enhanced biological phosphorus removal (EBPR). In the EBPR process, PAOs are exposed to alternating anaerobic and aerobic (oxygen as electron acceptor) or anoxic (inorganic compounds as electron acceptors e.g. nitrate) environments. During the anaerobic phase, PAOs take up volatile fatty acids (VFAs) such as acetate from the wastewater and store them as an intracellular polymer, polyhydroxyalkanoates (PHA). PAOs hydrolyse intracellular poly-P to derive ATP requirements for anaerobic uptake of VFAs. This results in a release of phosphate into the surrounding environment. When exposed to aerobic or anoxic conditions, PAOs take up phosphate from the wastewater and replenish their poly-P reserves. Intracellular PHA reserves are used as the carbon and energy source for this purpose and oxygen or nitrate is used as a final electron acceptor (Mino et al., 1998). The increase in PAO cell numbers and an incorporation of P back into PAOs facilitate removal of P from wastewater. Wasting a portion of biomass (Bio-P sludge) then facilitates a net removal of P from wastewater. To recover P entrapped in biomass, the Bio-P sludge can be diverted to anaerobic digesters where hydrolysis of Bio-P sludge could facilitate a concentrated P stream suitable for struvite precipitation. However, the requirement of anaerobic digesters,

limits P recovery to WWTPs that have anaerobic digesters. In Australia, a large number of WWTPs are not fitted with anaerobic digesters (e.g. only two out of 106 wastewater treatment plants in Western Australia have anaerobic digesters).

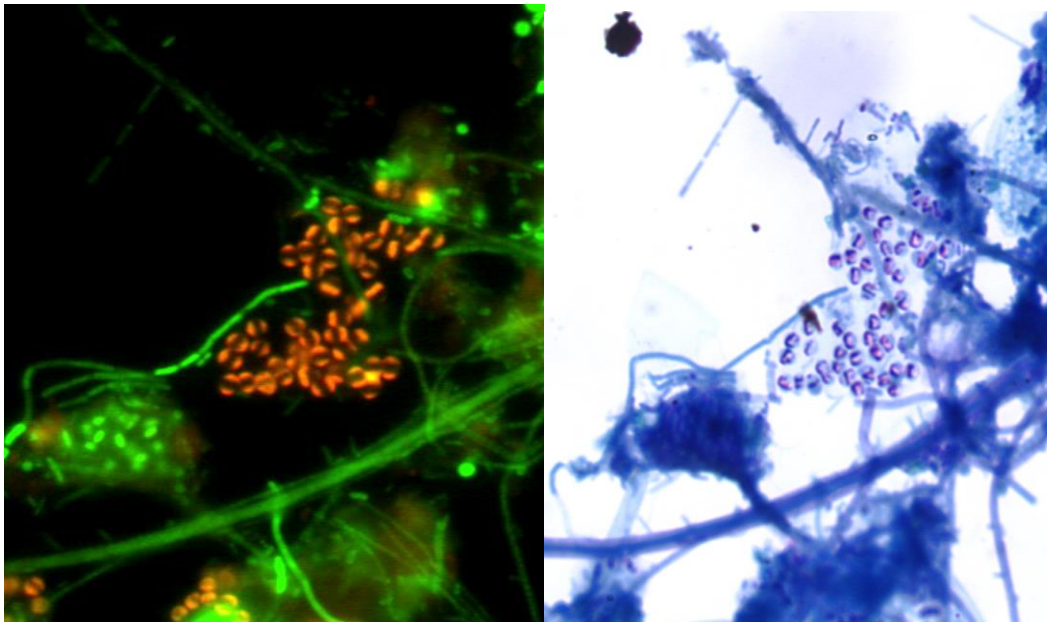

Figure 1.5. Fluorescence in situ hybridization (FISH) and post-FISH Methylene Blue stains of Noosa WWTP sludge. Panels on the left show FISH images with EUB338-FITC (green) and PAO651-Cy3 (orange). Panels on the right show Methylene Blue stains of identical fields as those on the left. Cells containing polyphosphate inclusions (purple) correspond to PAO651-binding cells in each example.

In the case of using nitrate or nitrite as an electron acceptor in an anaerobic/anoxic configuration, denitrifying-PAOs (DPAOs) couple denitrification and P uptake in a conversion that requires less carbon in comparison to the aerobic EBPR (Kerrnjespersen et al., 1994). The unique P uptake metabolism of PAO/DPAO has brought about several innovative approaches to overcoming the barrier of low concentration of P in the wastewater (Jenkins and Tandoi, 1991, Yuan et al., 2012). The potential for the accumulation of P within sludge can facilitate an opportunity for P release under anaerobic conditions. This anaerobic condition is known to occur along the sludge line of an EBPR system equipped with an anaerobic digester and causes unwanted precipitation (Adnan et al., 2003a, Cieslik and Konieczka, 2017). However, with the appropriate system design and the engineered control of the activity of the PAO, this provides an opportunity for recovering P from P-concentrated streams (Mehta et al., 2015). Examples of such systems are presented in Figure 1.3. The

potential for recovery of P depends on the concentration of P in the liquid. The addition of calcium or magnesium will result in precipitation of phosphate as calcium phosphate or struvite, respectively, provided the concentration of phosphate is greater than the saturation concentration of the desired precipitate.

1.10 *STRUVITE: THE PREFERRED END-PRODUCT*

Precipitation of P with magnesium is in the form of magnesium ammonium phosphate or struvite (Adnan et al., 2003b). Struvite is formed via the following reaction:

$$Mg^{2+} + NH_4^{+} + PO_4^{3-} + 6\,H_2O \rightarrow MgNH_4PO_4 \cdot 6\,H_2O$$

The reaction rate and maturity depend on a number of factors such as molar ratio (Mg:N:P), concentration of ions, pH, stirring rate, supersaturation and the influence of contaminants such as Ca or organic compounds, and temperature(Antakyali et al., 2012). Struvite crystallization can take place at a molar ratio of 1:1:1 for Mg:N:P, and a P concentration of greater than around 100 mg/L)(Rittmann et al., 2011). Struvite is an accepted slow release fertilizer with high plant uptake quality. Struvite formation represents a simple and economic approach for de-solubilizing P as well as nitrogen (N). The selective nature of the struvite crystallization process also rejects the heavy metals, pathogens, and other contaminants, allowing the end-product to meet the regulatory requirements for land application. Struvite crystals can be separated easily from water and particulates due to their high density. Struvite recovery for P recovery has been extensively applied, with numerous published case studies and commercial processes available in the market (Table 1.1).

Table 1.1. Technologies that recover P as Struvite

Approach	Scale	Efficiency (%)*		Reference
		Related to sludge input	Related to WWTP influent	
AirPrex®	Full-scale	80	10-15	(Heinzmann, 2009)
CSIR	Laboratory-scale	90	10-25	(Pinnekamp et al., 2007)
DHV Crystalactor®	Full-scale	85	Max 40	(Brett, 1997)
Trevisio (WWTP)	Full-scale	90	10–25	(Cecchi et al., 2003)
Kurita packed bed	Pilot-plant	90	10–25	(Pinnekamp et al., 2007)
Nishihara Reaktor	Full-scale	90	10–25	(Kumashiro et al., 2001)
NuReSys®	Full-scale	90	20-30	(Moerman et al., 2009)
NuReSys®	Full-scale	90	10-25	(Egle et al., 2015)
Ostara Pearl Reactor®	Full-scale	90	10–25	(Adnan et al., 2003b)
PECO	Pilot-plant	90	10–25	(Dockhorn, 2007)
PHOSIDIE	Laboratory-scale	90	10–25	(Egle et al., 2015)
Phosnix Unitika	Full-scale	90	10–25	(Ueno and Fujii, 2001)
Phospaq®	Full-scale	90	10–25	(Abma et al., 2010)
PRISA	Pilot-plant	90	Max 35	(Montag and Pinnekamp, 2008)
REPHOS®	Full-scale	90	10–25	(Lebek and Lohmar, 2013)
STRUVIA®	Full-scale	90	10–25	(Mele et al., 2014)

*P recovery potential of a technology based on P flow in the sewage sludge and the Waste Water Treatment Plants (WWTP) influent.

1.11 *AREAS THAT REQUIRE MORE ATTENTION*

Although it is favourable to recover P from the aqueous phase of municipal wastewater treatment plants and that as mentioned above established technologies are commercially available, their execution is limited to treatment plants configured for EBPR operation (Amann et al., 2018). In EBPR systems 90% of the P can be removed: the remaining P is released from P-rich sludge after sludge dewatering and stabilization in the anaerobic digesters. The P concentrated liquid stream then facilitates the conversion of soluble P to a fertilizer such as struvite. However, in the case of wastewater treatment facilities which are not equipped with anaerobic digesters, little

research has been carried out (Ye et al., 2016, Cieslik and Konieczka, 2017). The difficulties of implementation of a digester system in these plants, include the ecological impacts and the associated high capital costs. While there has been extensive research on the subject of P recovery from wastewater plants with anaerobic digesters, there are only a handful of studies that focus on strategies for P recovery from plants that do not use digesters(Wong, 2016, Strom, 2006)

1.12 *TECHNOLOGIES THAT DO NOT USE ANAEROBIC DIGESTERS TO RECOVER P*

Phosphorus recovery strategies that are proposed for biological P recovery in plants without anaerobic digesters can be divided into two groups. The first group involves strategies that use the EBPR process, as it is described in section 1.9 to facilitate P recovery from a P-rich side-stream after an anaerobic P release process: these kind of strategies enable P recovery from an existing treatment plant using a simple set-up that can be integrated into an existing wastewater treatment facility (e.g. the configuration proposed by Kodera et al. (2013) and Wong et al. (2013)); the second involves strategies that offer a novel P recovery tactic in the design of a new treatment plant system, such as the system that was suggested by Shi et al. (2012a), an anaerobic-anoxic/nitrifying (A_2N) configuration combined with induced crystallization process called A_2N-IC. The A_2N process generates a side stream that is fed to the crystallizer for P recovery as struvite. Such a crystallization step can reduce the P loading for biological removal process.

Recently, a novel modification to the EBPR process to facilitate both P recovery and denitrification has been proposed and validated in the laboratory (Wong et al., 2013). This novel post-denitrification configuration termed enhanced biological phosphorus removal and recovery (EBPR-r) consists of two steps where a PAO biofilm capable of reducing nitrate is employed to concentrate P from wastewater (Figure 1.6). During the first step, a PAO biofilm is exposed to a wastewater stream under aerobic or anoxic conditions, during which P is taken up by the biofilm and stored as poly-P using nitrate and residual dissolved oxygen as electron acceptors. During the second step, the poly-P enriched PAO biofilm is exposed to a separate smaller P recovery stream, to which an external carbon source (acetate) is added to trigger the release of phosphate under anaerobic conditions. In this mode of operation, the PAO biofilm is used as a carrier

of P from a dilute wastewater stream to a P recovery stream. The increase in P concentration in the P recovery stream is thus a result of the different volumes maintained between the P recovery and wastewater stream.

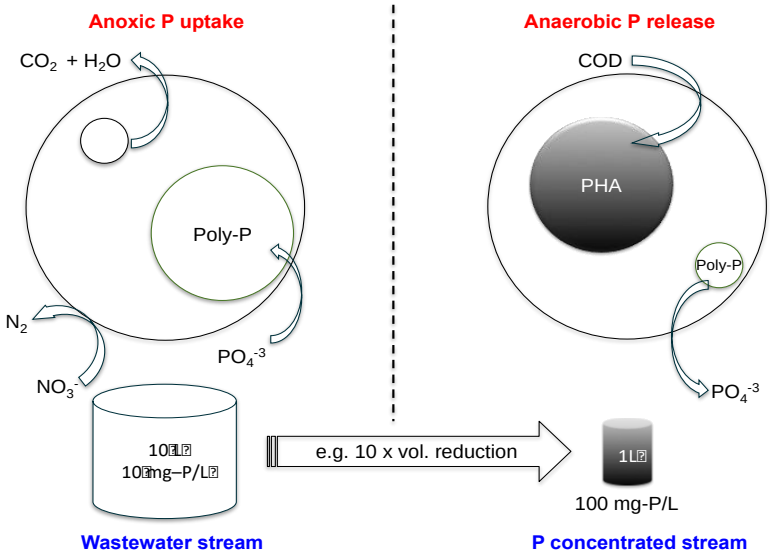

Figure 1.6. A novel method of using DPAOs to concentrate P from municipal wastewaters. During an anoxic phase, DPAOs accumulate P from a dilute P-containing wastewater stream as poly-P using the energy released from the stored polyhydroxyalkanoates (PHA). In a subsequent anaerobic phase, DPAOs release accumulated P into a recovery stream whilst taking up volatile fatty acids(Wong et al., 2013).

Wong et al. (2013) demonstrated that the EBPR-r process was able to generate a P recovery stream four times concentrated (28 mg-P/L) to that of the wastewater stream (7 mg-P/L). A four-time volumetric reduction to that of the wastewater stream facilitated a higher P concentration in the P recovery stream (Figure 1.7). A repeated release of P by a PAO biofilm (10 P-uptake and release cycles) to a recovery stream facilitated higher concentrations (~ 100 mg-P/L), allowing recovery of P in wastewater as a fertiliser (e.g. as struvite or calcium phosphate).

From a microbiology standpoint, the findings of Wong et al. (2013) is significant as it suggests that this distinct mode of operation (i.e. wastewater vs. P recovery stream) does not appear to interfere PAO metabolism. It also demonstrates PAOs ability to take up P from a dilute wastewater stream (< 10 mg-P/L) and release the same into a concentrated P recovery stream (100 mg-P/L). If a desired P concentration in the recovery stream could be achieved in a single uptake and release cycle, this proposed strategy could then be implemented in a fashion similar to a sequencing batch reactor

(SBR). Hence, further optimisation of this novel P recovery strategy by increasing the biomass density of PAOs will be a subject of further research.

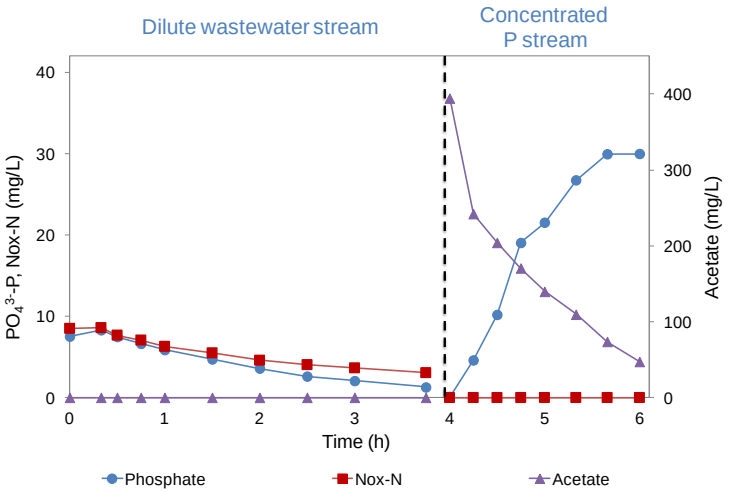

Figure 1.7. PAO biofilm could take up P from a synthetic medium with low P concentration and subsequently release the P into a separate, smaller volume using nitrate as electron acceptor, resulting in a concentration of P (Wong et al., 2013).

Similarly, with an anaerobic/aerobic trickling filter configuration, Kodera et al. (2013) achieved a recovery solution which was 25 times more concentrated than the initial wastewater stream (i.e. from an initial 5 to a final 125mg-P/L) simply by recirculating the recovery solution. In this kind of attached growth system, the bulkiness and rigidity of the media (biofilm carriers or trickling filters), places a limit on the achievable volume reduction ratio. Therefore, in order to achieve a high concentration of P, repeated recirculation of the recovery stream is required.

Acevedo et al. (2015) investigated two different strategies for P recovery from the P-rich stream created by adding carbon (C) to the sludge after aerobic P uptake. First, they released P by addition of a fixed amount of volatile fatty acids (VFAs) at the end of the aerobic stage of each cycle to trigger P sequestered sludge to release P under anaerobic conditions, which would facilitate a frequent recovery. In the second approach they increased the amount of VFAs in order to get more P released into the recovery stream, and as a consequence, P extraction had to be performed less frequently. The authors observed a shift in the dominance of microbial community from PAO's to GAO's after implementing the P extraction technique. The change in the population of organism was also confirmed by another study that explored the

effects of P recovery from an enriched stream after the anaerobic phase by the means of chemical precipitation using ferric chloride as the coagulant (Xia et al., 2014). Table 1.2 presents a summary of lab-scale studies for biological P removal that do not rely on anaerobic digesters. Among these strategies, Phostrip is the only process with potential for full-scale application. There is clearly a lack of research on developing P recovery for conventional wastewater treatment plants that are not equipped with anaerobic digesters.

Table 1.2. Phosphorus recovery strategies (lab-scale) that have been proposed for biological P recovery in plants without anaerobic digesters

Reference	P accumulation strategy	Pros	Cons
(Acevedo et al., 2015)	Treating anaerobic/aerobic EBPR sludge with external C created a P-rich recovery stream; 1) frequent dosage of a fixed amount of C, with frequent extraction, 2) increase in C led to greater P release and required less frequent P extraction	• Following this strategy, it was possible to recover up to 81% of the incoming P • The system recovered well from microbial shifts (from PAO to GAO) after P extraction	• No long-term data • A shift in the dominant microbial community from PAO to GAO after each high P extraction
(Xia et al., 2014)	Anaerobic/aerobic mixed liquor at the end of aerobic phase is removed to a batch reactor where C is added, P is precipitated, followed by filtering of the sludge and putting it back into the reactor	• No sludge production • P removal and recovery	• Some loss of P in the recovery stream • Method of sludge filtration is impractical on a large scale
(Shi et al., 2016, Shi et al., 2012b)	Anaerobic-anoxic/nitrifying (A_2N), a 2-sludge system configuration combined with induced crystallization. The A_2N process generates a side stream that is fed to the crystallizer for P recovery	• P-rich supernatant generated by DPAO • Anaerobic sludge digestion is avoided	• The need for several tanks and related configurations • Complexity • High installation costs
(Kodera et al., 2013)	Anaerobic- aerobic trickling filter; aerobic P uptake; anaerobic P release to a recirculated stream with addition of C; 50 % influent P is recovered in each cycle	• Little production of excess sludge • 25 times concentrated stream	• Only 50% P removal • Recirculation is needed • The difficulty to return all the recirculated solution • Limitation on volume reduction ratio
(Lv and Yuan, 2015)	In an anaerobic-aerobic SBR, half of anaerobic supernatant was transferred to a separate chemical basin and P was precipitated with ferric chloride, once a day	• Meeting discharge regulations for the systems with low COD/P ratio or with high P, N in the influent • Sludge settleability significantly enhanced	• Anaerobic P release decreased • P removal deteriorated • GAOs overtook the microbial community
(Wong et al., 2013)	Enriched DPAO biofilm was treated with external C to anaerobically release P; 1) into a smaller recovery volume; 2) recirculation of the recovery stream for multiple release to the same stream	• Lower energy consumption due to no aeration requirements for P uptake • Optimum use of the external carbon source to achieve both denitrification and P uptake	• Bulkiness of biofilm media imposes limitation on the volume reduction ratio • The need for re-circulation to obtain high P levels • Some precipitation of P occurred during multiple re-circulations
(Lu et al., 2016)	Mixed sludge was removed from the SBR and transferred to the batch reactor; external C added; sludge was filtered then returned to SBR during the aerobic period	• Incomplete N removal • The use of the non-woven cloth to filter sludge is impractical in large scale • Denitrification efficiency declined • Granular sizes reduced	• No sludge production • Target P removal from the final effluent was met
(Tian et al., 2016)	Anaerobic/aerobic biofilter was fed with concentrated acetate to force the release of P from sequestered biofilm which was then harvested and recovered	• Attached growth systems are good for P removal because of long SRTs • High adaptability under adverse growth condition • More suited for decentralized WWTPs or industrial	• Complicated configuration • Only 48% of influent P could be recovered

1.13 *IMPLICATIONS*

In terms of social and environmental benefits, the EBPR-r process could be considered as a cost effective and an environmentally friendly approach to remove and recover P from wastewater. At present water recycling is a major priority of the wastewater industry both in Australia and overseas and will be part of future water supply schemes across the world. With the realisation that P cannot be substituted and that its reserves are depleting (Cordell et al., 2011), P recovery is also gaining momentum as a priority for the wastewater industry worldwide. Water recycling processes to achieve potable water supplies rely heavily on secondary effluent quality to reduce operational costs. Although reverse osmosis membranes could remove phosphate and some nitrate, these nutrients contribute towards decrease of membrane life particularly by promoting biofouling. Hence, post-denitrification to reduce total nitrogen levels in secondary effluent is important and if post-denitrification is facilitated via EBPR-r, both nitrate removal and P recovery could be achieved with no additional external carbon requirements. Further, revenue from recovered P could offset operational costs and reduce costs associated with water recycling.

While revenue from recovered P would assist cost effective water recycling, the novel EBPR-r post-denitrification configuration could maximise the use of external carbon by not only facilitating nitrogen removal but also P recovery. The approach also encourages the industry to re-consider the use of chemical precipitants to remove P since the cost savings from not using chemicals and not needing to handle chemical sludge is likely to offset costs associated with simultaneous nitrogen removal and P recovery using this novel EBPR-r post-denitrification configuration. While the proposed post-denitrification configuration could facilitate integration of water recycling processes into existing treatment facilities by ensuring the removal of both nitrogen and P to very low levels, the ability to recover P without incurring additional financial constrains to the industry will assist the industry to move towards nutrient recovery from wastewater.

1.14 *ALTERNATIVE METHODS PROMISED TO FACILITATE P RECOVERY WITHOUT THE NEED OF ANAEROBIC DIGESTERS*

1.14.1 PHOSTRIP

A typical plug-flow activated plant is already equipped with the volume exchange opportunity between the conventionally designed aerobic treatment train (dilute) and the anaerobic return activated sludge (RAS) line (concentrated), as proposed by Wong et al. (2013). In addition, PAOs are part of the naturally occurring bacteria in raw activated sludge RAS, and even in conventional treatment plants these are active to some degree (Levin and Della Sala, 1987). Researchers have shown that by incorporating additional anaerobic units on the RAS line of a conventional plant, a side-stream EBPR process is attainable (Levin and Della Sala, 1987). The most common form of this technology is the Phostrip process, in which a part of the RAS is directed to anaerobic tank for P release. The advantages of such systems include: 1) being less vulnerable to the fluctuations and shock loads in the influent, in comparison to the mainstream processes; 2) lower construction costs; 3) better control over nitrate removal, both due to the extended solids retention time, and the enrichment of DPAOs; 4) better nutrient removal in comparison to the plug flow systems (Levlin and Hultman, 2003). Despite the theoretical benefits compared to conventional systems, there are still problems with the application of Phostrip, including inefficiency and low levels of P in the stripper tank (Arvin and Wiechers, 1983). The initial motive of this technology was to reduce P in the effluent of a conventional plant, rather than P recovery, and the emergence of the EBPR process to some extent superseded the Phostrip technology, leading to it becoming somewhat "forgotten". However now, with the increased understanding of the luxury P uptake mechanism, suitable engineering of this approach could offer opportunities for the design of a simple process for P recovery.

1.14.2 GRANULAR SLUDGE PROCESS

The granular sludge process is a recently developed technology that has a vast range of application in wastewater treatment processes (Kishida et al., 2006, Winkler et al., 2012, Pronk et al., 2015). Granules aggregate under controlled conditions causing a

diverse group of bacteria to flocculate and form dense and packed structures (Zheng et al., 2005). The occurrence of different layers within a granule allows for different biological conversions to occur simultaneously. Matured granules show excellent settling properties and high biomass density (Etterer and Wilderer, 2001). As discussed earlier, Wong et al. (2013) and Kodera et al. (2013) proposed technologies that use re-circulation or volume reduction strategies to increase P concentration in the recovery stream. In these kinds of attached growth systems, the bulkiness and rigidity of the media (biofilm carriers or trickling filters), places a limit on the achievable volume reduction ratio. Therefore, in order to get a high concentration of P, the recovery stream needs to be recirculated repeatedly. A suspended growth system like granular sludge technology with high biological conversion rates, and good settling properties is ideal for use in a volume reduction strategy: it is anticipated that densely packed granules would result in a bigger volume exchange ratio between the dilute and concentrated streams.

1.14.3 SIMULTANEOUS NITRIFICATION DENITRIFICATION AND P REMOVAL (SNDPR)

A relatively recent innovation in wastewater treatment termed simultaneous nitrification–denitrification and phosphorus removal (SNDPR) can perform biological nutrient removal with lower carbon demand than conventional processes (Zeng et al., 2003a). Simultaneous nitrification-denitrification (SND) is possible under low dissolved oxygen (0.5 mg/l) conditions by creating aerobic/anoxic zones based on diffusion-based oxygen flux through the flocs (Zeng et al., 2004). When SND is combined with EBPR, denitrifying phosphorus accumulating organisms (DPAO's) can simultaneously denitrify nitrate/nitrite while taking up P, using the same amount of C. This process saves capital cost since it has lower oxygen demand, C requirement and sludge production (Meyer et al., 2005). One of the promising techniques for attaining complete SNDPR is with the use of aerobic granular sludge. Densely packed granules suggest a high ratio of bacteria to space and excellent settling properties. Therefore, SNDPR via granular biomass can have the potential to be used as a platform for fostering a low-cost nutrient recovery stream (Lu et al., 2016).

1.15 *DEFICIENCIES IN METHODS THAT DO NOT USE DIGESTERS*

The current biological strategies for P recovery from the plants that do not use digesters are inadequate and these existing methods are indeed suffered from limitations therefore there is a need to develop more practical methods. The reasons for these limitations are due to a number of factors:

1. There are limited methods that focus on P recovery from the conventional wastewater treatment plants that are not furbished with EBPR. Phostrip is the only strategy that appears to have the potential to enable P recovery from non-EBPR plants, however it is not extensively used anymore.
2. Most of these strategies are reliant on the addition of external C, to facilitate anaerobic P release.
3. The complexity, high potential capital costs of implementation, and the restrictions imposed by the choice of the media/treatment process decreases the practicality of these strategies.
4. Only few studies had explored alternative procedures for increasing P concentration in the recovery stream.
5. There are no published long-term studies on the potential impacts of methods such as volume reduction or re-circulation (strategies where the aim is to enable a higher concentration of P in the recovery stream).

1.16 *THESIS AIM AND OBJECTIVES*

This thesis is aimed at developing simple and practical technologies that facilitate recovery of P from wastewater. The scope involves addressing the shortcomings of promising established technology, and developing adoptable, economical, and environmentally sound strategies for P recovery. The specific objectives are as follows:

1- To investigate and improve the established Phostrip process for P recovery from non-EBPR plants

2- To examine the capacity of the granular sludge process to produce P enriched liquor desirable for P recovery.
3- To develop a novel granular sludge-SNDPR technology for nutrient removal and P recovery without the addition of external C.
4- To study the robustness and long-term effects of using a volume reduction strategy for creating a highly P enriched stream.

1.17 *THESIS STRUCTURE*

Thesis structure: This thesis consists of six chapters: **Chapter 1** provides background on P recovery from municipal wastewater treatment plants, including the current state of knowledge on various approaches and the limitations of current practices. **Chapter 2** focused on the Phostrip process, with a systematic examination of the P release kinetics from return activated sludge (RAS). The experiments were designed to simulate the conditions typical of the RAS line of a conventional treatment plant that is not designed for biological P removal. The aim of the study was to determine whether Phostrip could be retrofitted to conventional activated sludge wastewater treatment plants to achieve an EBPR process at these plants. **Chapter 3** describes a preliminary investigation of the biological P accumulation and release mechanisms by PAO. This involved a study on the efficiency of granular sludge for P removal using a laboratory-scale sequencing batch reactor under alternating aerobic/anaerobic conditions. This approach succeeded in increasing the concentration of P by 45-fold, which would be sufficient for recovery of P by struvite precipitation. The study described in **Chapter 4** investigated the long-term effects of a volume reduction strategy in a denitrifying process, where the granular sludge was exposed to higher levels of volume reduction in a stepwise process in order to achieve a higher concentration of P in the recovery stream. In **Chapter 5** the SNDPR process was tested for its potential in creating a P-rich recovery stream, which uses the natural available C in the wastewater. It was shown that it is feasible to obtain P-enriched liquor (with concentrations up to 100 mg-P/L) using this granular SNDPR process, providing in-reactor DO is maintained at around 0.5 mg/L. The importance of denitrifying polyphosphate accumulating organisms (DPAOs) and glycogen accumulating organisms (DGAOs) to achieve complete removal of N from the effluent was shown. **Chapter 6** discusses and summarises the insights obtained in this thesis. Implication of the thesis and recommendation for future research directions are addressed.

2 RE-VISITING THE PHOSTRIP PROCESS TO RECOVER PHOSPHORUS FROM MUNICIPAL WASTEWATER

Extended from

SALEHI, S., CHENG, K. Y., HEITZ, A. & GINIGE, M. P. 2018. Re-visiting the Phostrip process to recover phosphorus from municipal wastewater. *Chemical Engineering Journal,* 343**,** 390-398.

CHAPTER SUMMARY

This study examined an innovative approach to make use of the Phostrip process to recover phosphorus (P) from municipal wastewater. Returned activated sludge (RAS) from a municipal wastewater treatment plant was systematically studied to examine P release kinetics of RAS in a recovery stream that contained high concentrations of phosphate (PO_4^{3-}-P). Findings suggested that the specific P release rate in RAS declined with increasing concentration of PO_4^{3-}-P in the recovery stream. However, there was a strong positive linear correlation between acetate consumed and P released by the RAS (Pearson product-moment correlation coefficient [$\underline{r}$ = 0.98, $\underline{n}$=45, $\underline{p}$<0.0005 (1.13e-31)]). The data also suggest that acetate concentration in the recovery stream was not a factor in the observed reduction of specific P release rate with increasing PO_4^{3-}-P in the recovery stream. When P release rates (poly-P hydrolysis rate) at different initial P concentrations were modelled using a modified Michaelis-Menten equation, a good fit was achieved between the experimental and the modelled data. According to the model, the maximum specific P release rate (18 mg-P/g-MLSS.h) halved when PO_4^{3-}-P concentration in the recovery stream reached approximately 83 mg-P/L. Additionally, the RAS demonstrated a $P_{release}/C_{acetate\ uptake}$ molar ratio of approximately 0.5. An application of the derived P release kinetics into an innovative side stream process configuration showed that the Phostrip tank (9 m^3) would only demand a small footprint to recover P from a wastewater treatment plant that receives 61 ML/d of influent.

2.1 *INTRODUCTION*

There are increasing pressures on wastewater utilities to remove phosphorus (P) from municipal wastewater to minimise environmental impacts on the downstream receiving water bodies (e.g. eutrophication). Most of the wastewater treatment plants (WWTPs) in Australia are not engineered to facilitate enhanced biological phosphorus removal (EBPR) and hence rely on chemicals (alum or ferric chloride) to precipitate P from wastewater. These chemicals are often added into the main wastewater stream and hence, the chemical sludge produced cannot be separated from activated sludge. As a consequence, the biologically active fraction in activated sludge decreases over time, impacting overall nutrient removal.

Typically, P concentration in municipal wastewater is low (7-10 mg-P/L) and the volumes of wastewater vary in different WWTPs (e.g. 1 – 100 million litres per day). When dealing with high volumes of wastewater with such low concentrations of P, chemical removal of P is highly inefficient and as such often leads to a noticeable wastage of chemicals. It is well accepted that the efficiency of chemical P precipitation from municipal wastewater can be significantly improved at a higher P concentration of at least 60 mg-P/L (Kaschka and Weyrer, 1999).

Phostrip is a strategy that has been widely used to create a P-enriched side stream liquor, thus improving the effectiveness of chemical P removal (Levin and Della Sala, 1987). Since chemical precipitation takes place in a separate side stream, the mainstream and the activated sludge remain isolated from the chemical sludge. In fact, this process, which was introduced in the early 1970s, makes use of biological phosphorus removal to create the side stream P rich liquor. Mainstream biological phosphorus removal is largely governed by a unique group of microorganisms, commonly referred to as polyphosphate accumulating organisms (PAOs) (vanLoosdrecht et al., 1997, Islam et al., 2017). PAOs are naturally abundant in activated sludge and these organisms are capable of storing phosphate (PO_4^{3-}) several times in excess of that required for growth. When exposed to aerobic conditions, PAOs oxidise their intracellular carbon reserves (polyhydroxyalkanoates (PHA)) to obtain energy to take up and store PO_4^{3-}-P as polyphosphate (poly-P). When exposed to anaerobic conditions, PAOs replenish the depleted carbon reserves by up taking organic carbon, particularly volatile fatty acids (VFAs). The energy requirement for

the storage of organic carbon is derived from the hydrolysis of the intracellular poly-P. This results in a release of PO_4^{3-}-P back into the environment. The side stream Phostrip process, facilitates this intracellular release of poly-P by exposing a fraction (15-30 %) of returned activated sludge (RAS) to an organic carbon source in a small volumetric flow (Table 2.1) (Baetens, 2001, Acevedo et al., 2015, Kaschka and Weyrer, 1999). The release of PO_4^{3-}-P into the small volumetric flow creates a side stream liquor that is rich in PO_4^{3-}-P. The concentration of PO_4^{3-}-P in the side stream liquor is then sufficiently high (>100 mg/L) to allow efficient chemical precipitation of PO_4^{3-}-P from wastewater.

In the 1970s Phostrip facilitated the achievement of low effluent discharge of PO_4^{3-}-P. However, with the development of new operational strategies (e.g. Bardenpho process, Phoredox process) the need to add chemicals (e.g. alum) to maintain a low effluent discharge of P was found unnecessary and as a consequence, Phostrip has not been further embraced since the 1970s (Baetens, 2001). With no added chemicals, an alternate sequential exposure of activated sludge to an anaerobic, anoxic and/or aerobic zone enabled maintenance of low effluent P concentrations by simply wasting excess activated sludge (rich in intracellular poly-P) (Mehta et al., 2015). However, recently there is renewed interest in developing Phostrip to facilitate P recovery, for example, as a beneficial product such as struvite (e.g. Ostara Pearl® and Wasstrip® process). The focus of the current study was to assess the potential for Phostrip to recover P from WWTPs that are currently either not removing P or using chemicals to remove P from wastewater.

Commercial recovery of P from municipal WWTPs is largely achieved through the production of struvite (NH_4MgPO_4). Struvite is a highly desired product because of its unique agronomic properties, such as ease of application to the field, the high P content and low solubility (Bhuiyan et al., 2007). Commercial large-scale struvite production processes (e.g. Ostara Pearl® process) are mostly designed to operate with influent containing PO_4^{3-}-P concentrations >100 mg/L (Kleemann, 2016). Application of Phostrip and PO_4^{3-}-P concentrations achieved in the sidestream effluent have varied considerably and Table 2.1 provides a summary of several key studies in the literature. Phostrip appears to have largely been used at WWTPs that were not configured to facilitate EBPR (Kaschka and Weyrer, 1999). However, Phostrip was still able to facilitate a side stream enriched in PO_4^{3-}-P and this confirms that, irrespective of

mainstream configuration, there is always a natural abundance of PAOs in the activated sludge. The abundance of PAOs in non-EBPR plants are likely due to anaerobic conditions that prevail in the RAS lines. The RAS line in this instance would act as a substitute for the anaerobic basin of an EBPR plant. Hence there is an opportunity to apply Phostrip at non-EBPR plants, to maintain a low discharge of PO_4^{3-}-P in effluent, and to recover P from municipal wastewater. In this instance, the anaerobic Phostrip tank would substitute for the anaerobic basin of an EBPR plant.

Although integration of Phostrip with non-EBPR plants resulted in side stream PO_4^{3-}-P liquor concentrations of only 20 – 40 mg/L (Table 2.1), inadequate to facilitate commercial recovery of P, usage of Phostrip in a repetitive process (i.e. multiple passes) could achieve higher PO_4^{3-}-P concentrations. Such an approach has previously been demonstrated in relation to a novel post-denitrification configuration (EBPR-r) (Wong et al., 2013). Wong et al. (2013) however, noted difficulties to maintain a linear increase of PO_4^{3-}-P with repeated re-use of a P recovery stream.

Hence, the aim of this study was to develop a deeper understanding of the P release kinetics from RAS from a non-EBPR plant. Our specific objectives were (i) to understand conditions that govern the release of P from RAS into a recovery stream; and (ii) to design a side stream configuration based on the P release kinetics data obtained from the study in (i). The study was carried out using RAS from a full-scale wastewater treatment plant not configured for EBPR. Fresh RAS was subjected to a range of batch studies to examine the influence of PO_4^{3-}-P (in recovery stream) and external carbon concentrations on the release of P from RAS. P release rates (poly-P hydrolysis rate) at different initial P concentrations were modelled using a modified Michaelis-Menton equation.

Table 2.1. Phostrip and conditions of its application to create a side-stream P concentrated liquor

Reference	Mainstream			Phostrip process					
	Process configuration	Scale	Influent PO_4^{3-}—P (mg/L)	Ratio of RAS directed to the stripper (%)	HRT* (h)	SRT#	Source of COD! for Phostrip	COD (mg/L)	PO_4^{3-}-P (mg/L) in side stream
(Kim et al., 2000)	anoxic/aerobic	pilot	3-6	30 & 15	12-15	12-15	Internal	-	10-20
(Rensink et al., 1997)	anaerobic/aerobic /anoxic/aerobic	pilot	10	33	4	4	External (acetate)	10-20	20-50
(Miyamoto mills et al., 1983)	aerobic/side stream anoxic	pilot	10	14-30	5-9	8-10	Elutriate^ +internal	-	25-40
(Brdjanovic et al., 2000)	anoxic/aerobic	lab tests on full-scale samples	6.4	5	3	-	External (acetate)	110	25
(Mclaren, 1979)	anoxic/aerobic	laboratory	10	20	6.72	-	Internal	-	11
(Islam et al., 2017)	anoxic/aerobic	laboratory	7.2	30-35	2.5-14	-	External (acetate)	-	41
This Study	anoxic/aerobic	lab tests on full-scale samples	8	0.51	1.36	-	External (acetate)	-	100

*Hydraulic retention time; #Sludge retention time; !Chemical oxygen demand; ^ primary or secondary Effluent, or P-stripper supernatant

2.2 *MATERIALS AND METHODS*

RAS from a municipal WWTP in Western Australia (capacity 61 ML/d) was used for all experiments carried out in this study. A schematic of the plug-flow configuration of the activated sludge plant is detailed in Figure 2.1. The treatment train was composed of only an anoxic and an aerobic zone. The lack of an anaerobic zone in the treatment train confirms that the WWTP is not designed to facilitate EBPR. All batch experiments in this study were carried out using freshly collected RAS.

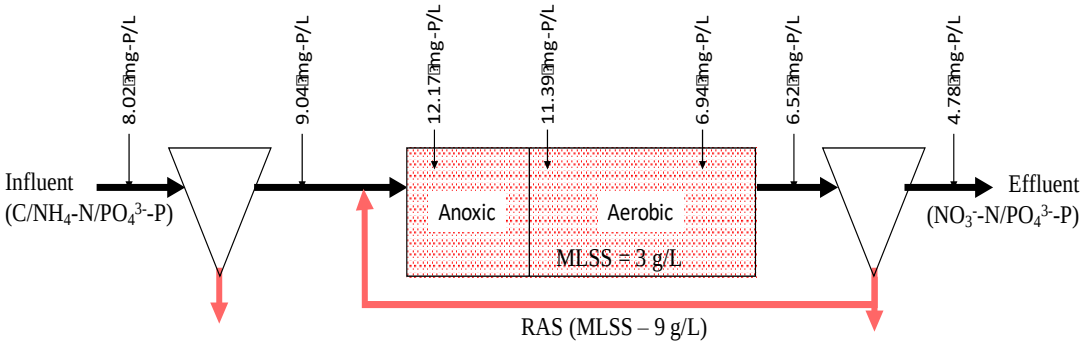

Figure 2.1. Process flow diagram of the municipal wastewater treatment plant from which the returned activated sludge used in this study originated from. The values above the treatment line are phosphate concentrations recorded at the respective locations.

2.2.1 DETERMINATION OF THE INDIGENOUS DENITRIFYING ABILITY OF RAS

Since the presence of nitrate in the Phostrip tank could affect the release of P from RAS (Barnard, 1983, Chuang et al., 1996), it is important to ensure that nitrate is exhausted or maintained at minimal level in the RAS line. Field measurements suggested that although the final effluent of the WWTP contained a NO_3^--N concentration of 4.78 mg/L, there was no measurable NO_3^--N in the RAS. To determine if denitrification could be efficiently carried out during the settling of biomass in the secondary sedimentation tank (SST), a specific experiment was carried out simulating the condition of a SST. A column reactor with an internal diameter (D) of 7.5 cm and a height of (H) 150 cm was constructed to mimic the settling condition in the SST. The column reactor was fitted with evenly spaced (10 cm apart) sampling ports along the height of the reactor. Mixed liquor (5 L) from the WWTP was collected from the end of the aerobic tank and was transported to the laboratory within 1 h. Subsequently 2.8 mL of a 1 M sodium nitrate solution was added into the mixed liquor to ensure the concentration of nitrate was not limiting. After mixing vigorously (by repeatedly inverting the container), the mixed liquor was placed in the column and was allowed to settle. Immediately after the mixing step, liquid samples were withdrawn from two sampling ports (the bottom and the topmost) at regular time intervals (0, 15, 30, 45 and 60 min) to monitor NO_3^--N concentrations at the top and bottom of column reactor. The collected samples were immediately filtered through 0.22 µm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia),

and were then stored at 4°C until analyses. To confirm the reproducibility of results, the experiment was repeated twice over a period of two weeks.

2.2.2 INVESTIGATING THE IMPACT OF ACETATE ON THE RELEASE OF P FROM RAS

The release of P in freshly collected RAS was examined in a separate experiment. A container (5L) was completely filled with fresh RAS, minimising intrusion of oxygen from the atmosphere in order to maintain anaerobic conditions that prevailed in the RAS line. The RAS was then immediately (within less than 1 h of collection) transported to the laboratory and 150 mL aliquots of the well-mixed RAS were placed into each of six 160 mL anaerobic bottles. The anaerobic bottles were subsequently capped (using butyl rubber stoppers) and crimped with aluminium seals and the liquor was purged with N_2 for 3 min. Defined volumes of a 1 M solution of sodium acetate (1M) were injected separately to the anaerobic bottles to give a series of subsamples containing initial acetate concentrations at 0, 100, 150, 200, 250 and 300 mg/L, respectively. The bottles were then placed on a shaker (170 rpm) and were incubated at 22 °C for 120 min. At regular time intervals (0, 5, 10, 15, 25, 30, 40, 50, 60, 75, 90 and 120 min) liquid samples (1.5 mL) were withdrawn from each of the bottles and were immediately filtered through 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia) and the filtrates were stored in 2 mL Eppendorf tubes at 4°C until analysis.

2.2.3 MULTIPLE ADDITION VS BATCH DOSING OF ACETATE FOR TRIGGERING RELEASE OF P FROM RAS

In this experimental step, the P release rates from the RAS were determined under two carbon dosing regimes: (1) aliquot (i.e. multiple additions), and (2) batch dosing (i.e. one-off addition). Two anaerobic bottles each containing 150 mL of RAS were prepared as detailed in Section 2.2.2 Both bottles were placed on a shaker (170 rpm) at 22 °C for 120 min. Identical quantities of acetate (approximately 250 mg/L) were added to each bottle: one bottle received all of the acetate at the start of the experiment (batch delivery), the other received the same quantity in 15 equal aliquots over a period of 120 min. At regular intervals (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 120 min) liquid (1.5 mL) samples were withdrawn from each of the bottles and

immediately filtered through 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia). The filtrates were stored in 2 mL Eppendorf tubes at 4°C until analysis.

2.2.4 INVESTIGATING THE IMPACT OF BULK WATER P CONCENTRATIONS ON RELEASE OF P FROM RAS

In this experiment, fresh RAS collected from the WWTP was exposed to different initial PO_4^{3-}-P concentrations. Aliquots of RAS (150 mL) were placed in 6 anaerobic bottles and the bottles were assembled as detailed above. Different volumes of K_2HPO_4 stock solution (1M) were introduced into the respective anaerobic bottles such that bottles contained 20, 60, 100, 150, 200 or 250 mg/L PO_4^{3-}-P, respectively. Finally, to trigger P release a non-limiting concentration of acetate (~300 mg/L) was introduced into all 6 anaerobic bottles. Thereafter, the bottles were incubated on a shaker (170 rpm) at 22 °C for 180 min and liquid samples (1.5 mL) were withdrawn from the bottles at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 180 minutes. The samples were immediately filtered through 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia) and the filtrates were stored in 2 mL Eppendorf tubes at 4°C until analysis.

2.2.5 ANALYTICAL METHODS

The concentrations of soluble PO_4^{3-}–P, NO_3^--N and acetate in the filtrates were determined using ion chromatography (ICS-3000, DIONEX). A Dionex ICS-3000 reagent-free ion chromatography (RFIC) system was fitted with an IonPac® AS18 4 x 250 mm column. Potassium hydroxide was the eluent for anion separation at a flow rate of 1 mL min^{-1}. The eluent gradient was as follows: 12-45 mM from 0-5 min, 45 mM from 5-8 min, 45-60 mM from 8-10 min and 60-12 mM from 10-13 min. The temperature of the column was maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppression® recycle mode).

The mixed liquor suspended solids (MLSS) in all anaerobic bottles were determined according to the Standard Methods for Water and Wastewater Analysis (Rice et al., 2012).

2.2.6 MODELLING

The P release rate (poly-P hydrolysis rate) versus initial P concentration data were modelled using a modified Michaelis-Menten equation as detailed in Varela & Harrison (L'Helguen et al., 2008, Varela and Harrison, 1999).

$$V = V_{max} * \left[1 - \frac{I_{max}\,[P]}{K_i + [P]}\right] \quad (1)$$

Where V is the specific P release rate (mg-P/g-MLSS.h); V_{max} is the maximum specific rate (mg-P/g-MLSS.h) of P uptake at undetectable concentration of PO_4^{3-}-P (mg/L) in the bulk solution; K_i (mg/L) is the PO_4^{3-}-P concentration at which V_{max} is reduced by half; I_{max} is the maximum realisation inhibition (values from 0 to 1); and [P] is the initial concentration of P in the bulk solution (mg/L).

2.3 RESULTS AND DISCUSSION

2.3.1 EVIDENCE OF PAO ACTIVITY IN THE RAS LINE OF A NON-EBPR PLANT

A mass balance between the influent and effluent showed that some overall removal of PO_4^{3-}-P occurred within the WWTP studied (Figure 2.1). The wastewater influent had an approximate PO_4^{3-}-P concentration of 8 mg/L which increased slightly along the treatment train, reaching a maximum of 12.2 mg/L at the upper anoxic zone before declining to a PO_4^{3-}-P concentration of 4.78 mg/L in the final effluent. The effective net removal of PO_4^{3-}-P between influent and effluent was therefore 3.22 mg/L.

Since P is an essential element for microbial growth, some of the removed PO_4^{3-}-P could have been consumed for the assimilation of new biomass. However, considering that PO_4^{3-}-P concentration in the primary sedimentation tank outlet was 9.04 mg/L, the elevated PO_4^{3-}-P concentration (12.2 mg/L) at the beginning of the anoxic tank must be attributed to the extra loading of P from the RAS line. For example, if the re-cycle ratio of the RAS was 100 %, to facilitate a PO_4^{3-}-P of 12.2 mg/L at the beginning of anoxic zone, the RAS line should contain a PO_4^{3-}-P concentration of 15.3 mg/L. If the re-cycle ratio was only 20 %, then a higher PO_4^{3-}-P concentration of 27.8 mg/L must be available in the RAS line. Such an elevated level of P in the RAS line implies that the WWTP already permitted a natural presence of PAOs in the activated sludge even though the plant was not operated to facilitate EBPR.

2.3.2 DENITRIFICATION OCCURRED NATURALLY WITHIN THE SECONDARY SEDIMENTATION TANK CREATING A NITRATE-FREE RAS STREAM

An effective and economically viable Phostrip process typically requires an absence of nitrate in the RAS line. In the presence of nitrate, the externally dosed carbon may not be fully used for P release but may be 'wastefully' used for denitrification (Barnard, 1983, Chuang et al., 1996). The negative impact of nitrate is often mitigated with the aid of a Pre-stripper (located upstream of Phostrip) that makes use of residual carbon present in Phostrip liquor to denitrify nitrate present in RAS (Kaschka and Weyrer, 1999).

In this study, although the final effluent of the WWTP contained nitrate, there was no measurable concentration of nitrate in the RAS line. As hypothesised and demonstrated in a lab-scale experiment with sedimentation of biomass, the nitrate in the sedimented biomass declined rapidly at the bottom of the sedimentation column reactor (Figure 2.2). Mixed liquor samples obtained from the end of the aerobic tank had a nitrate concentration of 3.5 mg/L and the nitrate concentration reduced to 1.4 mg/L between collection and transportation into the laboratory. Nitrate was manually added to the mixed liquor, increasing the concentration to 8.2 mg/L at the beginning of the experiment. Within an hour of the biomass settling, the concentration of nitrate in subsamples taken from the bottom of the reactor had decreased to 0.8 mg/L (i.e. 7.4 mg/L NO_3^--N had been removed from the bottom of the column reactor). However, the nitrate concentration at the top of column reactor remained at a higher level (7 times). Since this denitrification occurred without the external addition of carbon, it is likely that such a process was driven by intracellular or residual carbon in the wastewater.

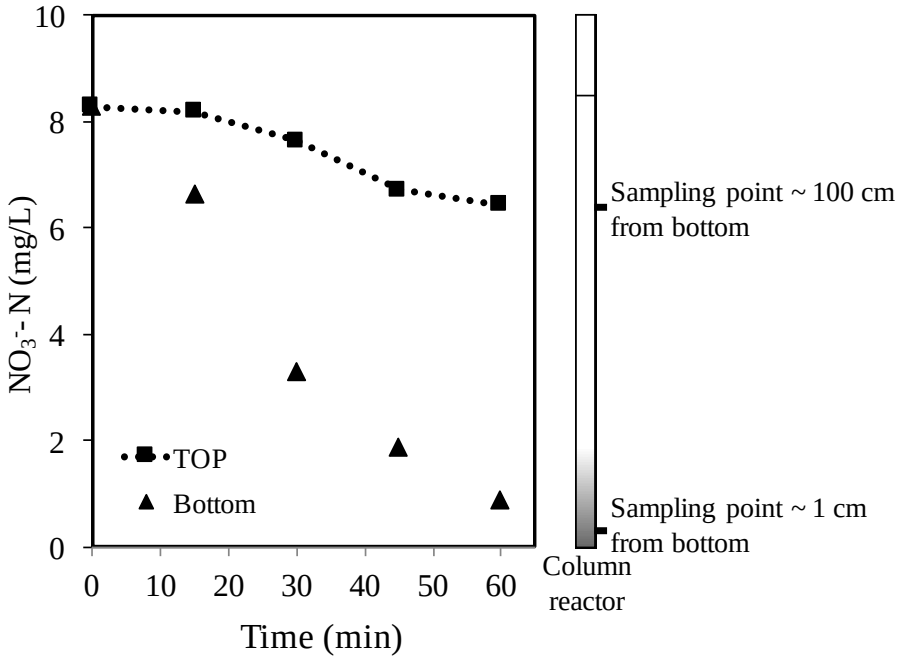

Figure 2.2. Nitrate reduction at the top and the bottom of the column reactor during settling of biomass

Clearly, this result suggests that there is sufficient carbon associated with activated sludge to remove nitrate from the sedimented sludge. However, increasing the sedimentation time to beyond 1 h remarkably affected the sedimentation due to ebullition of nitrogen gas (produced during denitrification), which was trapped in the sedimented biomass. Nevertheless, since the ebullition of nitrogen was a function of sedimentation time, and with proper management of sedimentation time, a sedimented biomass with low nitrate is achievable not requiring a Pre-stripper to remove nitrate in sedimented biomass.

2.3.3 P RELEASE RATE WAS NOT IMPACTED BY THE CONCENTRATION OF ACETATE

Since the release of P by PAOs under anaerobic conditions is accompanied with the uptake of VFAs (e.g. acetate), understanding the influence of acetate concentrations on the release of P from the RAS was considered useful. Hence, an experiment was carried out to examine this by exposing the RAS to a range of initial acetate concentrations (Figure 2.3). The results showed that irrespective of the initial acetate concentrations, P was released almost identically at the same rate (15.26 mg-P/g-MLSS.h) during the first 30 min of the experiment (Figure 2.3A). After 30 min, P

release slowed down to approximately 50% of the initial rate (7.17 mg-P/g-MLSS.h), depending on the availability of acetate (Figure 2.3A, B). During the final 60 min of experiment, the P release rate further decreased to 2.37 mg-P/g-MLSS.h. These reduction in rates were identical in all samples that had non-limiting concentrations of acetate (e.g. samples with initial acetate concentrations of 250 and 300 mg/L). This suggests that the reduction in P release rates did not occur as a result of the initial acetate concentrations used in this study. In addition, a positive linear correlation (Pearson product-moment correlation coefficient [$\underline{r}$ = 0.98, $\underline{n}$=45, $\underline{p}$<0.005 (1.13e-31)]) between the acetate consumed and P released by the biomass, suggested that the release of P with exposure to different initial concentrations of acetate was only dependent on the amount of acetate up taken rather than the concentration of acetate in the bulk (Figure 2.3E). This linear relationship (i.e. slope of the linear regression line) provided the $P_{release}/C_{acetate\text{-}C\ consumed}$ molar ratio for the biomass of this WWTP. As previously mentioned, this WWTP is not engineered for EBPR and yet its biomass demonstrated a P/C molar ratio of 0.66, which is high when compared to P/C ratios of 0.28 (Kerrnjespersen et al., 1994), 0.35 (Zeng et al., 2003b), and 0.84 (Hu et al., 2003) observed with activated sludges that were dominated by PAOs. This indicates that the activated sludge from this WWTP may also have a relatively high abundance of PAOs and a limited abundance of glycogen accumulating organisms (GAOs).

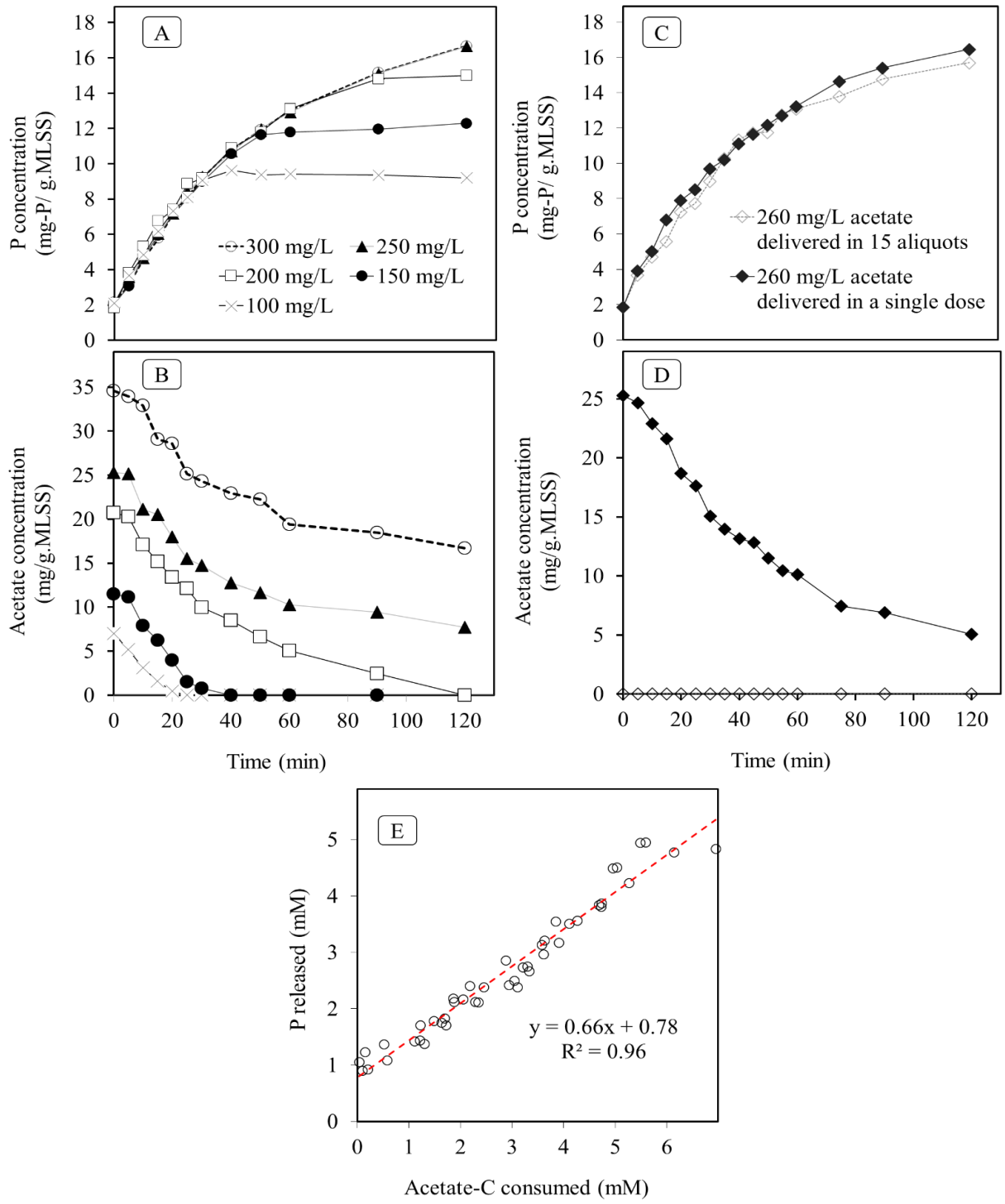

Figure 2.3. Influence of carbon (acetate) dosing on P release from RAS: (A, B) effect of initial acetate concentration; (C, D) effect of acetate delivery method; (E) relationship between P released and acetate consumed

To further confirm that a low concentration of acetate could enable P release rates that are comparable to those observed with high initial acetate concentrations, P removal was examined under the following two scenarios (a) a single dose delivery of 260 mg/L (i.e. a high initial acetate concentration) and (b) delivery of 260 mg/L of acetate via 15 equivalent aliquots (i.e. consistently maintaining a low acetate concentration throughout P release) (Figure 2.3C). The results showed that there was instantaneous utilisation of acetate during aliquot delivery of acetate with no measurable concentration of acetate detected throughout the experiment (Figure 2.3D). Interestingly, P removal with both a single delivery and aliquot delivery of acetate resulted in a similar trend of P release (Figure 2.3C). This demonstrates that low acetate concentrations have a negligible impact on P release from the RAS. In fact, compared to the earlier experiment (i.e. Figure 2.3A) a similar linear P release rate was observed during the initial 30 min of experiment (Figure 2.3C) and the change of rate during the remaining 90 min of the experiment was also similar.

In summary, the change of P release rate is independent of acetate concentration. Specifically, it is not a result of enzyme saturation (caused by exposure to higher concentrations of acetate) or due to a change in affinity with a decreasing concentration of acetate. However, with much higher initial concentrations of acetate, an enzyme saturation could take place, impacting PO_4^{3-}-P release. Future studies should examine enzyme saturation and inhibition kinetics to elucidate the highest initial PO_4^{3-}-P release rate that could be achieved with acetate, when bulk water is deficient of residual PO_4^{3-}-P.

2.3.4 ELEVATED PHOSPHATE CONCENTRATIONS IN BULK WATER INHIBITED P RELEASE FROM RAS

Studies discussed in Section 2.3.3 suggested that a low bulk water acetate concentration did not hamper the rate of P release. Hence, an experiment was also carried out to examine the effect of increased P concentrations in the bulk water on P release from RAS. In this experiment, the RAS was exposed to an acetate concentration of approximately 300 mg/L (which was shown in section 2.3.3 to be non-limiting and not saturating the enzyme that hydrolyses poly-P). The RAS was treated with different initial P concentrations and the P release and acetate consumption were measured (Figures 4). Irrespective of exposure to different initial

bulk water PO_4^{3-}-P concentrations, a positive linear correlation (Pearson product-moment correlation coefficient [r = 0.98, n=60, p<0.005 (4.14e-43)]) and a P/C molar ratio of 0.52 were observed as per before (Figure 2.4C). The minor variation of P/C ratio could be a result of experimental error and the high P/C ratio observed, once again confirms the effective use of carbon for P release even with an increasing concentration of P in the P recovery stream.

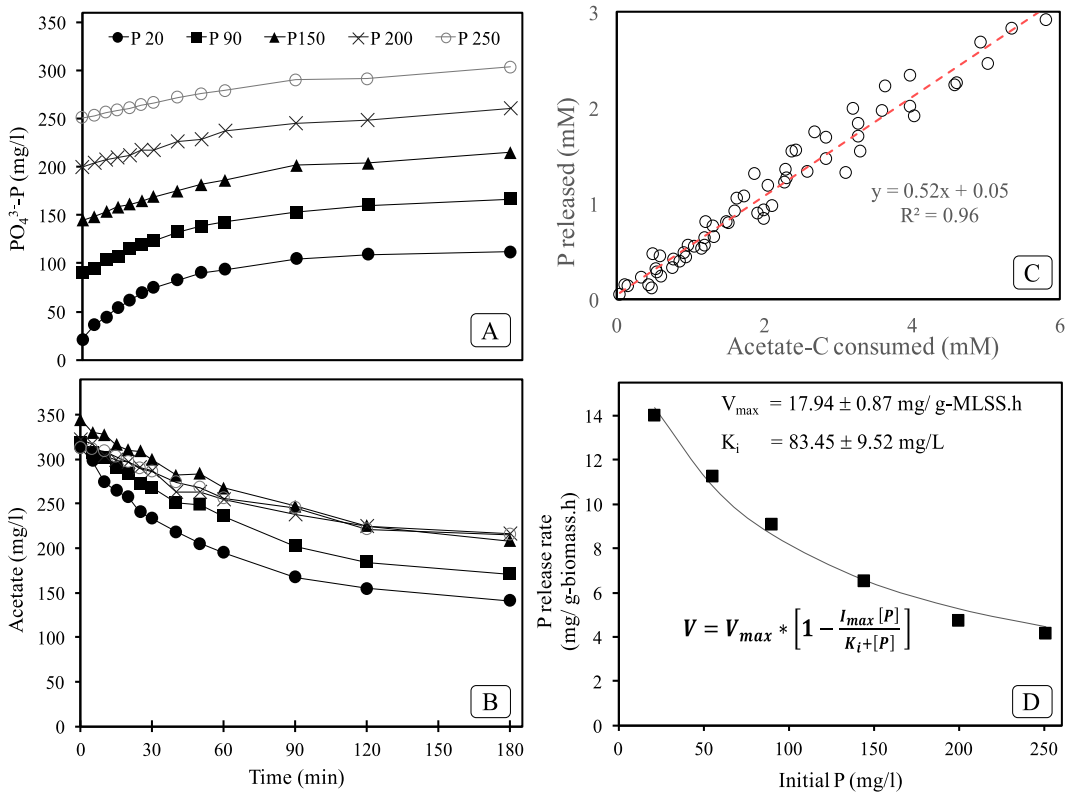

Figure 2.4. Phosphate release (Poly-P hydrolysis) by RAS exposed to different initial bulk water concentrations of PO_4^{3-}-P. [A] Increase of PO_4^{3-}-P concentrations in bulk water with release of P from RAS. [B] Acetate taken up during P release from RAS. [C] Amount of P released for acetate-C consumed [D] Specific rate of P release with increasing initial bulk water concentrations of phosphate.

The results also suggested that unlike the case with varying acetate concentrations, the specific rate of P release (calculated based on the first 30 min of P release) decreased with increasing concentrations of bulk water P (Figure 2.4D). Since all batch experiments were carried out using sub-aliquots (150 mL) of a well-mixed freshly collected sample of RAS, the poly-P content in each batch experiment could be considered identical. As can be observed from Figure 2.4B, all batch experiments

received a near constant non-limiting amount of acetate at the start of the experiment. Given that in this experiment only the bulk water PO_4^{3-}-P concentration was varied, it can be concluded that elevating the bulk water PO_4^{3-}-P concentrations can drastically affect the specific rate of P release from the RAS.

To further elucidate the inhibitory effect of bulk water PO_4^{3-}-P concentrations on the specific rate of P release, the specific rate of P release versus initial bulk water PO_4^{3-}-P concentration data was fitted to a modified Michaelis-Menten model (Figure 2.4D). The best fit to the data resulted in a V_{max} (± SE) of 17.94 ± 0.87 mg/g-MLSS.h and a K_i (± SE) of 83.5 ± 9.52 mg/L. Assuming a maximum inhibition of up to 100 % at higher bulk water PO_4^{3-}-P concentrations, the model was executed by constraining I_{max} to 1 (range for I_{max} is from 0 to 1 representing an inhibition of 0 to 100 %). The model fitting clearly affirmed the inhibitory effect of bulk water PO_4^{3-}-P concentrations on the specific rate of P release. As shown in Figure 2.4D, the maximal specific rate of P release (17.9 mg-P/g-MLSS.h) at 0 mg/L PO_4^{3-}-P concentrations in bulk water, rapidly reduced with release and accumulation of PO_4^{3-}-P in the bulk water. Specifically, the maximal specific rate halved once PO_4^{3-}-P concentrations in the bulk water reached 83.5 mg/L. This form of inhibition is similar to a product inhibition of an enzyme, and in this case PO_4^{3-}-P (the product of poly-P hydrolysis) appears to be inhibiting the enzymatic hydrolysis of poly-P. The inhibition of the enzymatic hydrolysis of poly-P should be a focus of a future systematic investigation. Since the specific P release rate decreased with accumulation of PO_4^{3-}-P in the bulk solution, the contact time for P release invariably needs to be increased to reach a higher concentration of PO_4^{3-}-P in the bulk solution (the P recovery stream).

2.3.5 A POTENTIAL APPLICATION OF PHOSTRIP FOR CONTINUOUS SIDE STREAM RECOVERY OF P FROM NON-EBPR PLANTS

If the Phostrip is to be applied to recover P from WWTPs (that have not been engineered to facilitate biological P removal), it could be technically viable and cost-effective if it is implemented as a continuous side stream P recovery process. Figure 2.5 details a process flow diagram that can facilitate a continuous recovery of P using the Phostrip process. Here, a portion of RAS is channelled through the Phostrip process to maintain a desirable (e.g. 100 mg-P/L) influent PO_4^{3-}-P concentration for a

subsequent P precipitation step. The continuous inflow of RAS to the Phostrip reactor would lead to a dilution of the PO_4^{3-}-P concentration. Hence, to maintain a steady concentration of PO_4^{3-}-P in the reactor, a steady release of P from the RAS should be achieved. For a steady release of P from RAS, a continuous dose of an appropriate quantity (based on the P/C ratio of RAS) of a carbon source such as acetate is required. As demonstrated in this study, the strategy of dosing acetate in multiple aliquots not only had negligible impact on the specific P release from RAS but also would enable maintenance of a very low (non-measurable) concentration of acetate within the Phostrip reactor. On the other hand, the hydraulic retention time (HRT) of the Phostrip reactor needs to be properly maintained based on the specific P-release rate of RAS in order to achieve a desirable concentration of PO_4^{3-}-P in the Phostrip reactor.

Design parameters of a Phostrip reactor for P recovery based on the results obtained from the WWTP examined in this study are listed in Table 2.2. P is a major essential element required for the growth of bacteria in activated sludge. Hence, not all P in wastewater can be recovered and approximately 58 % of P (0.28 t/d) that enters this plant can be recovered. Magnesium- and calcium-based precipitation products are the most common fertilizers considered when recovering P, and P concentrations of 100 mg-P/L or higher are considered economically essential to minimise chemical cost (Rittmann et al., 2011). Therefore, the in-reactor P concentration in the Phostrip reactor was assumed to be maintained at 100 mg/L. To recover 58 % of P at a concentration of 100 mg-P/L, the RAS needs to be loaded to the Phostrip reactor at a flow rate of 6.7 kL/h (Table 2.2). Since the P release rate when exposed to a bulk water concentration of 100 mg-P/L is only 8.16 mg-P/g-MLSS.h, the HRT required to counter the dilution effect caused by the inflow of RAS is 1.36 h. To maintain this HRT at the above RAS flow rate, the size of the Phostrip reactor is estimated to be only 9 m^3 (Table 2.2). In terms of external carbon requirements, considering the P/C ratio of the RAS of this plant, 1 t-acetate/d is required to recover the P. Based on the current market price of glacial acetate, the acetate cost for the wastewater treatment plant would be approximately AUD 525/d. This cost is lower compared with the chemical cost required for removing P using alum, with which the daily cost would be approximately AUD 3,100. The cost of acetate accordingly is only 17 % of the cost of alum.

Other potential costs such as chemical and infrastructure requirements for the precipitation of P as a beneficial end-product (e.g., hydroxyapatite or struvite) were not considered in the economic evaluation. The focus of the paper was the recovery of P by municipal wastewater utilities in a form that could be readily converted to a commercial product by, for example, a third party. The primary benefits for water utilities would include removal of P for environmental/regulatory purposes at lower cost than chemical addition. Negating the requirement to use alum would also avoid excess sludge volume and sludge handling issues and any other associated detrimental impacts on the WWT processes such as requirements for pH control in some circumstances.

Table 2.2. Design parameters for a continuous side stream recovery of P

Parameter	Unit	Phostrip P recovery process
C:N:P ratio needed for effective wastewater treatment		100:10:01
C in a typical WWTP (350 mg/L BOD)	mM	10.94
P that needs to be made available to remove this C	mM	0.11
Conc of PO_4^{3-}-P that is needed for biomass growth	mg-P/L	3.39
Influent PO_4^{3-}-P concentration [@]	mg-P/L	8.00
Influent PO_4^{3-}-P that can be recovered	mg-P/L	4.61
Influent flow rate [#]	ML/d	61
RAS flow rate [#]	ML/d	31
Maximum PO_4^{3-}-P that can be recovered daily	mg-P/d	281,372,031
Conc of P to be maintained in the Phostrip tank [P]	mg-P/L	100
V_{max} of RAS [@]	mg-P/g-biomass.h	17.94
K_i of RAS [@]	mg-P/L	83.46
I_{max} [@]		1
Specific P release rate in Phostrip tank (V) [$]	mg-P/g-biomass.h	8.16
Dry biomass weight of RAS [@]	g-biomass/L	9
Volumetric P release rate in Phostrip tank	mg-P/L-RAS.d	1,763
RAS directed to Phostrip tank	ML/d	0.16
Ratio of RAS directed to the stripper	%	0.51
The Phostrip reactor		
RAS flow rate into Phostrip tank	L/h	6,650
HRT (P conc / (P release rate x MLSS))	h	1.36
Size of Phostrip tank	L	9,054
	m^3	9.05
Cost of removing / recovering P by dosing glacial acetic acid		
P/C molar ratio for P release from RAS [@]		0.52
Glacial acetic acid requirement	g/d	524,589
Price of glacial acetic acid [*]	AUD/t	500
Cost of glacial acetic acid for removal/recovery of P	AUD/d	262.29
Cost of removing P dosing alum		
P/Al molar ratio for removal of P [!]		0.5
Al requirement	t/d	0.49
Price of alum [*]	AUD/t	250
Purity of alum	% (W-$Al_2(SO_4)_3$/W-Sol)	25
Content of Al in alum	% (W-Al/W-Sol)	4
Amount of alum required to remove the P	t/d	12.43
Cost of alum to remove P	AUD/d	3,107
Cost saving (glacial acetic acid vs alum)	%	8

[#] Obtained from wastewater treatment plant operator; [@] Experimentally derived in this study; * As quoted in https://www.alibaba.com; [!] Özacar and Şengil (2003); [$] Derived using the modified Michaelis-Menten equation $\boldsymbol{V = V_{max} * \left[1 - \frac{I_{max}\,[P]}{K_i + [P]}\right]}$

2.3.6 PHOSTRIP OFFERS BENEFICIAL USE OF ORGANIC CARBON FOR BOTH P RECOVERY AND DENITRIFICATION

In the proposed process flow diagram (Figure 2.5), after Phostrip, the RAS is returned back into mainstream treatment. The acetate used to trigger the release of P is intracellularly stored as PHA in the RAS. Once this RAS is returned to the anoxic basins of main stream, this additional carbon, which is conserved as PHA will be oxidised not only to uptake P (stored intracellularly as poly P) from the influent, but also to remove nitrate (with the assistance of denitrifying PAOs and GAOs, which uses nitrate as a final electron acceptor) in the anoxic tanks of mainstream (Zhao et al., 2016). Often, influent carbon is a limiting factor for many WWTPs to successfully remove nitrogen, and post denitrification with external carbon dosage has been commonly practiced to comply with stringent discharge limits of nitrogen (Aesoy et al., 1998). Post denitrification only facilitates removal of nitrogen and the demand for external carbon, imposes a significant operational cost to WWTPs. Wong et al. (2013) attempted to maximise the use of the added carbon by developing an innovative post denitrification configuration (EBPRr) not only to remove nitrogen but also to recover P from wastewater. Operation of this novel post denitrification configuration, however, is complex and maintenance of a two-sludge system can be technically demanding. On the other hand, the proposed side stream Phostrip P recovery process may be less challenging, as only a single sludge is used in both the mainstream and sidestream. Also, since the Phostrip process indirectly enhances nitrate removal in mainstream, the intrusion of nitrate into the RAS line can also be minimised, facilitating an overall better settling of biomass in the secondary sedimentation tanks. Overall the proposed Phostrip P recovery process provides for a cost-effective use of acetate by facilitating both recovery of P and removal of nitrate from the final effluent.

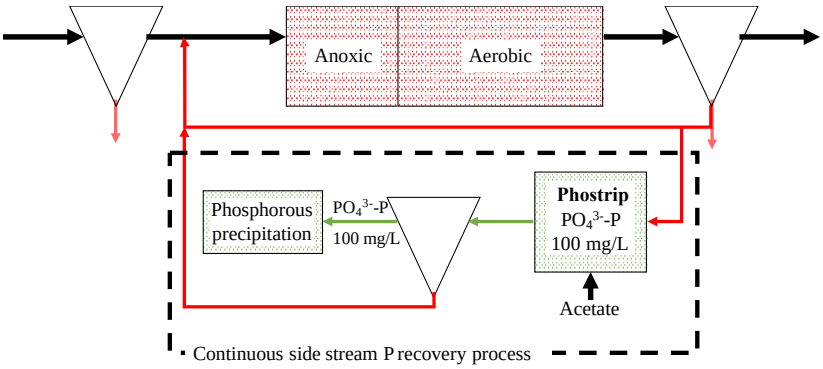

Figure 2.5. Phostrip as a continuous side stream P recovery process to recover P from non-EBPR plants.

2.4 *IMPLICATIONS OF THIS STUDY*

This study demonstrates the potential to recover P from non-EBPR wastewater treatment plants using Phostrip. We also demonstrate operational cost savings a WWTP can achieve by embracing P recovery over chemical precipitation of P using alum. In this study, a P/C molar ratio of approximately 0.5 was observed and at current market prices of acetate and alum, facilitating P recovery using acetate is more economical than removing P using alum. Even at a very low P/C molar ratio of 0.09, P recovery remains more economical than direct removal of P using alum precipitation (Shu et al., 2006). Lab-scale studies of EBPR demonstrate that continuous use of a volatile fatty acid such as acetate could increase the abundance of PAOs in activated sludge (Dou et al., 2007). Accordingly, long-term application of Phostrip could potentially increase the P/C ratio of activated sludge in a wastewater treatment plant. This is important as any increase of P/C ratio decreases the demand for acetate, reducing the operational cost of recovering P from wastewater. This study for the first time also proposed a novel sidestream reactor configuration, the feasibility of which has been demonstrated on the basis of the P release kinetics from RAS into a P concentrated stream. This side stream Phostrip reactor configuration only demands a small reactor footprint (only 9 m^3 for a relatively a large municipal wastewater treatment plant receiving 61 ML/d of wastewater) and is anticipated to enhance denitrification and increase settleability of RAS by minimising evolution of nitrogen gas in secondary sedimentation tanks (due to an overall reduction of nitrate in the effluent).

2.5 *CONCLUSIONS*

Detailed study of the P release kinetics of RAS collected from a non-EBPR WWTP showed that PO_4^{3-}-P release (Poly-P hydrolysis) from the RAS was negatively impacted as the concentration of PO_4^{3-}-P increased in the bulk water (recovery stream). The hindrance to PO_4^{3-}-P release was not due to acetate or its bulk concentration in the recovery stream but was the result of a competitive inhibition caused by the concentration of PO_4^{3-}-P in the recovery stream. The P release rate (18 mg-P/g-MLSS.h, when PO_4^{3-}-P concentration in the recovery stream was negligible) declined gradually with increasing PO_4^{3-}-P concentration in the recovery stream and the rate was reduced by half when the PO_4^{3-}-P concentration approached 84 mg-P/L. This finding enabled estimation of HRT and size of a tank to facilitate a Phostrip P recovery process to recover influent P received by a wastewater treatment plant. A small reactor footprint of 9 m^3 was found to be adequate to recover 0.3 t/d of P received at a treatment plant that had an influent flowrate of 61 ML/d. The above theoretical estimations of this study need to be demonstrated with confirmatory experiments in lab, pilot and full-scale.

3 INVESTIGATING THE EFFICIENCY OF GRANULAR SLUDGE FOR RECOVERY OF PHOSPHATE FROM WASTEWATER

Extended from the conference paper

SALEHI, S., CHENG, K. Y., HEITZ, A. & GINIGE, M. 2017. Investigating the Efficiency of Granular Sludge for Recovery of Phosphate from Wastewater. *World Academy of Science, Engineering and Technology, International Journal of Environmental and Ecological Engineering,* 4.

CHAPTER SUMMARY

An aerobic granular biomass was enriched to facilitate removal of phosphorus (P) from a dilute wastewater stream and to recover the removed P as a concentrated liquor in a separate recovery stream. The sequencing batch reactor was primarily exposed to an aerobic phase where polyphosphate accumulating organisms (PAOs) in the aerobic granules were made to uptake and intracellularly store PO_4^{3-}-P as polyphosphate (poly-P). The PAOs in the granules used oxygen as the final electron acceptor and oxidised their internal carbon reserves to fulfil energy requirements to uptake P. Subsequently the SBR was decanted and the granular biomass was exposed to an external carbon source in a small recovery volume under anaerobic conditions. PAOs under anaerobic conditions replenished its depleted carbon reserves and the energy requirements to uptake carbon was derived by hydrolysing poly-P. This hydrolysis resulted in a release of PO_4^{3-}-P back into the environment. Since the P is now in a small recovery volume the concentration of P is increased.

Using granular biomass, this study was able to achieve a very low wastewater/recovery stream volumetric ratio. As a consequence, it was possible to concentrate P in wastewater almost 45 times, enabling achievement of a PO_4^{3-}-P concentration of approximately 457 mg/L. Such high concentrations have never been previously reported in literature and the study for the first-time reports possibilities of achieving such concentrations with the assistance of granular biomass.

Furthermore, granules of different sedimentation rates were found to have different P releasing abilities. Hence, the study suggests the need to develop strategies to selectively retain granules of interest to further increase P recovery concentrations.

3.1 *INTRODUCTION*

Phosphorus is fundamental to all living things (Cordell et al., 2011). It is also vital for food production and is one of the three nutrients (nitrogen, potassium, and phosphorus) used in commercial fertilizers. Accordingly, global food security is dependent on the availability of P. The 'geochemical realities' of limited phosphate reserves and its impact on global food security can be managed to an extent with capture and reuse of P that is used in agriculture (Tiessen et al., 2011, Smil, 2000). Municipal wastewater contains approximately 8 – 10 mg/L of P and although it represents only a small proportion of the total P used in agriculture, recovering this P is important for a sustainable management of our remaining phosphorus reserves (Brett, 1997). The P concentration in wastewater, however is low and hence it is not economically viable to chemically precipitate this P (e.g. as struvite). P recovery from wastewater therefore only could be facilitated with creation of P concentrated streams in municipal wastewater treatment plants.

Currently many municipal wastewater treatment plants use enhanced biological P removal (EBPR) to biologically remove P received in influent wastewaters (Yuan et al., 2012). In EBPR a group of microorganisms referred to as phosphate-accumulating organisms (PAOs) are alternatively exposed to an aerobic and an anaerobic environment to facilitate removal of P from wastewater (Valverde-Perez et al., 2015). PAOs uptake orthophosphate from bulk water under aerobic and/or anoxic conditions and intracellularly stores it as polyphosphate (poly-P). The energy requirements for this process is derived by oxidising internal carbon storage polymers (e.g. Poly (3-hydroxyalkanoates) (PHA)) abundant in PAOs. PAOs replenish their internal carbon reserves by up taking volatile fatty acids (VFAs) under anaerobic conditions. The energy requirements for the uptake of VFAs are derived by hydrolysing poly-P (Mino et al., 1998). Hence, a release of P can be observed when the biomass is exposed to anaerobic conditions.

Wong et al. (2013) made use of PAO metabolism to develop a novel post-denitrification process that not only removed nitrogen but also facilitated recovery of P from secondary effluent. The strategy termed EBPR-r used a denitrifying-PAO biofilm to uptake P from a dilute wastewater stream. The biofilm was subsequently exposed to a smaller recovery stream together with VFA and the P captured (in the biomass) from secondary effluent now was in the small recovery stream facilitating a concentrated P liquor. Wong et al. (2013) was able to concentrate P in wastewater by a factor of four. With repeated release of P to the same recovery stream, the authors were also able to achieve a P concentration of 100 mg-P/L in the recovery stream.

Due to the rigid biofilm carrier media, it was not feasible for Wong et al. (2013) to further reduce volume of recovery stream. Hence, they were only able to achieve higher concentrations of P in the recovery stream with a repeated re-use of the recovery stream to release P. A potential alternative, which obviates the barriers of biofilm carriers, is granular biomass. Granular biomass not only enables maintenance of high biomass densities it also brings in superior compaction and settling properties. Accordingly, it was hypothesized that granular biomass could facilitate a higher volumetric ratio between the wastewater and the recovery stream enabling recovery of P at a much high concentration. In this study, granular PAOs were enriched in a SBR reactor, with application of a typical anaerobic/aerobic cycle. The study specifically examined the ability of granular biomass to generate a highly concentrated P liquor with application of volume reduction. Additionally, based on sedimentation rate different granular biomass fractions were collected and examined for their efficiencies to release P into a recovery stream.

3.2 *MATERIALS AND METHODS*

3.2.1 SEQUENCING BATCH REACTOR AND GENERAL OPERATION

A laboratory scale sequencing batch reactor (SBR) with a working volume of 4 L was operated under alternating aerobic/anaerobic conditions to enrich a granular biomass capable of up taking and releasing P (Figure 3.1). The reactor was seeded with activated sludge (1 L) collected from a local municipal wastewater treatment plant. The inoculum had a mixed liquor suspended solids (MLSS) concentration of approximately 4.0 g/L. An operational cycle of the SBR included a 2 h anaerobic

period with 5 mins of feeding (synthetic medium), 2 h of aerobic period, 20 min settling and 10 min decanting. Acetate and propionate were alternately used (bi-weekly) as the carbon source to facilitate the enrichment of PAO's (Lu et al., 2006). The reactor was operated for over 100 days and once a stable performance was achieved, the sludge was used in a series of batch experiments (detailed below, Figure 3.1).

3.2.2 FEED COMPOSITION

The 3.3 L synthetic feed was composed of 40 mL of solution A, 200 mL of solution B and 200 ml of solution C. The composition of these stock solutions are as followed: Stock solution A (per L) : 25.63 g CH_3COONa or 17.15 g CH_3CH_2COONa; Stock solution B (per L) : 0.9 g $MgSO_4$, 0.765 g NH_4Cl, 5.96 mg allyl-N thiourea, 7.5 mg Peptone, 7.5 mg Yeast extract, 142.5 mg $CaCl._2H_2O$, 30 mg ethylenediaminetetraacetic acid (EDTA),4.5 mg $FeCL_3.6H_2O$, 0.36 mg $ZnSO_4.7H_2O$, 0.36 mg $MnCl_2.4H_2O$, 0.18 mg $Na_2MoO4.2H_2O$, 0.09 mg $CuSO_4.5H_2O$, 0.45 mg CoCl2.6H2O, 0.54 mg KI, 0.45 mg H3BO3; Stock solution C (per L): 371 mg KH_2PO_4, 647 mg K_2HPO_4. The synthetic feed contained 10 mg/L of phosphate-P and 200 mg/L of chemical oxygen demand (COD).

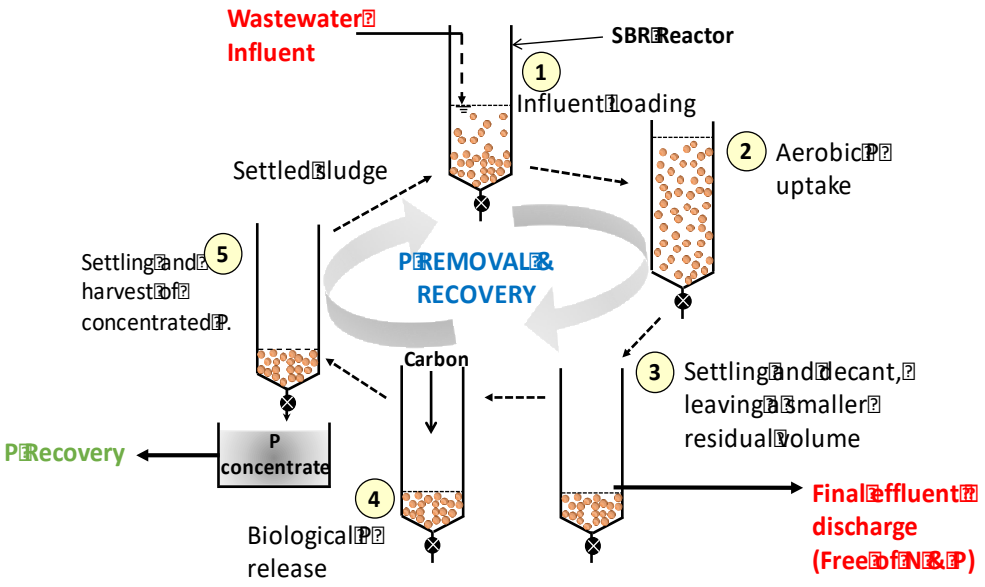

Figure 3.1. Operational strategy for the sequencing batch reactor.

3.2.3 PROCESS MONITORING AND CHEMICAL ANALYSES

The SBR operation was automated using National Instruments (USA) software (LabVIEW) and data acquisition & control hardware. Online monitoring of dissolved oxygen (DO), pH and redox potential (ORP) was carried out using a luminescent DO probe (PDO2, Barben Analyzer Technology, USA), an intermediate junction pH probe (Ionode IJ44, Ionode Pty Ltd, Australia) and an intermediate junction redox probe (Ionode IJ64, Ionode Pty Ltd, Australia), respectively.

Cyclic studies were performed to evaluate the kinetics of P uptake and release of the granular biomass. Each cyclic study involved withdrawing 2 ml of sample from the reactor every 15–30 min over the entire 6-h cycle. Each sample was immediately filtered using a 0.22 μm pore size syringe filter (Acrodisc® PF, Pal Corporation, UK). The concentrations of soluble PO_4^{3-}–P and acetate in the filtrates were determined using ion chromatography (ICS-3000, DIONEX).

3.2.4 BATCH EXPERIMENTS WITH THE ESTABLISHED GRANULAR PAO BIOMASS

Two batch experiments were carried out in this study.

3.2.4.1 Experiment (1) Evaluation of the P release ability of the established sludge with a decreasing volume of medium;

At the end of aerobic P-uptake phase, well mixed biomass was transferred to 20 ml anaerobic bottles. After allowing the biomass to settle for 20 mins, supernatant was discarded from the top of the bottles to obtain 1, 2, 4 and 5 times wastewater/recovery stream volumetric ratios. The bottles were sealed with aluminium caps, and nitrogen was sparged to the bottles to create anaerobic condition. Finally, an identical quantity of COD (200 mg) was added to all the bottles. Bottles were shaken for 2 hrs and at the end of the experiment liquid samples were taken using a syringe and filtered and were stored at 4°C until analysed.

3.2.4.2 Experiment (2) Evaluation of the P release ability of four different fractions of the granular sludge;

Once the granular SBR reactor achieved a steady state, experiments were carried out to examine whether different granular biomass fractions with different sedimentation rates had different P releasing capabilities. For this purpose, 1 L of well mixed biomass

was taken from the master reactor at the end of aerobic phase and was placed in a cone-shaped imhoff vessel. After settling for 20 minutes the settled biomass was divided equally into four parts (each part 50 ml) from the bottom to the top and was placed in well mixed jars. The fifth jar contained the mixed biomass taken directly from the master reactor and was used as a control.

To investigate impact of volume reduction on P release, the aliquots were transferred to 20 ml anaerobic bottles and different volumes of supernatant was discarded from the top without disturbing the settled biomass to impose different recovery volumes. Then all the bottles were sealed with anaerobic caps and was sparged with nitrogen for 3 min to create anaerobic conditions. 200 mg of COD was then injected into each bottle and mixed for two hours. Samples were taken out at the end of 2 hours and were immediately filtered and were stored at 4°C until analysed. The mixed liquor suspended solids (MLSS) in all anaerobic bottles were determined according to the Standard Methods for Water and Wastewater Analysis (Rice et al., 2012).

3.3 *RESULTS AND DISCUSSION*

The activated sludge inoculum was successfully acclimated as a granular biomass after approximately 110 days of reactor operation and the biomass exhibited a PAO phenotypic behaviour and was capable of releasing a PO_4^{3-}–P concentration of approximately 100 mg/l during the anaerobic phase of reactor operation.

When the granular biomass was subjected to various wastewater/recovery stream volumetric ratios, a near linear increase of P concentration was recorded (Figure 3.2). With volume reduction a P concentration of approximately 457 mg/L was achieved in the recovery stream. The P concentration in the influent wastewater (i.e. for aerobic P uptake) was only 10 mg/L and with volume reduction it was possible to concentrate P in the recovery stream almost 45 times (Figure 3.2). Such a higher concentration of P is highly desirable for a downstream chemical precipitation of P. Such a high concentration was only feasible due to the superior compaction properties of granular biomass.

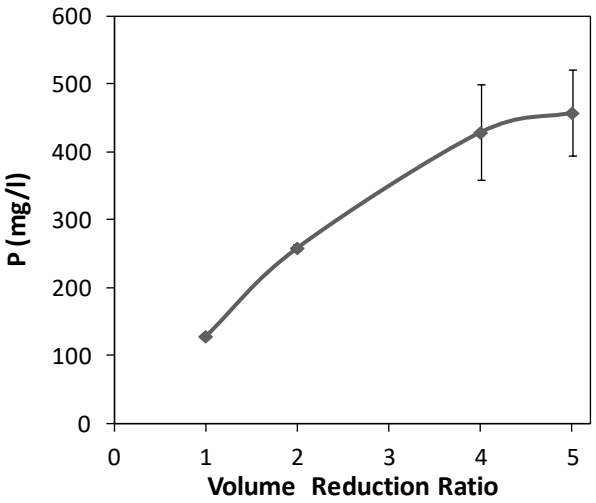

Figure 3.2. P concentration after 2 hr anaerobic phase at different volume reduction ratios.

To further elucidate the ability of the granular biomass to concentrate P, another series of experiments were conducted with biomass derived based on the different sedimentation rates of the granular sludge. At the time of executing these experiments, the total P release end of an anaerobic phase was marginally lower due to a loss of biomass from the reactor (Figure 3.3). The fractions were segregated based on their rate of sedimentation and the highest rate of sedimentation was observed with fraction 1 and the lowest was with fraction 4. According to Figure 3.3A, when comparing against the control, all fractions except fraction 4 appear to have an equal ability to release P into the recovery stream. However, the MLSS concentrations of each of the fractions were noted to be different. Hence to facilitate a meaningful comparison, the P concentrations obtained for each individual fraction was normalised with their respective P concentrations obtained without volume reduction (i.e. at a volume reduction ratio of 1), as shown in Figure 3.3B. This enabled comparison of the effectiveness of different fractions. The results showed that the performance of fractions 3 and 4 were close to the control. Volume reduction ratio does not appear to have any inhibitory effect on the P release ability of these two fractions.

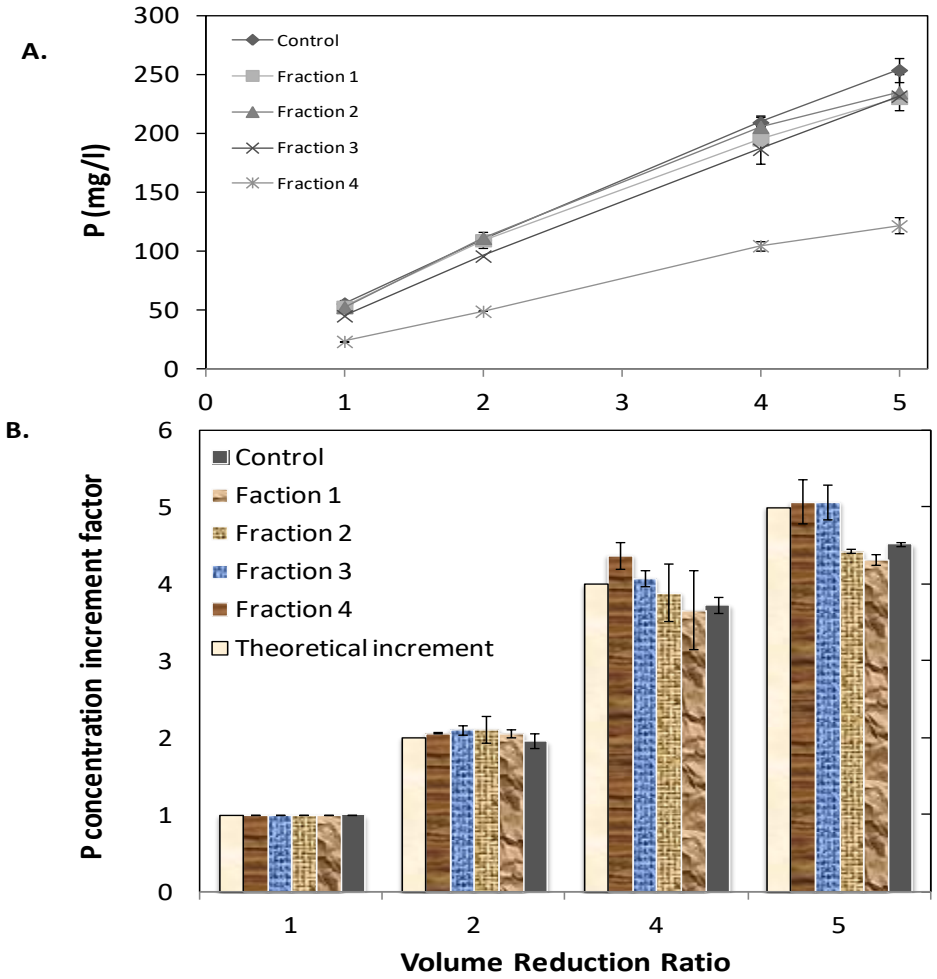

Figure 3.3. Effect of volume reduction on biological P release from various fractions of the established granular sludge: (A) P concentration vs. volume reduction ratio; (B) P concentration increment vs. volume reduction ratio.

To realize the role of different fractions of the granular biomass and their individual contributions towards generating a concentrated stream of P, specific P release efficacies (mg.P/g.MLSS) of different fractions were also calculated. On comparing specific P release efficacies (mg.P/g.MLSS), fraction 3 appeared to be the most active fraction and its specific P release efficacy appears to have not been affected by volume reduction (Figure 3.4). Further studies should examine microbial community differences of each of these fractions.

This study provides insight towards P release efficacies of different fractions and such knowledge can be utilised to selectively retain specific granules by manipulating the settling time of the SBR.

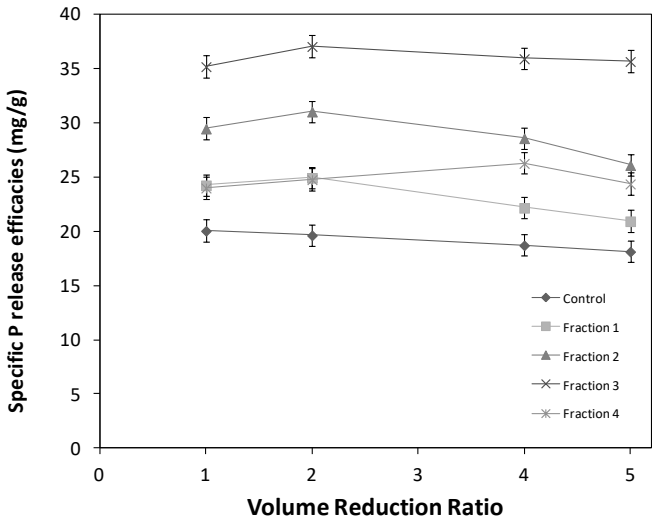

Figure 3.4. Specific P release efficacies (mg.P/g.MLSS) of different fractions of the granular sludge as a function of volume reduction ratio.

3.4 *CONCLUSIONS*

This study for the first time demonstrates that granular biomass enables achievement of higher wastewater/recovery stream volumetric ratios. As a consequence, it was possible to concentrate the influent P 45 times enabling achievement of a 457 mg/L P concentration in the recovery stream.

This study also reveals the differences of P release abilities of granular biomass fractions that show different sedimentation rates. Certain fractions appear to have superior P releasing capabilities over others. Future research should examine phylogenetic differences among these different granules and develop strategies to selectively retain granules of interest in the SBR.

4 A NOVEL STORAGE DRIVEN GRANULAR POST DENITRIFICATION PROCESS: LONG-TERM EFFECTS OF VOLUME REDUCTION ON PHOSPHATE RECOVERY

Extended from

SALEHI, S., CHENG, K. Y., HEITZ, A. & GINIGE, M. P. 2018. A novel granular post denitrification EBPR process: Long-term effects of volume reduction on phosphate recovery. *Chemical Engineering Journal*.

CHAPTER SUMMARY

Anoxic granular biomass with enhanced biological phosphorus (P) removal was used in a post-denitrification configuration to concentrate P in wastewater. The study examined the use of anoxic granules to facilitate application of volume reduction to create a P-enriched stream (>100 mg-P/L). The results indicated the importance of maintaining a food to microorganism (F/M) ratio of ~0.124 g-COD/g-MLSS.d to achieve P and nitrogen (N) removal close to 100 %. While granulation required a short settling time and a high-volume exchange ratio, biomass wasting was essential to control the F/M ratio to maintain a suitable microbial diversity and abundance. Diversity and abundance were also impacted by volume reduction, but the effect of this was marginal compared with the effect of decreasing F/M ratio. Furthermore, a decrease in the F/M ratio enhanced sedimentation (SVI5 decreased from 55.5 to 32.0 ml/g-MLSS) but potentially decreased dewaterability (capillary suction time increased from 15.5 s to 19.4 s). Recovery of P as a concentrated liquor had minimal impact on the bacterial diversity.

4.1 *INTRODUCTION*

Phosphorus (P) is a non-renewable element that is essential for life. With increasing demand for P fertilisers, there is a push towards recycling of P from waste streams such as municipal wastewater (Mayer et al., 2016). However, as P concentration in municipal wastewater is typically low (< 10 mg/L), recovering P directly from the water is economically challenging (Egle et al., 2015). To achieve economies of scale,

the P concentration in wastewater needs to be elevated. Recently, biological strategies have been developed to create P concentrated side streams, enabling recovery of P as a chemical precipitant such as struvite (NH4MgPO4) or calcium phosphate (Ca3(PO4)2) (Cieslik and Konieczka, 2017).

The biological P-concentrating strategies developed so far have exploited the metabolic pathways of a group of microorganisms naturally abundant in activated sludge (Tarayre et al., 2016). This group of microorganisms, which can store P in excess of their growth and metabolic requirements, are broadly termed phosphate-accumulating organisms (PAOs). Under aerobic (oxygen as a final electron acceptor) or anoxic (nitrate or nitrite as final electron acceptors) conditions, PAOs are able to take up and store P as polyphosphate (poly-P). The energy requirements for this process is derived from the oxidation of intracellular carbon reserves (polyhydroxyalkanoates (PHA)) (Ni et al., 2015, Jenkins and Tandoi, 1991). PAOs that utilise nitrate or nitrite as final electron acceptors are often called denitrifying-PAOs or DPAOs. When PAOs/DPAOs are exposed to anaerobic conditions in the presence of volatile fatty acids (VFAs, e.g. acetate), they can take up the VFAs and replenish their carbon reserves. The beneficial aspects of using VFAs over other carbon to facilitate anaerobic P release is well demonstrated (Chen et al., 2015). The energy requirements to uptake carbon are fulfilled by poly-P hydrolysis, which releases P as phosphate (PO43-) to the surrounding water. The P uptake-release ability of PAOs/DPAOs has been exploited to concentrate P in wastewater, improving the economies of scale to recover P from municipal wastewater (Zeng et al., 2003b, Filipe and Daigger, 1999, Wong et al., 2013).

Wong et al. (2013) exploited the metabolism of DPAOs, demonstrating a new post denitrification configuration to facilitate removal of nitrate and recovery of P from municipal wastewater. This new configuration, termed as Enhanced Biological Phosphorus Removal and Recovery (EBPR-r), unlike conventional post denitrification processes utilised intracellular carbon reserves of a biofilm to remove P and nitrate from wastewater. This utilisation of internal carbon was advantageous in preventing discharge of carbon with effluent. The replenishment of internal carbon reserves and recovery of phosphate from the biofilm were facilitated by allowing the biofilm to release P into a separate recovery stream, which is several times smaller in volume compared to the wastewater stream. This enabled Wong et al. (2013) to recover P in a

separate liquor where P was 4 times higher in concentration compared to the P concentration in the wastewater.

Recently, Kodera et al. (2013) demonstrated a similar volume reduction approach with an aerobic PAO biofilm grown in a trickling filter reactor. They successfully created a P recovery stream that was 25 times the concentration of P in the influent wastewater. Increasing the P recovery concentration further was challenging for Wong et al. (2013) and Kodera et al. (2013) primarily due to the large volume occupied by the biofilm carrier media. The large carrier media volume prevented a further reduction of recovery volume and averted achievement of much higher P concentrations in the recovery stream. As such, the only viable option for both studies was to repeatedly re-use its recovery volume to continue capture P released from the biofilm, until such time the desired P concentration was achieved in the recovery liquor.

A possible strategy to overcome the abovementioned limitation of biofilm carrier media to concentrate P is to utilise granular biomass. The high biomass densities and the excellent settling and dewatering properties of granular biomass may help minimise the recovery volume enabling recovery of P at elevated concentrations. The separation of the concentrated liquor from the granular biomass was also expected to be effective (Gao et al., 2011). Additionally, the high biomass density in granular sludge may facilitate a higher P and N removal efficiency, enabling treatment of a large volume of wastewater with a single exposure to the granular sludge. While this would facilitate a smaller footprint, it is also likely to further increase opportunity to achieve a highly concentrated P liquor in the recovery stream without the need for a repeated re-use of the recovery volume to capture P.

Having realised the potential of granular sludge, Lu et al. (2016) operated a granular sludge reactor to facilitate simultaneous nitrification, denitrification and P removal (SNDPR), and attempted to recover P from the granular biomass by exposing part of the biomass to acetate under anaerobic conditions. The granular biomass was subsequently separated from the concentrated P liquor and returned back into the main reactor with the use of a woven cloth (Lu et al., 2016). Although a technology based on a woven cloth appears impractical for full-scale implementation, the study clearly demonstrated the ease of dewatering granular biomass to recover the concentrated P liquor. Without considering volume reduction, several other studies also have

examined P recovery from wastewater using granular biomass (Liu et al., 2017, Tervahauta et al., 2014, Angela et al., 2011).

To our knowledge, no study has explored the use of volume reduction to recover P as a concentrated liquor using granular sludge. The feasibility of this approach and its long-term implications on granular sludge warrant investigation if the wastewater industry is to capitalise from the beneficial properties of granular biomass to recover P from municipal wastewater. Therefore, this study investigates P recovery with post denitrification, using a granular biomass. A granular sequencing batch reactor (SBR) was operated to facilitate P and nitrate removal from an influent wastewater volume that was maintained constant throughout the study. The anaerobic recovery of P from the granular biomass into a small recovery stream was systematically studied over a long-term period (250 days), by gradually increasing the volumetric ratio between the wastewater and the recovery stream. The long-term performance and stability of the reactor was monitored using physiochemical and microbiological measurements.

4.2 *MATERIALS AND METHODS*

4.2.1 REACTOR START-UP AND SBR OPERATION

The SBR used in this study had a working volume of 3.7 L and was operated at room temperature (20 – 22°C). The operational cycle (8 h) of the SBR is depicted in Figure 4.1 [A] and consisted of (1) a 5 min nutrient loading, (2) a 6 h anoxic P uptake and denitrification period, (3) a 20 min settling and a 5 min period of decant (2.8 L), (4) a 1 min feed of carbon source and a subsequent 2 h period of anaerobic P release, and (5) a 10 min settling and recovery of P as a concentrated liquor (recovery of P was carried out only during Phase 5 of the long term reactor operation). At the beginning of steps (2) and (4), nitrogen was sparged as required into the reactor to create anaerobic conditions (dissolved oxygen (DO) 0 mg/L). Apart from during settling and decanting, the liquor in the reactor was gently mixed (100 rpm) using an overhead stirrer (RZR2020, Heidolph, Germany). The hydraulic retention time of the SBR reactor was maintained at 10.2 h. The granulation of biomass was facilitated by applying a high volumetric exchange ratio (78 %) at the end of the anoxic period of reactor operation. During the course of operation we applied a SRT of 28 days for the

initial 115 days, from then we stopped wasting of the biomass up to day 176 after which the 28 days SRT was resumed.

The SBR operation was automated using National Instrument hardware (CompactRIO) and software (Labview). Online monitoring and control of DO set point (at 0 mg/L) was facilitated by using a luminescent DO probe (PDO_2; Barben Analyser Technology, USA) and a feedback controllable solenoid valve that controlled the flow of nitrogen gas from a nitrogen generator to the reactor. The pH in the reactor was monitored online using an intermediate junction pH sensor (Ionode IJ44, Ionode Pty Ltd, Australia). An intermediate junction redox sensor (Ionode IJ64, Ionode Pty Ltd, Australia) was also used for online monitoring of redox changes in the reactor. Although pH and redox were monitored online, these measurements were not used for any kind of direct online control of the reactor.

The seed sludge for the start-up of the reactor was sourced from a municipal wastewater treatment plant (WWTP) in Perth, Western Australia. This WWTP utilises SBRs to treat 160 ML/d of municipal wastewater. The mixed liquor suspended solids (MLSS) of the return activated sludge (RAS) collected from the WWTP was approximately 6 g/L. A uniform mixture of RAS collected was introduced into the laboratory scale SBR to facilitate an initial MLSS of 4 g/L in the reactor.

4.2.2 COMPOSITION OF SYNTHETIC INFLUENT FOR POST DENITRIFICATION AND EXTERNAL CARBON SOURCE FOR RECOVERY OF P

The synthetic feed introduced at the beginning of the anoxic period contained solution A (200 mL), solution B (200 mL) and reverse osmosis (RO) water, giving an initial in reactor PO_4^{3}-P concentration of 7 mg/L and a NO_3^{-}-N concentration of 16 mg/L for each cycle. Solution A (per L) contained 1.8 g $NaNO_3$, 0.9 g $MgSO_4$, 3.05 g NH_4Cl, 7.5 mg Peptone, 7.5 mg Yeast extract, 142.5 mg $CaCl_2 \bullet 2H_2O$, 30 mg ethylenediaminetetraacetic acid (EDTA), 4.5 mg $FeCl_3.\bullet 6H_2O$, 0.36 mg $ZnSO_4 \bullet 7H_2O$, 0.36 mg $MnCl_2 \bullet 4H_2O$, 0.18 mg $Na_2MoO_4 \bullet 2H_2O$, 0.09 mg $CuSO_4 \bullet 5H_2O$, 0.45 mg $CoCl_2 \bullet 6H_2O$, 0.54 mg KI and 0.45 mg H_3BO_3; Solution B contained (per L) 24 g KH_2PO_4 and 42 g K_2HPO_4. The volume of RO water introduced at the beginning of the anoxic period (to make up a final anoxic reactor volume of 3.7

L) varied based on the operational phase of the SBR and was proportional to the anaerobic volume (Table 4.1).

During step (4) of the reactor cycle (Figure 4.1 [A]), solution C (20 mL) was added to facilitate anaerobic P release. Solution C contained CH_3COONa (25.63 g/L) and in-reactor CH_3COONa concentration varied based on the liquid volume of the reactor in step (4) of SBR operation (Table 4.1).

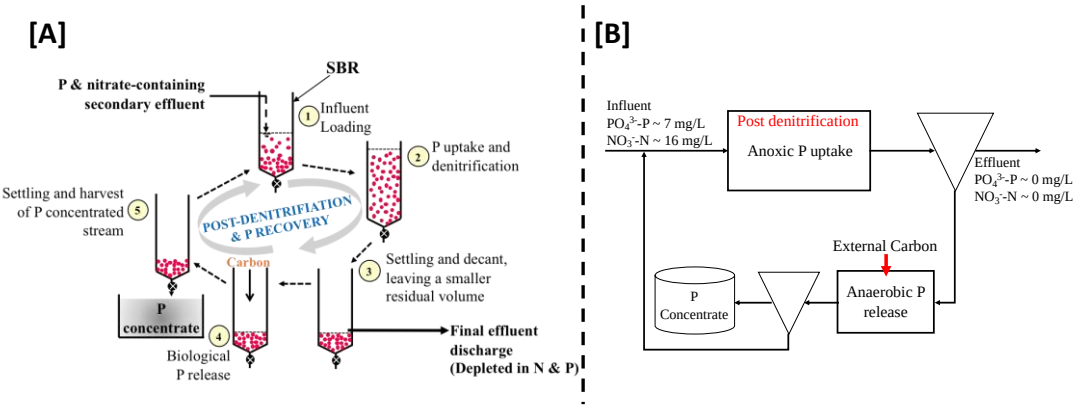

Figure 4.1. [A] A schematic illustration of the post denitrification and P recovery process. [B] A step-wise illustration of an operational SBR cycle.

4.2.3 REDUCING VOLUME FOR ANAEROBIC P RELEASE TO INCREASE P CONCENTRATION, FAVOURING P RECOVERY

To avoid carryover of residual nitrate into the anaerobic zone and to create a high-volume exchange ratio between the P uptake and release phases, 2.8 L of reactor volume was decanted at the end of the anoxic zone. Subsequently, solution C and RO water were introduced into the reactor to facilitate each of the anaerobic reactor volumes maintained at different phases of reactor operation (Table 4.1).

At the end of the final phase (5) of reactor operation, in addition to the 2.8 L of effluent that was discharged during step 3 (Figure 4.1[A]), an aliquot of 100 mL was removed to recover the amount of P that had been loaded (as influent) into the reactor as a concentrated liquor. This volume was recovered after allowing the reactor to settle for 10 min.

Figure 4.1. SBR operation during entire period of study

Phase of SBR operation	No of days of SBR operation	Times volume reduced during anaerobic P release	Volume (L)			
			Anoxic volume during P uptake	Effluent decanted	Anaerobic volume during P release	Volume of recovery stream
1	1 - 72	1	3.7	2.8	3.7	0
2	73 - 101	1.7	3.7	2.8	2.2	0
3	102 - 138	2.5	3.7	2.8	1.5	0
4	139 - 219	4	3.7	2.8	0.9	0
5	219 - 250	4	3.7	2.8	0.9	0.1

4.2.4 PROCESS MONITORING AND CHEMICAL ANALYSES

Daily influent and effluent samples were collected from the SBR to monitor the stability of the reactor during each phase of SBR operation. Once stable operation of SBR was observed, a minimum of 2 cyclic studies were carried out in each phase of SBR operation. During an 8 h cyclic study, mixed liquor samples (2 mL) were withdrawn at regular time intervals to enable measurement of PO_4^{3-}-P, NO_3^--N, NO_2^--N and acetate transformations during a reactor cycle. All samples collected both daily and during cyclic studies were immediately filtered through 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia) and the filtrates were stored in 2 mL Eppendorf tubes at 4°C until analysed.

The concentrations of soluble PO_4^{3-}–P, NO_3^--N, NO_2^--N and acetate in the filtrates were determined using ion chromatography (ICS-3000, DIONEX). A Dionex ICS-3000 reagent-free ion chromatography (RFIC) system was fitted with an IonPac® AS18 4 x 250 mm column. The eluent for anion separation was potassium hydroxide at a flow rate of 1 mL/min. The analysis was carried out using gradient elution as follows: the concentration was increased from 12 - 45 mM from 0 - 5 min, maintained at 45 mM from 5 - 8 min, increased from 45 - 60 mM between 8 - 10 min and decreased

from 60 - 12 mM between 10 - 13 min. The temperature of the column was maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppression® recycle mode). The detection limits of the instrument for soluble PO_4^{3-}-P, NO_3^--N, NO_2^--N and acetate are 0.9 mg-P/L, 0.14 mg-N/L, 0.20 mg-N/L, and 0.59 mg/L, respectively.

The MLSS concentration was determined after each cyclic study as detailed in Standard Methods for Water and Wastewater Analysis (Rice et al., 2012).

4.2.5 MICROBIAL COMMUNITY ANALYSIS AND MICROSCOPIC IMAGING

Once stable operation was achieved during each phase of SBR operation, a mixed liquor sample was collected to characterise microbial diversity in the reactor. Upon collection, the sample was immediately stored in a freezer at -80°C. Prior to analysis, the sample was thawed at room temperature and DNA was extracted using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., USA) in preparation for 454 pyrosequencing. DNA sequencing was carried out as detailed in Nagel et al. (2016). Post sequence analysis was carried out using the Quantitative Insights Into Microbial Ecology (QIIME) software package version 1.9.1 (Caporaso et al., 2010). The specific relative abundance of PAOs and GAOs in the RAS was derived by blasting all representative sequences against reference sequence databases of PAOs and GAOs, created from the MiDAS taxonomic database (McIlroy et al., 2015).

During phase 5 of SBR operation, microscopic imaging of the granules was carried out on a wet mount using an upright Fluorescence Microscope (Zeiss AxioImager M1). Light microscopic images were captured using an AxioCam HRm (Carl Zeiss) high resolution camera.

4.3 RESULTS AND DISCUSSION

4.3.1 RAPID GRANULATION OF BIOMASS DURING A SHORT ACCLIMATISATION OF ACTIVATED SLUDGE TO A POST-DENITRIFICATION CONFIGURATION

A schematic representation of the operational mode of this post denitrification reactor is given in Figure 4.1[B]. In this operational mode, post denitrification was primarily

driven using the carbon storage polymers in the biomass. Since carbon storage polymers were produced with the carbon that was added during anaerobic P release, no externally dosed carbon would come into contact with the wastewater stream. In our volume reduction technique, the external carbon was specifically supplied to the settled sludge embodied in a small volume of liquid so as to facilitate recovery of P as a concentrated liquor. Since the carbon was dosed under anaerobic conditions, limited wastage of carbon via aerobic oxidation was expected.

After approximately 50 d of operation, a near 100 % P and N removal efficiency was achieved (Figure 4.2[A]) and this coincided with a stable MLSS of 3 g/L in the reactor (Figure 4.2[C]). At the beginning of the study, the reactor was inoculated with activated sludge (approximately 4 g/L). During the initial 115 d of reactor operation, a sludge retention time (SRT) of 28 d was maintained in the SBR with wasting of biomass just before the end of the anoxic period. This SRT and the high-volume exchange ratio enabled biomass granulation, with removal of poorly settling biomass from the reactor. Within 19 d, the MLSS in the reactor reduced to approximately 3 g/L and from day 19 until early Phase 3 (approximately 110 d) of reactor operation, the MLSS remained steady (2.98 ± 0.3 g/L). The initial reduction of MLSS resulted in an increase of food to microorganisms (F/M) ratio from 0.11 to 0.14 g-COD/g-MLSS.d (Figure 4.2[F]): an increase of F/M ratio is known to favour granulation due to an enhanced production of extracellular polymeric substances (Li et al., 2011). During this period of steady state, large granules (diameter of approximately 3 mm) were observed in the reactor (Figure 4.3[A]) and the biomass showed good and stable dewaterability (CST 15.9 ± 0.49 sec) and settling properties (SVI_5 56.3 ± 1.12 mL/g-MLSS).

The use of granular sludge to facilitate post-denitrification is well reported in the literature (Bhuvanesh and Sreekrishnan, 2016). However, storage driven post-denitrification with granular biomass is yet to be comprehensively studied. In this study, the successful start-up of the granular reactor affirms that stable granules with good settling properties could also be achieved with anoxic/anaerobic cycling of biomass, which is important to facilitate both recovery of P and post-denitrification. With aerobic granules, stable granulation is reported with maintenance of a F/M ratio of 0.33 g-COD/g-MLSS.d (Tay et al., 2004). In this study however, stable granules were observed at a much lower F/M ratio of 0.14 g-COD/g-MLSS.d. This suggests

that in addition to F/M ratio, conditions of reactor operation may also influence the stability of granules. Future studies should examine F/M ratios and its impact on granular stability under various reactor operational conditions.

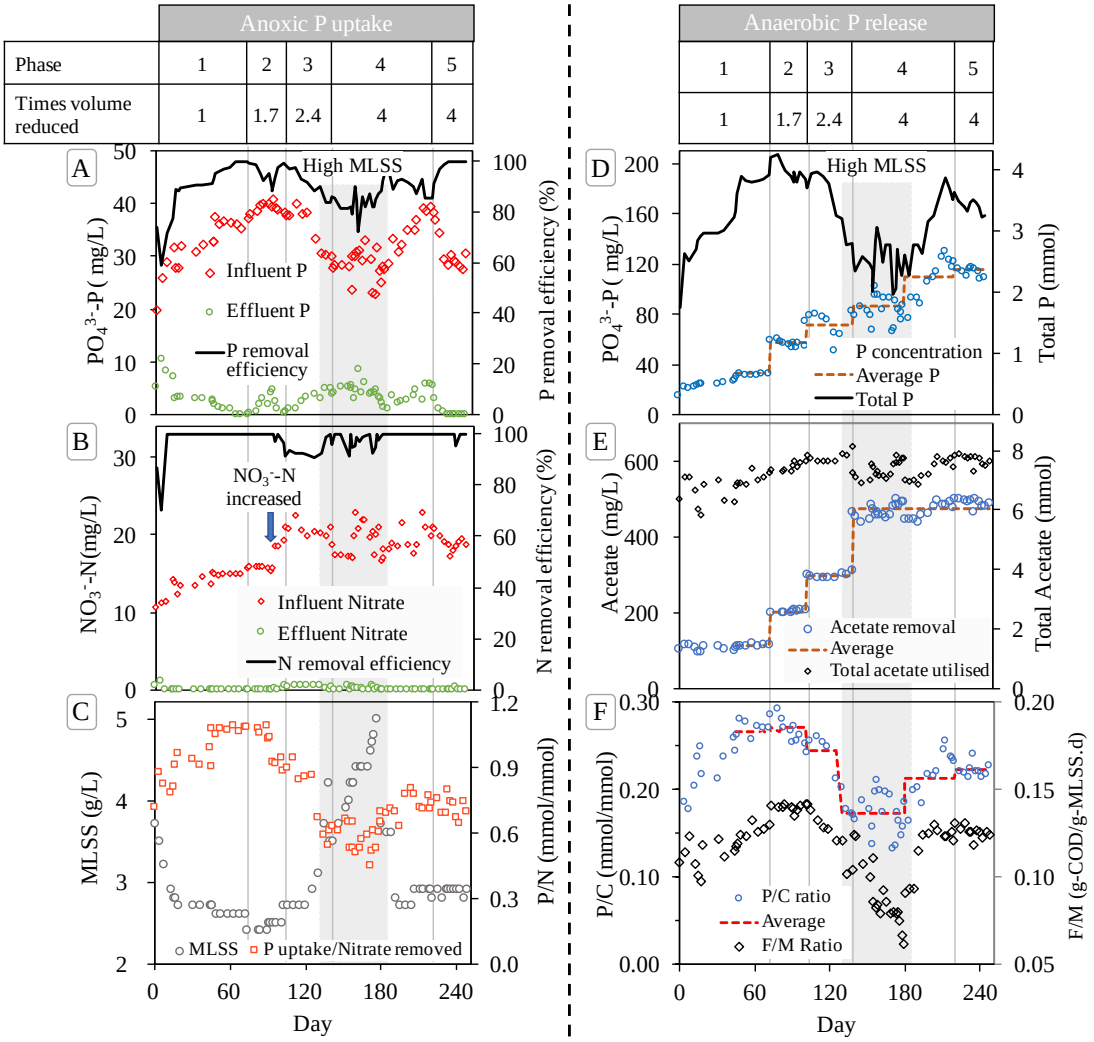

Figure 4.2. Impact of biomass wasting and volume reduction on long-term performance and stability of reactor. Implication on [A] Anoxic P removal; [B] Anoxic nitrate removal; [C] MLSS and P/N ratio; [D] Anaerobic P release; [E] Anaerobic acetate consumption; [F] P/C and F/M ratios.

When volume reduction was applied during steady operation of SBR (Phase 2 & early Phase 3), the P concentration in the anaerobic zone increased linearly (R^2 = 0.96), proportional to the volume reduction that was enforced in the anaerobic zone. With a reduction in volume of just 2.4-fold, a P concentration of 79.3 ± 3.8 mg/L was achieved with a single anaerobic release of P into the recovery stream (Figure 4.2[D]). In contrast, even with a 4-fold reduction of volume, Wong et al. (2013) only achieved a low P concentration of 28 mg/L in the recovery stream.

Interestingly, the specific P uptake rate (2.36 ± 0.15 mg-P/g-MLSS.h), P release rate (7.71 ± 0.54 mg-P/g-MLSS.h), nitrate removal rate (1.09 ± 0.11 mg-N/g-MLSS.h) and acetate utilisation rate (27.84 ± 1.18 mg-Ac/g-MLSS.h) observed during steady state of operation were comparable with the findings of Wong et al. (2013). Hence, the higher P concentration achieved with a 2.4-fold volume reduction was due to the higher biomass density that prevailed in the granular sludge. The notable difference, however was with P/C and P/N molar ratios during this stable period of operation, which were 0.26 ± 0.01 mmol-P/mmol-C and 0.99 ± 0.10 mmol-P/mmol-N, respectively. These ratios were consistent with observations reported in previous literature (Kapagiannidis et al., 2013, Lanham et al., 2011) but were noticeably different to observations made by Wong et al. (2013) (The P/C molar ratio (0.08 mmol-P/mmol-C) was approximately 3 times lower than that observed in this study). This suggests that wasteful utilisation of carbon (acetate) by GAOs in our study was relatively low, as supported also by a low relative abundance of DGAOs (Figure 4.4[C]). The high P/N molar ratio (2.90 mmol-P/mmol-N) observed by Wong et al. (2013) was likely a result of aerobic P uptake in the reactor due to the prevalence of residual oxygen in the wastewater. The 0.99 ± 0.10 mmol-P/mmol-N observed in this study, on the other hand, is comparable with Kuba et al. (1996) who observed a P/N molar ratio of 0.7 mol/mol with the use of DPAOs to facilitate P removal.

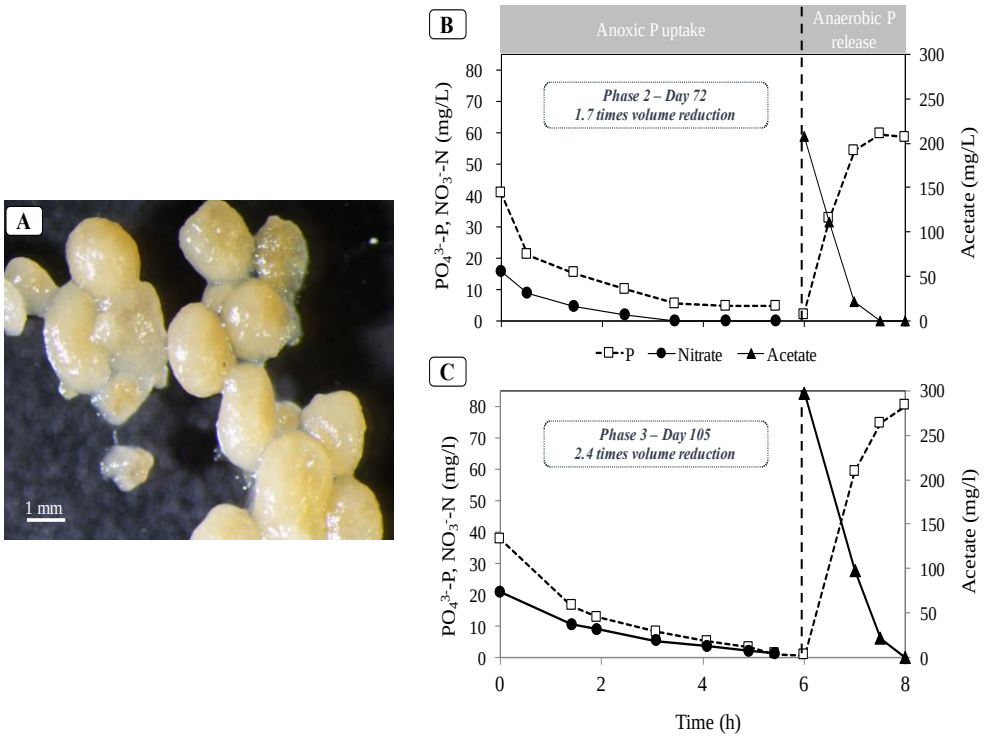

Figure 4.3. The granular biomass and its ability to effectively remove nutrients (nitrate and P) from wastewater and facilitate recovery of P. [A] Light microscopic image of the granular biomass in the reactor; [B] A cyclic study carried out on day 72 of reactor operation; [C] A cyclic study carried out on day 105 of reactor operation.

4.3.2 OPERATIONAL CHANGES IMPACTED MICROBIAL COMMUNITY STRUCTURE IN THE REACTOR

A cyclic study carried out during steady state of operation in Phase 2 (day 72) revealed that nitrate in the reactor was completely removed within 3.5 h of the anoxic period (Figure 4.3[B]). Due to non-availability of nitrate for the remainder (2.5 h) of the anoxic period, P removal was incomplete resulting in a notable residual (~ 5 mg-P/L) at the end of the anoxic period. In order to facilitate the uptake of this residual quantity of P, the influent nitrate for the anoxic period was increased by 5 mg-N/L starting from day 94 onwards. As expected, the additional supply of nitrate facilitated a complete removal of P (Figure 4.3[C]) and at the end of the anoxic period no measurable P and N were detected in the effluent discharged from the reactor.

The impact of volume reduction on both acetate and P concentration in the anaerobic period of the reactor cycle is noteworthy (Figs. 3[B] & [C]). Compared to Phase 2, there was a further (1.4-fold) reduction of anaerobic volume in Phase 3 of reactor

operation. The acetate concentration at the start of the anaerobic zone of the cyclic study reported on day 72 was 208 mg/L (Figure 4.3[B]). When considering the volume reduction, the acetate concentration at the start of the anaerobic zone of the cyclic study on day 105 (Fig 4.3[C]) should theoretically be 293 mg/L which compared well with the actual measured concentration of 297 mg/L. Similarly, P concentration at the end of the anaerobic zone of the cyclic study recorded on day 72 (Figure 4.3[B]) was 58.6 mg/L and the P concentration at the end of the anaerobic period recorded on day 105 (Figure 4.3[C]) was 80.2 mg-P/L, which was close to the theoretical value (82.7 mg-P/L) estimated based on volume reduction ratio. This suggests a linear increase of P could be achieved by merely reducing the liquid volume of the anaerobic zone. Nonetheless, according to Salehi et al. (2018) this linear increase of P would be impaired with further increase of P in the anaerobic recovery stream as a result of a diminishing specific P release activity of the microbes possibly caused by some form of a product (PO_4^{3-}) inhibition of an enzyme.

The microbial community structure during the long acclimatisation period (Phase 1) remained relatively steady (Figure 4.5). Compared to the microbial community structure of the inoculum used, the community structure that prevailed in Phase 1 appeared different (Figure 4.5). This is likely to be a result of operational conditions imposed on the lab-scale reactor. During the later stage of Phase 2 (94 d), influent nitrate was marginally increased and soon after (i.e. 101 d), a further volume reduction was also imposed to instigate Phase 3 of reactor operation. A microbial community characterisation carried out at the end of Phase 2 (101 d) revealed that yet another community shift had occurred in the reactor (Figure 4.5) and that there was a clear differentiation of microbial diversity between Phase 1 and 2. This change of microbial diversity could be in response to both increase of nitrate and volume reduction.

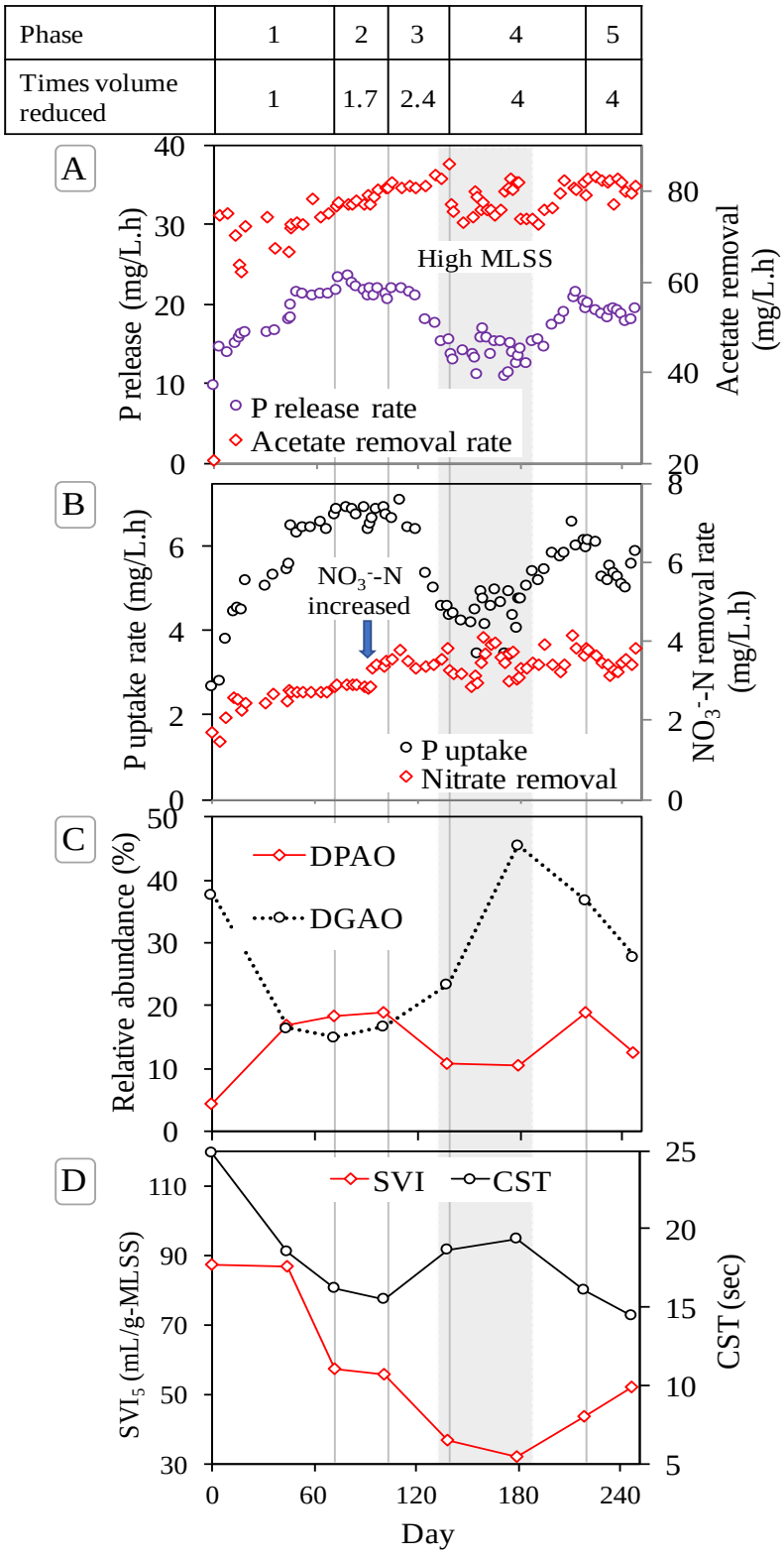

Figure 4.4. Operational changes and its influence on [A] Anaerobic P release and acetate uptake rates; [B] Anoxic P uptake and nitrate removal rates; [C] DPAO and DGAO abundance; [D] Sludge stability (reflected as sludge volume index) and dewaterability properties

4.3.3 TERMINATION OF BIOMASS WASTING NEGATIVELY IMPACTED P REMOVAL AND RELEASE

By the end of Phase 2, there was near 100 % removal of P (Figure 4.2[A]), the total P recovered was high (Figure 4.2[D]), N removal efficiency was near 100 % (Figure 4.2[B]), P/N molar ratio was 0.94 mmol/mmol (Figure 4.2[C]), P/C molar ratio was 0.27 mmol/mmol (Figure 4.2[F]) and MLSS was steady at approximately 3 g/L (Figure 4.2[C]). The P release rate (20.61 ± 1.72 mg/L.h), acetate uptake rate (81.75 ± 0.21 mg/L.h) (Figure 4.4 [A]), P uptake rate (6.33 ± 0.75 mg/L.h) and nitrate removal rate (3.47 ± 0.14 mg/L.h) (Figure 4.4[B]) were all high and stable and DPAO and DGAO abundance in the reactor remained steady at 18 % each.

The cyclic study carried out on day 105 (Figure 4.3[C]) suggested that long periods of anoxic (6h) and anaerobic conditions (2h) were required to achieve complete removal of both N & P and recovery of P, respectively. The cycle length of the SBR was 8 h but a cycle of this length is a hindrance to achieve higher nutrient loading. One strategy to overcome this hindrance is to increase the biomass concentration in the reactor. Hence, in the beginning of Phase 3, biomass wasting was terminated in the reactor to increase the MLSS concentration.

MLSS in the reactor started to increase drastically from ~ day 120 of Phase 3 and peaked in Phase 4 reaching an approximate MLSS concentration of 6 g/L in the reactor. However, this increase in MLSS did not result in the anticipated increase in the overall performance of N & P removal and P recovery. Rather, both removal and recovery of P were negatively impacted. With the increase in MLSS, P removal efficiency declined to approximately 80 % and P release declined to 37 %. N removal efficiency which was impacted with increasing nitrate (mid Phase 2), improved with the increase in MLSS, to become marginally below 100 % at the end of Phase 3. The increase in MLSS also negatively impacted P/N (0.53 mmol/mmol), P/C (0.17 mmol/mmol) and F/M (0.079 g-COD/g-MLSS.d) ratios (Figure 4.2[C] & [F]). The reduced P uptake and release rates (4.39 ± 0.51 mg/L.h and 13.90 ± 1.62 mg/L.h, respectively) (Figure 4.4[A] & [B]) indicated that the elevated MLSS was detrimental to P removal and release. Interestingly, the increase of MLSS appeared to have had minimal impact on both acetate uptake rate (77.85 ± 3.07 mg/L.h) and N removal rate

(3.39 ± 0.36 mg/L.h) when compared with the rates recorded before the increase in MLSS (81.75 ± 0.02 mg/L.h and 3.47 ± 0.14 mg/L.h, respectively).

The biomass in the SBR reactor had minimal exposure to dissolved oxygen and therefore, the final electron acceptor available was mainly nitrate. Since there was complete uptake of acetate during the anaerobic period of a reactor cycle (Fig 4.3[B] & [C]), the reduction of nitrate in the anoxic period was predominately driven by microbial storage. During Phases 1 and 2 of reactor operation, there was a decline of DGAO and an increase of DPAO population (Figure 4.4[C]): by the end of Phase 2 the DPAO and DGAO population had attained stability (at around each 18 %). Once wasting of biomass was terminated, with increase of MLSS in the reactor, there was a rapid increase of DGAOs and this coincided with a decline of DPAOs (Figure 4.4[C]). The decline of DPAO population is also evident from the observed decline of P release and uptake rates. Since the increase of DGAOs coincided with a decrease of DPAOs, no decline of N removal rate was observed during the increase of MLSS in the reactor. Even with an increase of MLSS, an increase of N removal rate in the reactor was not observed, perhaps due to a low N removal rate by the DGAOs.

The increase of MLSS also induced a major change in the microbial community structure (Figure 4.5) and this perhaps was a result of the reduced F/M ratio (Figure 4.2[F]). The reduced F/M ratio is likely to have triggered dominance of different bacterial species of DPAOs and DGAOs. An eco-physiological study carried out by Ginige et al. (2007) demonstrated how F/M ratios caused dominance of different bacterial species. During the period (Phase 3 and 4) where MLSS was above 3 g/L, the F/M ratio in the reactor ranged between 0.077 ± 0.014 and 0.0875 ± 0.011 g-COD/g-MLSS.d and during Phase 1 and 2, the F/M ratio ranged between 0.133 ± 0.007 and 0.140 ± 0.002 g-COD/g-MLSS.d. This minor change of F/M ratio might have induced this significant change in the microbial diversity in the SBR reactor.

The higher MLSS however, further enhanced the settling properties of the biomass with SVI_5 decreasing from 55.5 ml/g-MLSS at the end of Phase 2 to 32.0 ml/g-MLSS just prior to re-instating biomass wasting at mid Phase 4. Visually, the granules also appeared to be larger and these large granules were denser, contributing towards the reduced SVI_5 values that were observed. Interestingly, the dewaterability of the biomass continued to decrease (CST increased from 15.5 s to 19.4 s) with the increase

in MLSS (Figure 4.4[D]), despite a decrease of SVI_5 (from 55.5 to 32.0 ml/g-MLSS) and this perhaps was a result of an increase in loosely bound extracellular polymeric substances (LB-EPSs) in the bulk water. These observations are consistent with Zhang et al. (2016). However, the results appear contradictory with the findings of Li and Yang (2007) and Yang and Li (2009). Accordingly, there appear to be some inconsistencies between previous studies and hence, providing a satisfactory clarification to our observations based on past literature is not feasible. We therefore suggest the need for future studies to understand the reasoning behind the opposing trends of SVI_5 and dewaterability observed in this study.

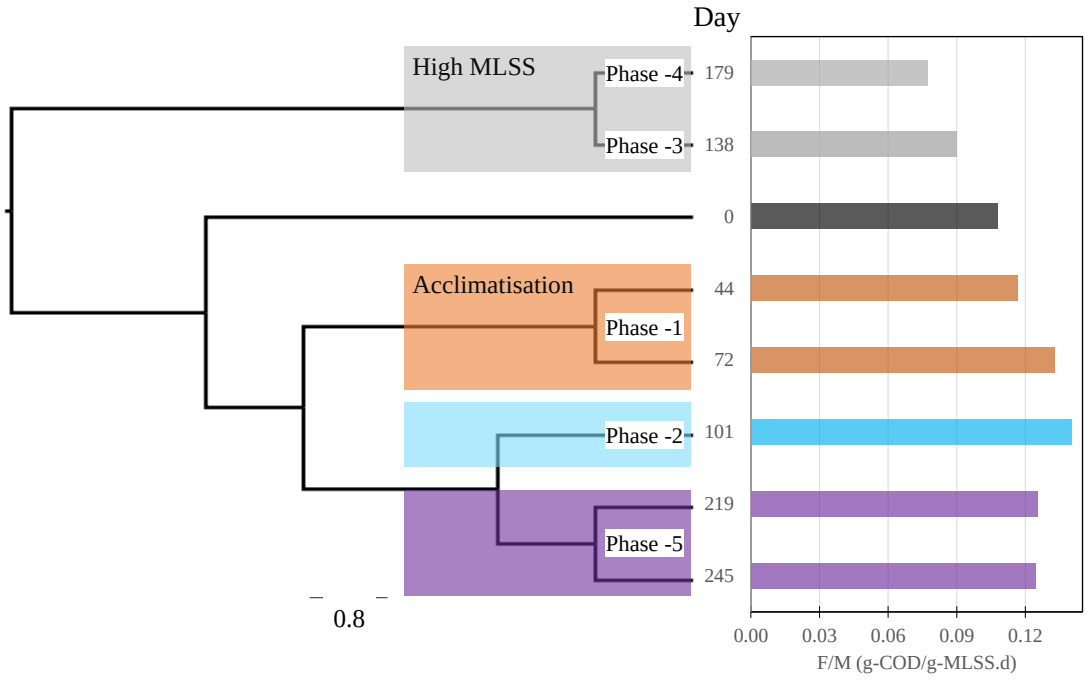

Figure 4.5. A comparison of phylogenetic diversity of each phase of reactor operation represented as a weighted UniFrac, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendogram of jackknifed phylogenetic resampling of the rarified OTU table. Branches are colour coded to represent each phase of reactor operation and are shown alongside the F/M ratio that was applied.

4.3.4 BIOMASS WASTING IS NOT NECESSARILY ONLY TO FACILITATE GRANULATION BUT TO SUSTAIN THE CORRECT MICROBIAL DIVERSITY FOR POST-DENITRIFICATION AND P RECOVERY

Due to poor P removal and release that was observed with increasing MLSS, from day 176 onwards, biomass wasting was once again reinstated to enforce a SRT of 28 d. As a consequence, the MLSS in the reactor rapidly decreased to 3.12 ± 0.10 g-MLSS/L at the end of Phase 4. The reduction of MLSS increased F/M ratio and by the end of Phase 4 (or beginning of Phase 5) it reached a value of 0.0125 ± 0.004 g-COD/g-MLSS.d (Fig 2[F]). With increasing F/M ratio, by the end of Phase 4 (or beginning of Phase 5) the bacterial diversity appeared to have reverted back close to the diversity that prevailed during Phase 2 of reactor operation (Figure 4.5). The uptake (6.07 ± 0.08 mg-P/L.h) and release (20.27 ± 0.86 mg-P/L.h) rates of P also increased and were comparable to those observed at the end of Phase 2. The N removal rate continued to remain steady and, as anticipated, the DPAO population once again increased and this coincided with a gradual decrease of DGAO population (Figure 4.4[C]). At the end of Phase 4 (or beginning of Phase 5) the P removal efficiency was also observed to be increasing (Figure 4.2[A]) and total P release reached a value similar to that observed at the end of Phase 2 (Fig 2[D]). These positive changes resulted primarily from wasting biomass, which appears to be critically important to maintain the right balance of bacteria in the reactor.

This change of performance also coincided with improved dewaterability of sludge (a decrease of CST from 19.4 to 16.1 s). The SVI_5 was observed to be increasing and was trending towards values that were observed during Phase 2 (increased from 32.0 to 43.6 ml/g-MLSS by end of Phase 4). The increase of SVI_5 suggested that biomass wasting impacted the density of granular biomass. Considering that DGAOs decreased and DPAOs increased in the reactor (Figure 4.4[C]) it is unclear whether the change of density in the granules was a result of this bacterial population change. Future studies should examine the bacterial population change and its impact on the density of granular biomass.

4.3.5 P RECOVERY IMPACTS THE POLY-P POOL OF DPAOS

In Phase 5 (i.e. starting on day 217) P recovery was implemented. The results showed that approximately 70 % of influent P was recovered as a concentrated liquor (recovery concentration 115.4 ± 5.21 mg-P/L) at the end of the anaerobic period (Figure 4.2[D]). During Phase 5, P was removed from the reactor both by wasting biomass (end of anoxic period) and recovery of P (end of anaerobic period). Recovery of P reduced the poly-P pool in the biomass as reflected by the declining total P measurement over Phase 5 of reactor operation (Figure 4.2[D]). The decline of poly-P was also indicative by the P concentration at the onset of the anoxic period. At the beginning, P in the anoxic period is composed of both the P that was released by biomass during the anaerobic period and P that was received in the influent. Due to a reduction of the internal poly-P pool (as a result of P recovery), the P released during the anaerobic period declined causing a lower anoxic P concentration (Figure 4.2[A]). However, during Phase 5, a 100 % P removal efficiency was achieved and this resulted in an effluent that contained a negligible P (< limit of detection) concentration at the end of the anoxic period (Figure 4.2[A]). The decline of the poly-P pool impacted removal rates of P & N, the release rate of P (Figure 4.4[A] & [B]), the P/N ratio and the P/C ratio (Figure 4.2[C] & [F]). During Phase 5, the DGAOs continued to decline (from 37 to 28 %) and DPAOs, which increased during the later stages of Phase 4, also declined. Hence, the decline of P release (from 20.23 ± 0.86 to 18.64 ± 0.67 mg/L.h) and uptake rates (from 6.07 ± 0.08 to 5.40 ± 0.41 mg/L.h) were attributed to a reduction of DPAOs (from 19 to 12 %). The decline of both DPAOs and DGAOs possibly impacted the N removal rate (a marginal decline from 3.73 ± 0.09 to 3.56 ± 0.18 mg/L.h) reducing the P/N molar ratio from 0.74 ± 0.03 to 0.69 ± 0.04. During Phase 5 there was a continuous improvement in the dewaterability of sludge (CST declined from 16.1 to 14.5 s). The SVI_5 also continued to increase and reached a similar value (52 ml/g-MLSS) to that observed at the end of Phase 2.

Considering that all of the changes observed during Phase 5 of reactor operation (i.e. with implementation of P recovery) were due to the depletion of the internal poly-P pool of the biomass, the daily total P loading into the reactor could be increased to counter the reduction of the poly-P pool. While it appears logical to reduce/ or stop biomass wasting to minimise depletion of internal poly-P pool, our study demonstrated the implications of not maintaining a steady F/M ratio by wasting biomass. Future

research should examine the extracellular polymeric substances and the implications of increasing daily total P loading into the granular post denitrification reactor by increasing influent P concentration and by reducing cycle length.

4.4 *IMPLICATIONS OF THIS STUDY*

This study demonstrates for the first time the potential to recover P using a granular post denitrification configuration. This study is also the first to demonstrate the long-term impact of volume reduction on the stability of anoxic granular biomass. Based on the findings, operational conditions that facilitate granulation do not always enable selection of the right microbial diversity required for a process, here the post denitrification and P recovery process. Our study also highlighted the importance of maintaining an appropriate F/M ratio for the process. In our study, maintaining an approximate F/M ratio of ~0.124 g-COD/g-MLSS.d was found to be essential to achieve good uptake and release of P. Such a F/M ratio promoted the growth of DPAOs and kept the DGAO population at a low level, enabling achievement of a good P/C ratio that maximised the use of carbon for both P and N removal. Hence, F/M ratio appears to be an important parameter that requires careful maintenance to facilitate P recovery from secondary effluent using this novel granular post denitrification configuration. This study was also the first to show that P can be recovered at concentrations above 100 mg-P/L with just a 4-fold volume reduction using a granular post denitrification configuration. The long-term study also demonstrates the robustness of the granular sludge to the mode of operation (i.e. volume reduction) that facilitated recovery of P from secondary effluent.

4.5 *CONCLUSIONS*

This study was conducted to determine the long-term implications of volume reduction on a granular post denitrification process that facilitates P recovery. The novel granular post denitrification process enabled P to be recovered at concentrations above 100 mg-P/L in a single sequencing batch cycle. According to the findings, an effective removal of P and N from wastewater and recovery of P require a steady maintenance of a F/M ratio of approximately 0.124 g-COD/g-MLSS.d and this required wasting of biomass from the reactor. Volume reduction impacted microbial diversity in the reactor but this impact was not as significant as the effect of reducing

F/M ratio. Although a low F/M ratio impacted P removal, interestingly it enhanced settling (or compaction) of granular solids. Although SVI_5 decreased with a low F/M ratio, the dewaterability of sludge declined (the CST measurement increased) suggesting a possible increase of LB-EPS in bulk water with a decrease of F/M ratio. Recovery of P reduced the poly-P pool of biomass and as a consequence the DPAO abundance decreased, impacting P removal & release rates and this also marginally impacted the N removal rate. The depletion of the poly-P pool, however, had a minimal impact on bacterial diversity in the reactor. Overall, these results increase our understanding of the granular post denitrification process proposed to facilitate P recovery from secondary effluent.

5 SIMULTANEOUS NITRIFICATION, DENITRIFICATION AND PHOSPHORUS RECOVERY (SNDPR) - AN OPPORTUNITY TO FACILITATE FULL-SCALE RECOVERY OF PHOSPHORUS FROM MUNICIPAL WASTEWATER

Extended from

SALEHI, S., CHENG, K. Y., HEITZ, A. & GINIGE, M. P. 2018. Simultaneous nitrification, denitrification and phosphorus recovery (SNDPr) - An opportunity to facilitate full-scale recovery of phosphorus from municipal wastewater. *Journal of environmental management*.

CHAPTER SUMMARY

Sewage treatment plants are a potential point source for recycling of phosphorus (P). Several technologies have been proposed to biologically recover P from wastewater. The majority of these technologies are side-stream processes and rely on an external source of carbon to facilitate P recovery. To date, no studies have demonstrated the potential to facilitate main-stream recovery of P, using carbon that is naturally present in wastewater. Simultaneous nitrification, denitrification and phosphorus removal (SNDPR) is an elegant process that can uptake influent carbon and effectively remove both nitrogen (N) and P from wastewater. SNDPR studies to date, however, have failed to facilitate a P rich liquor end-of-anaerobic-phase, that enables economies of scale to recover influent P. Therefore, this study examined the feasibility of achieving a P rich liquor (i.e. > 70 mg-P/L) with SNDPR. A synthetic influent that replicated the nutrient and carbon concentrations of municipal wastewater was used to investigate whether carbon in the influent wastewater could enable both nutrient removal and P recovery from wastewater. Our granular SNDPR process was able to facilitate a P rich liquor of approximately 100 mg-P/L end-of-anaerobic-phase. A dissolved oxygen (DO) concentration of 0.5 mg/L in a sequencing batch reactor (SBR) was found to be essential to achieve complete nutrient removal and a high P concentration at the end of the anaerobic phase. The study also demonstrated the importance of denitrifying polyphosphate accumulating organisms (DPAOs) and glycogen accumulating

organisms (DGAOs) to achieve complete removal of N from the effluent. Compared to nitrifying bacteria, the polyphosphate accumulating organisms (PAOs) had a higher affinity towards DO. This study, for the first time, showed that the mainstream recovery of P is feasible using a SNDPR process.

5.1 *INTRODUCTION*

Modern agricultural practices are highly reliant on phosphorus (P) to achieve high crop yields. P however, is a non-renewable resource and depletion of P reserves is likely in the next 50 – 100 years (Shu et al., 2006). With the aim of reducing pressures on mining, there has been considerable interest on processes for P recycling in the recent past. Municipal wastewater treatment plants are a key point source for recycling of P (Cordell et al., 2009). However, the economics of P-recovery from this source are not encouraging due to the low concentrations typically found in influent wastewater (< 10 mg-P/L) (Cieslik and Konieczka, 2017). Current P recovery techniques (e.g. as struvite) require concentrated streams containing at least 50 mg/L of P and research thus far has focused on developing strategies to concentrate P within wastewater treatment processes (Yuan et al., 2012).

All biological strategies developed to generate a concentrated stream of P from municipal wastewater, have thus far exploited the metabolic processes of polyphosphate accumulating organisms (PAOs). PAOs, are the driving force in enhanced biological phosphorus removal (EBPR) and have a unique metabolism. When exposed to aerobic or anoxic conditions, the PAOs uptake orthophosphate (PO_4^{3-}) from the surrounding environment and store it intracellularly as poly-P (Tarayre et al., 2016, Yuan et al., 2012). The P uptake takes place with a simultaneous oxidation of intracellular polyhydroxyalkanoates (PHA) (Lee et al., 2001). This results in a net removal of PO_4^{3-}-P from municipal wastewater. When PAOs with intracellular poly-P are exposed to a carbon (C) source (e.g. acetate) under anaerobic conditions, carbon reserves are replenished, utilising energy derived from hydrolysis of the stored poly-P (Kapagiannidis et al., 2013). As a consequence, PO_4^{3-}-P is released back into the environment (Chuang et al., 1996). Wong et al. (2013) strategically facilitated this second step of the EBPR process in a separate smaller volume of liquid and, based on this principle, they developed a method to achieve a concentrated P stream. With repeated use of this recovery stream in the EBPR process, the authors were able to

achieve a P concentration of up to 100 mg-P/L. Wong et al. (2013) combined this P recovery strategy with post denitrification to maximise the use of carbon, not only to promote nitrate removal but also for the recovery of P from wastewater.

The need to add external carbon to facilitate post-denitrification presents a significant operational cost to the wastewater industry. Hence, the wastewater industry is constantly examining strategies to maximise the use of naturally abundant carbon in municipal wastewater to remove both nitrogen (N) and P from wastewater. Simultaneous nitrification-denitrification and P removal (SNDPR) is an elegant process that can achieve biological nutrient removal from wastewater at a lower carbon demand (Zeng et al., 2003a). In SNDPR, N removal largely takes place via the nitrite pathway. It has been demonstrated that both denitrification and P removal take place with the aid of denitrifying PAOs (DPAOs, use nitrate or nitrite as final electron acceptors for P uptake) or denitrifying glycogen accumulating organisms (DGAOs, use nitrate or nitrite as final electron acceptors). The abundance of both the nitrite pathway (for N removal) and DPAOs (for both denitrification and P removal) in SNDPR, significantly reduces the demand for oxygen, decreasing aeration costs. This, in turn, helps to conserve naturally occurring carbon, enabling its use to successfully remove both N and P from wastewater (Zeng et al., 2003a, Wang et al., 2015).

There are many studies that have demonstrated the effectiveness of SNDPR to remove C, N, and P to very low levels (Table 5. 1). However, none have demonstrated that SNDPR could facilitate P recovery, using the carbon naturally present in wastewater. Among lab-scale studies carried out, there are only a handful of studies that have used a synthetic feed that resembled municipal wastewater in terms of C, N and P concentrations. However, these studies only achieved low P concentrations at the end of the anaerobic phase (P release). Further, the incomplete removal of N and P also raise questions on whether P recovery from municipal wastewater containing low C concentrations could actually be achieved without the need for external C. For example, even with a higher concentration of C (chemical oxygen demand (COD) 400 mg/L) in the influent, Wang et al. (2016b) only managed to achieve a P concentration of ~ 25 mg/L at the end of the anaerobic phase of their reactor cycle. Similarly, Jia et al. (2013b) only achieved a P concentration of 17 mg/L. Nonetheless, several non-SNDPR lab-scale studies have proven the feasibility of achieving higher P concentrations (~ 100 mg-P/L) using low COD (e.g. 200 mg/L) and P (10 mg/L)

concentrations in the influent (Barat et al., 2008). Since these studies ignored nitrogen removal (by using a nitrification inhibitor (allyl-N thiourea)), it still remains unclear whether low COD concentrations could facilitate simultaneous N removal and P recovery.

The aim of this study was to explore the use of a granular SNDPR process to recover influent P in a very small volume as a highly-concentrated P liquor (i.e. recovery of the approximately 10 mg-P in one litre of wastewater treatment plant influent within a volume of 100 mL, thereby giving a concentration of 100 mg-P/L, or a 10-fold concentration factor). There is already a natural release and uptake of PO_4^{3-}-P during SNDPR operation and the study aimed to optimise the SNDPR process to maximise PO_4^{3-}-P release during the anaerobic phase of the sequencing batch reactor (SBR) cycle. As previously mentioned, SNDPR studies with C, P and N ratios of a typical wastewater influent have only managed to achieve PO_4^{3-}-P concentrations of 70 mg-P/L during anaerobic P release. Higher P concentrations have only been achieved with influent C concentrations greater than 400 mg-COD/L (Jia et al., 2013b, Zeng et al., 2003a). Such high concentrations of C in the influent are not observed in typical municipal wastewater (Azizi et al., 2013). Hence, this study specifically examined the feasibility to use typical municipal wastewater carbon concentrations to achieve higher release of PO_4^{3-}-P during the anaerobic phase of reactor operation.

A laboratory-scale SBR reactor was operated for a period of 4 months under alternating anaerobic / aerobic conditions. A synthetic medium, replicating concentrations of ammonia, P and carbon typical of municipal wastewater influent was used as influent to the SBR reactor. Dissolved oxygen (DO) concentrations, mixing, volume exchange ratio, and biomass wasting was carefully managed to promote the growth of granular biomass. The performance of the reactor was closely monitored in terms of aerobic / anoxic P, N removal, anaerobic P release and microbial community changes.

5.2 *MATERIALS AND METHODS*

5.2.1 SEQUENCING BATCH REACTOR OPERATION

A laboratory-scale SBR reactor with a working volume of 4 L was operated at room temperature (20 - 22˚C) under alternating anaerobic / aerobic conditions. The reactor

was seeded with waste activated sludge (WAS, 2 L) collected from a local municipal wastewater treatment plant (Subiaco, WA, Australia). The inoculum had a mixed liquor suspended solids (MLSS) concentration of approximately 4.0 g/L. The operational cycle included a 2 h anaerobic period with 5 min of feeding (synthetic medium), 2 h of aerobic period, 20 min settling and 10 min decanting. Acetate and propionate were alternately used (bi-weekly) as the carbon source to facilitate the enrichment of PAOs (Lu et al., 2006). At the beginning of the anaerobic phase, 2.8 litres of synthetic wastewater was pumped into the reactor, enabling a volume exchange ratio of 70 %, which was considered desirable for the enrichment of the granules. The hydraulic retention time (HRT) and solid retention time (SRT) were maintained at 9 h and 20 days, respectively. The reactor was operated for a period of 110 days.

National Instrument hardware (CompactRIO) and software (Labview) were used to control, monitor and automate the operation of the reactor. Mixing was achieved at 50 rpm using an overhead stirrer (RZR2020, Heidolph, Germany). Maintenance of DO at set point was achieved using a luminescent DO probe (PDO_2; Barben Analyser Technology, USA) and the Labview software by switching on and off a solenoid valve connected to a compressed air outlet. The DO level was maintained between 0.30 and 0.8 mg/L. An intermediate junction pH sensor (Ionode IJ44, Ionode Pty Ltd, Australia) and an intermediate junction redox sensor (Ionode IJ64, Ionode Pty Ltd, Australia) were also fitted into the reactor and their outputs were recorded online. The pH in the reactor was not controlled.

5.2.2 SYNTHETIC MEDIUM

The 2.8 L synthetic feed was composed of 40 mL of solution A, 200 mL of solution B, 200 mL of solution C and 2.36 L of deionised water. The composition of these three stock solutions were as follows: Stock solution A (per L): 25.63 g CH_3COONa or 17.15 g CH_3CH_2COONa; Stock solution B (per L): 0.9 g $MgSO_4$, 3.05 g NH_4Cl, 7.5 mg Peptone, 7.5 mg Yeast extract, 142.5 mg $CaCl_2.2H_2O$, 30 mg ethylenediaminetetraacetic acid (EDTA), 4.5 mg $FeCl_3.6H_2O$, 0.36 mg $ZnSO_4.7H_2O$, 0.36 mg $MnCl_2.4H_2O$, 0.18 mg $Na_2MoO_4.2H_2O$, 0.09 mg $CuSO_4.5H_2O$, 0.45 mg $CoCl_2.6H_2O$, 0.54 mg KI, 0.45 mg H_3BO_3; Stock solution C (per L): 0.37 g KH_2PO_4, 0.65 g K_2HPO_4. The introduction of the feed at the beginning of the cycle, imposed a

PO_4^{3-}-P concentration of 10 mg/L, an NH_4-N concentration of 40 mg/L and a COD concentration of 200 mg/L in the reactor.

5.2.3 PROCESS MONITORING AND CHEMICAL ANALYSIS

Long-term term performance monitoring of the reactor was carried out with routine influent and effluent sampling and mixed liquor suspended solids (MLSS) measurements. Influent and effluent samples were immediately filtered using 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia) and stored at 4°C. The concentration of MLSS in the reactor was determined according to Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012).

The reactor performance was monitored by conducting cyclic studies. The cyclic studies facilitated the monitoring of N removal and P uptake/release kinetics of the biomass. Each cyclic study involved withdrawing 2 ml of sample from the reactor every 15–30 min over the entire 6-h cycle. Each sample was immediately filtered using a 0.22 μm pore size syringe filter (Cat. No. SLGN033NK, Merck Pty Ltd, Australia). At the end of each cyclic study the MLSS of the reactor was determined in accordance to the Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012).

The PO_4^{3-}-P, NH_4-N, NO_3^--N, NO_2^--N, Mg^{2+} and acetate concentrations in the filtered samples were determined using ion chromatography (ICS-3000, DIONEX). A Dionex ICS-3000 reagent free ion chromatography (RFIC) system equipped with an IonPac® AS18 4 x 250 mm column was used to measure acetate, nitrite and nitrate concentrations in liquid samples. Potassium hydroxide was used as an eluent at a flow rate of 1 mL/min. The potassium hydroxide concentration was 12-45 mM from 0-5 min, 45 mM from 5-8 min, 45-60 mM from 8-10 min and 60-12 mM from 10-13 min. Ammonium (NH_4^+-N) and Mg^{2+} were measured with the same RFIC but with a IonPac® CG16, CS16, 5 mm column. Methansulfonic acid was used as the eluent at a flow rate of 1 mL/min and a 30 mM concentration was maintained for 29 min. The temperature of the two columns were maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppression® recycle mode).

5.2.4 MICROBIOLOGICAL ANALYSIS

Biomass samples were taken from the reactor for microbiological analysis. Upon collection, the biomass samples were immediately stored in a -80 °C freezer. Subsequently, the samples were thawed at room temperature in preparation to extract DNA for 454 pyrosequencing. DNA extractions were carried out using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., USA) and stored at -20 °C until sequenced. DNA sequencing was carried out as previously described (Nagel et al., 2016).

In brief, the extracted DNA was quantified using a Qubit fluorometer, and 1-ng samples were amplified using the 16S ribosomal ribonucleic acid (rRNA) gene V4/5 primers (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT) (Caporaso et al., 2010). Specifically, the above gene-specific primers were used with gene-specific primers tagged with Ion Torrent-specific sequencing adaptors and barcodes. The tagged and untagged primers were mixed at a ratio of 90:10. Amplification of all samples were restricted to 18–20 cycles minimising primer-dimer formation. Amplification was confirmed by agarose gel electrophoresis, and amplified products were quantified by fluorometry. Subsequently up to 100 amplicons were diluted to equal concentrations and adjusted to a final concentration of 60 pM. Templated Ion Sphere Particles (ISP) were then generated and loaded onto sequencing chips using an Ion Chef (Thermofisher Scientific) and sequenced on a PGM semiconductor sequencer (Thermofisher Scientific) for 650 cycles using a 400 bp sequencing kit that yields a modal read length of 309 bp. Data collection and read trimming/filtering was performed using TorrentSuite 5.0.

5.2.5 BIOINFORMATICS PIPELINE

The Quantitative Insights into Microbial Ecology (QIIME) software package version 1.9.1 (Caporaso et al., 2010) was used for processing of the sequenced data. Three main files (454-machine generated FASTA file & quality score file and user generated mapping file) were used for downstream analysis in QIIME. The split_libraries.py script was used to separate reads in the FASTA file according to the mapping file. Chimeric sequence reads were thereafter identified and filtered using USEARCH61 and an unaligned reference SILVA database (Version 128) 97_otus_16S.fasta (Quast et al., 2013). Subsequently, operational taxonomic units (OTUs) were assigned at 97

% sequence similarity using the same reference database file from SILVA. Once a representative sequence was appointed for each OTU picked, a taxonomic assignment was carried out using the RDP classifier version 2.2 (Wang et al., 2007) in reference to MiDAS taxonomic database version 2.1 (McIlroy et al., 2015). The bacteria directory at MiDAS taxonomic database was further used to determine various metabolic groups.

5.3 *RESULTS AND DISCUSSION*

5.3.1 LONG-TERM PERFORMANCE OF REACTOR

Upon inoculation with activated sludge, approximately 3 months was required to develop a granular biomass that satisfactorily maintained low N, P and C (0 mg NH_4-N/L, 0.8 mg NOx-N/L, 0 mg PO_4^{3-}-P/L and 0 mg Acetate/L) concentrations in the effluent. During the first 62 days, there was an attempt to maintain a DO level ranging from 0.4 to 0.6 mg/L in the aerobic period. However, the system failed to maintain the desired DO set point as over 2/3 of the cycle, the DO fluctuated between 0 and 0.2 mg/L (Figure 5.2a). Nonetheless, this mode of operation still enabled a rapid improvement in the aerobic P uptake and anaerobic P release activity of the biomass. Specifically, a near identical linear increase of activity (approximately 2 to 20 mg PO_4^{3-}-P/g-MLSS) was recorded for both aerobic P uptake and anaerobic P release (Figure 5.1a) during the period of 0 – 62 days. During this period, nitrification and denitrification activities also showed a gradual increase. Compared to the increase of aerobic P uptake and anaerobic P release activities, the increase of nitrification and denitrification activities were approximately 6 times lower (Figure 5.1a). The MLSS only marginally fluctuated (4.18 ± 0.62 g/L) throughout the entire period of reactor operation (Figure 5.1a) and this suggests only a minor change in biomass concentration in the reactor (since suspended solids in the synthetic feed is negligible, the MLSS reflects the biomass concentration in the reactor). Hence, the increase of nutrient removal observed can be assumed to be a result of an increase in either the abundance or the enzymatic activities of specific microbial communities.

Figure 5.1d illustrates a large microbial community shift in the reactor between 7 and 34 days of operation. There was a gradual reduction in the relative abundance of the members of order Rhodocyclales, which consists of microorganisms such as *Candidatus Accumulibacter* (a well-known PAO), *Propionivibrio* (a well-known

GAO), *Thauera*, *Dechloromonas* and *Sulfuritalea* (which are known denitrifiers/aerobic heterotrophs) (Lu et al., 2006, McIlroy et al., 2016, Coyotzi Alcaraz, 2014). The relative abundance of glycogen accumulating organisms (GAOs) and other heterotrophs decreased and the relative abundance and / or activity of PAOs increased during this period (Figure 5.1e). The result also corroborated with the rapid increase of P uptake/release activity recorded during this period (Figure 5.1a).

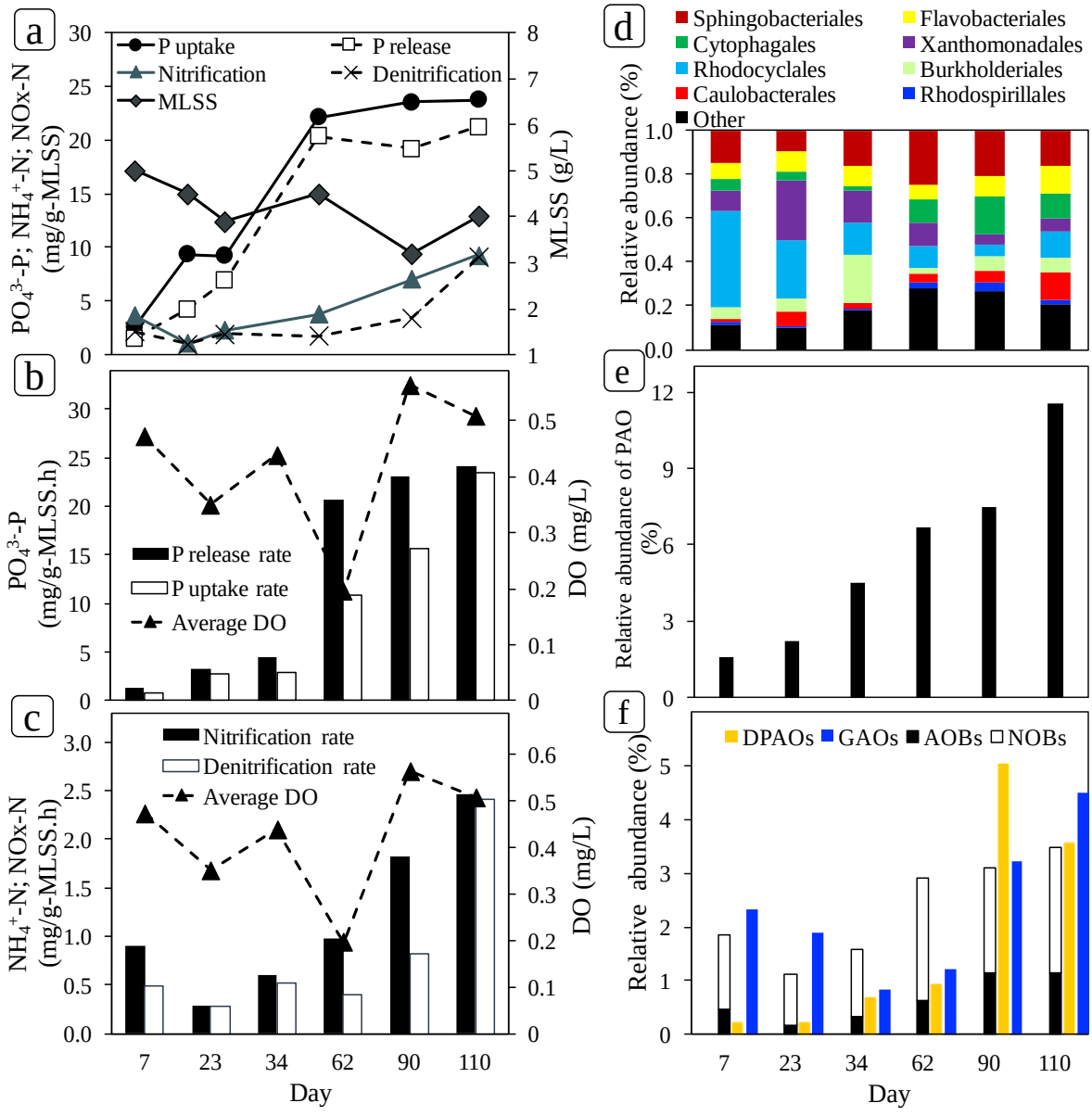

Figure 5.1. (a) P uptake/release and nitrification/denitrification activity changes in the reactor; (b) P uptake and release rates in the reactor; (c) Nitrification and denitrification rates in the reactor, (d) The abundance and shift of bacteria classified to the order level; (e) Relative abundance of PAOs; (f) Relative abundance of DPAOs, GAOs, AOBs, NOBs.

On the other hand, the decrease in the relative abundance of the members of order Rhodocyclales coincided with an increase in abundance of the members of order Burkholderiales. Bacterial genera such as *Rhodoferax* and *Acidovorax* of Burkholderiales are known for their ability to denitrify (Thomsen et al., 2007). Hence, the increase in abundance of the members of order Burkholderiales may suggest an overall increase of heterotrophic denitrifiers and there was also a gradual increase of denitrification observed between 7 and 34 days of reactor operation (Figure 5.1c). From 62 to 110 days of operation, a marginal community shift was observed at an order level and the overall bacterial community in the reactor remained stable.

Parallel to the increase of biological activity, the specific release and uptake rates of P also increased in the reactor (Figure 5.1b). However, an increasing difference was noted between specific P release and uptake rates (Figure 5.1b). The low specific P uptake rate, necessitated a prolonged exposure of biomass to aerobic conditions to enable a complete removal of P. Similarly, during the first 62 days, although nitrification and denitrification activities increased, specific nitrification and denitrification rates declined or remained analogous to day 7 (Figure 5.1c). The low specific P uptake and the nitrification rates were probably a result of the low DO concentration that prevailed during the aerobic period of the reactor. Hence, from day 62 onwards, the DO set point of the reactor was increased to maintain a DO concentration of 0.3 – 0.6 mg/L throughout the aerobic period. This resulted in a gradual increase of specific P uptake rate (Figure 5.1b). The increase of DO also enriched the PAOs (i.e. both aerobic and DPAOs) and by day 110, the overall PAO abundance reached 12 % (Figure 5.1e). The increase of PAOs and DO facilitated similar specific P release / uptake rates (i.e. 24.14 and 23.52 mg-P/g.MLSS.h respectively) and this enabled a rapid removal of P during the aerobic/anoxic period of the reactor cycle.

The increase of DO also increased the specific nitrification and denitrification rates, which were 2.41 and 2.46 mg-N/g.MLSS.h respectively by day 110 (Figure 5.1c). A marginal increase of the ammonia oxidising bacteria (AOB) and the nitrite oxidising bacteria (NOB) population was also observed once the DO concentration was increased in the reactor (Figure 5.1f). The increase of denitrification rate correlated with an increase of DPAO and DGAO abundance in the reactor, suggesting that DPAOs and/or DGAOs were responsible for the observed increase of denitrification.

An overall decrease in abundance of order Burkholderiales (a bacterial order known to contain denitrifiers (Thomsen et al., 2007)) from 22.5 to 7.25 % between days 34 and 110 (Figure 5.1d) suggest a possible decrease in abundance of heterotrophic denitrifiers. The marginal increase of abundance and/or community shift of the order Rhodocyclales (a bacterial order known to contain DPAOs and DGAOs (Zhang et al., 2018)), on the other hand coincided with an increase of DPAOs and/or DGAOs in the reactor. Overall, the increase of nitrification, denitrification and P removal resulted in a low nutrient content in the reactor effluent.

5.3.2 THE PAOS HAVE A HIGHER AFFINITY TOWARDS OXYGEN THAN AOBS

Two cyclic studies carried out on days 62 and 110 were compared to understand how an increase of DO would impact the overall performance of the reactor. The cyclic study carried out on day 62 (Figure 5.2a) revealed a poor removal of NH_4^+-N. In this cyclic study, a clear bending point is visible in the NH_4^+-N profile during the aerobic phase of the reactor cycle (Fig 2a). During the initial 2 h of the aerobic cycle, the NH_4^+-N removal rate was 0.7 mg/g-MLSS.h. After 2 hours into the aerobic cycle, P was completely up taken by the PAOs and thereafter a higher NH_4^+-N removal rate of 1.35 mg/g-MLSS.h was observed (Figure 5.2a). Although the reactor was aerated during the entire aerobic phase, there was no measurable concentration of DO during the first 30 min of reactor operation (Figure 5.2a). During this period, the removal of NH_4^+-N was insignificant when compared with the removal of PO_4^{3-}-P (Figure 5.2a). This suggests that biological ammonia oxidation (driven by AOB) was compromised during this period, and PAOs appeared to have preferentially utilised all of the supplied oxygen to uptake P. An increase of DO in the reactor was only noted once PO_4^{3-}-P concentration decreased to approximately below 40 mg/L and this coincided with an increased AOB activity as reflected by the increased removal of NH_4^+-N. This observation implies that the acclimatised PAOs in the biomass had a higher affinity towards oxygen when compared with AOBs. Blackburne et al. (2008) however, showed that AOBs have a higher affinity towards oxygen in a study that was conducted to examine whether NOBs could be washed out in a continuous-flow reactor using DO concentration as the only selection factor. Similarly, Carvalheira et al. (2014) showed that PAOs also have a higher affinity towards oxygen in a study they carried out to examine the impact of aeration on PAOs and GAOs. Although both

AOBs and PAOs are known to have higher affinities towards oxygen, to our knowledge no study has examined which of these two has the highest affinity towards oxygen. This study for the first time provides indirect evidence that PAOs have a higher affinity towards oxygen compared to AOB, although to be conclusive, the oxygen Monod half saturation constants (K_o), for both AOBs and PAOs should be determined.

Increasing the DO concentration in the reactor increased the availability of oxygen for both P uptake and nitrification to simultaneously occur from the start of the aerobic phase of the reactor cycle (Figure 5.2b). An unchanged rate of NH_4^+-N reduction before and after completion of P uptake, suggests that nitrification occurred at its maximum rate during the entire aerobic period, implying that AOBs were not limited by oxygen. While the bulk of the NOx-N produced from nitrification was simultaneously removed, a small concentration was observed accumulating in the reactor (Figure 5.2b). This small increase of NOx-N confirms that the denitrification rate was marginally lower than the nitrification rate in this reactor. Since the nitrification process was completed approximately 30 min prior to the end of the cycle, the remaining 30 min of the cycle was sufficient to completely remove NOx-N from the final effluent.

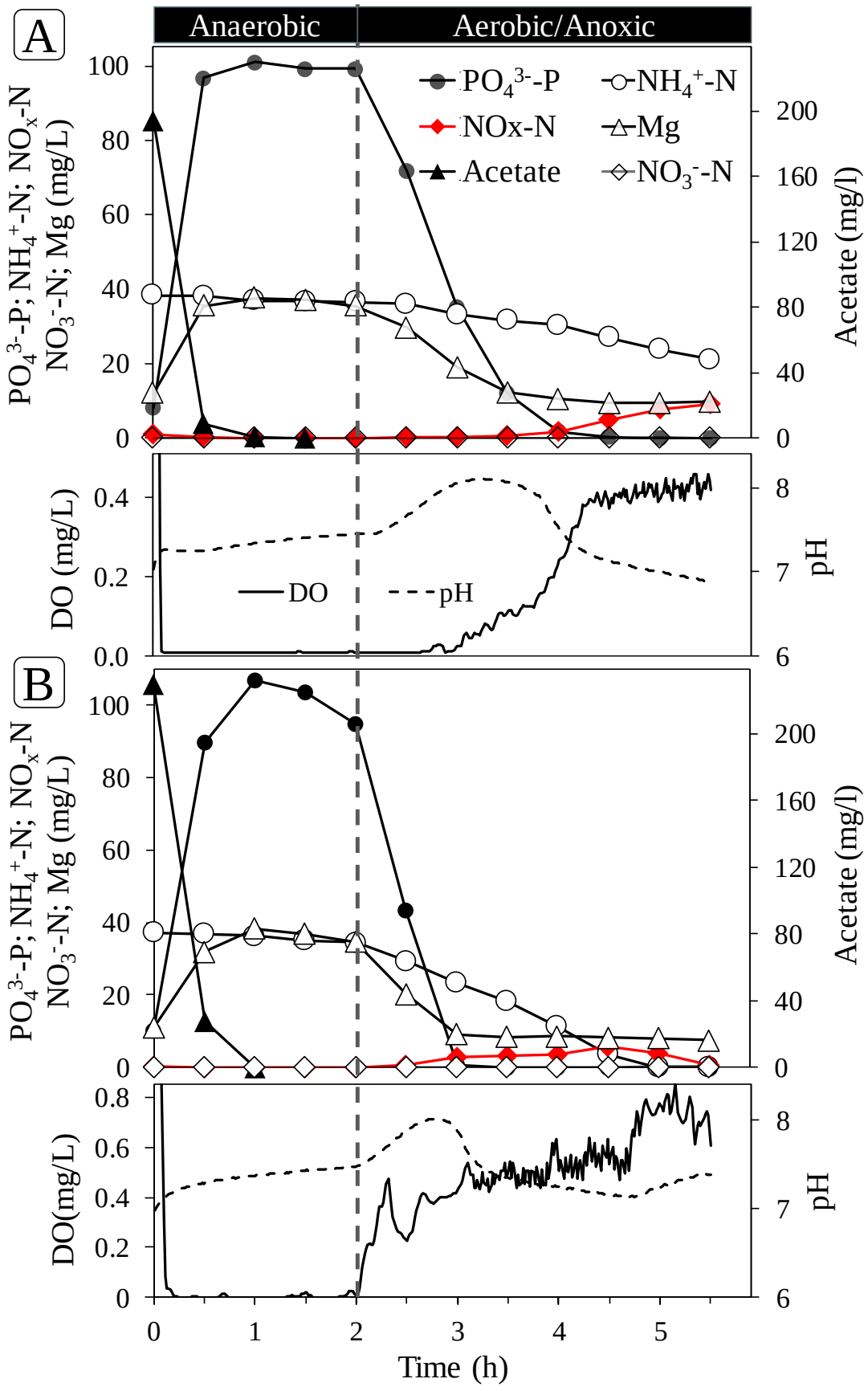

Figure 5.2. Cyclic studies carried out on (a) 62 d and (b) 110 d

Maintaining a suitable oxygen gradient within the granule is essential for the described SNDPR process to occur. In this study, a DO concentration of approximately 0.5 mg/L (during the aerobic phase) was found to be suitable to achieve a similar nitrification and a denitrification rate. The bulk water DO concentration enabled both oxygen-demanding (i.e. P uptake by PAOs and nitrification by AOB) and denitrification reactions to simultaneously take place, enabling an efficient SNDPR process. The results further revealed that an increase in DO concentration to approximately 0.5 mg/L (due to a completion of P uptake), was not detrimental for a complete removal of N. This finding is noteworthy because DO concentrations in excess of 0.5 mg/L have been demonstrated as detrimental to the SNDPR process (Meyer et al., 2005). Specifically, elevated levels of DO were thought to further oxidise nitrite (NO_2^--N) into nitrate (NO_3^--N), increasing carbon requirements to remove P and NO_x^--N from the final effluent (Meyer et al., 2005, Zeng et al., 2004).

5.3.3 A LOW C:N RATIO SIMILAR TO THAT OF MUNICIPAL WASTEWATER CAN FACILITATE A HIGH CONCENTRATION OF P RELEASE ENABLING P RECOVERY

The influent used in this study had C and N contents similar to that of a typical municipal wastewater, as characterised by a low COD:N ratio (here approximately five). To determine whether the SNDPR process had become more efficient at releasing P during the anaerobic phase, the specific P release rates were calculated (Figure 5.1b). Clearly, the rate of P release during the anaerobic phase of the process increased gradually over the entire period of the study, reaching a maximal rate of 24 mg/g.MLSS.h at day 110 (Figure 5.1b). Further, during the initial days of reactor operation, the COD: $P_{released}$ ratio was high, approximately 25 (at day 7). However, a more than 10-fold decrease of this ratio was achieved after 110 days of operation (to 2.35), signifying that the biomass became more efficient in using the influent carbon to facilitate P release (Figure 5.2a & b). Upon achieving this low COD: $P_{released}$ ratio, the PO_4^{3-}-P concentration increased to 100 mg/L at the end of the anaerobic phase of the cycle (Figure 5.2a & b). Given that the influent PO_4^{3-}-P concentration was low (10 mg/L), the ability of the described process to increase the PO_4^{3-}-P concentration by 10-fold (i.e. reaching ~100 mg P/L at end of the anaerobic phase) creates an

opportunity to recover influent PO_4^{3-}-P in a smaller volume as a concentrated PO_4^{3-}-P liquor.

To our knowledge, this is the first study to demonstrate that a high concentration of PO_4^{3-}-P (up to 100 mg-P/L) can be achieved in a SNDPR process using a wastewater influent with a low COD/N ratio of five (COD concentration of 200 mg/L and an NH_4^+-N concentration of 40 mg/L) (Table 5. 1). This is a notable finding, as earlier studies with similar low COD/N ratios have only demonstrated a low PO_4^{3-}-P release of approximately ~40 mg/L (Wang et al., 2015, Wang et al., 2016b) (Table 5. 1). Given that municipal wastewater typically contains only low concentrations of biodegradable COD, the results of this study highlight the potential of using SNDPR to promote P recovery while achieving excellent removal of nutrients and C from municipal wastewater. Nonetheless, further studies using real municipal wastewater as influent are required to validate the current findings.

5.3.4 ARE DGAOS PRIMARILY RESPONSIBLE FOR DENITRIFICATION?

In the SNDPR process, organic carbon is introduced into the reactor during the anaerobic phase of the cycle. In this study, the organic carbon (acetate or propionate) in the synthetic wastewater was fully consumed and stored by the granular biomass (Figure 5.2) in the complete absence of any electron acceptor. Hence, PAOs, DPAOs, GAOs and/or DGAOs are the likely organisms that stored the majority of the COD that prevailed in the influent.

The observed denitrification in the described process took place both during and after P uptake was completed (Fig 2). In the absence of P and a source of carbon, denitrification could only take place with the assistance of DGAOs, whereas denitrification during P uptake may have been a result of both DPAOs and DGAOs. Fig 5.3 shows a gradual increase in the denitrification rates before and after P exhaustion (i.e. in the presence and absence of P) during the acclimatisation period in the reactor. During the early operation of the reactor (i.e. 62 d), the denitrification rates observed in the presence of P were an order of magnitude higher compared to the denitrification rates observed in the absence of P (Figure 5.3). According to the cyclic study on day 62 (Figure 5.2a), the accumulation of NOx-N specifically after exhaustion of P, was a result of the low denitrification rate and/or a higher nitrification

rate. The nitrification rate, however, only increased marginally (0.2 mg/L.h) after exhaustion of P (Figure 5.2a) and hence, the accumulation of NOx-N resulted from the reduction of the denitrification rate. This suggests that in addition to DGAOs, DPAOs were also likely to be contributing towards denitrification when P was present in the reactor. The reduced denitrification rate observed in the absence of P (i.e. after all the PO_4^{3-}-P was up taken by PAOs or DPAOs) perhaps was due to the inability of DPAOs to denitrify and this also suggests DPAOs reliance on P to remain active in the reactor (Figure 5.3). During the final days of reactor operation, the denitrification rates observed in the absence of P far exceeded denitrification rates observed in the presence of P (Figure 5.3). This is a result of an increased DGAO activity and/or an increased DGAO abundance. A microbial analysis confirmed an increase of DGAO abundance from approximately 1.2 to 4.5 % between 62 and 110 days of reactor operation. Although chemical data does not suggest an increase or a decrease of DPAO activity, the microbial analysis indicated a 1.5 % reduction in the abundance of DPAOs during the final 20 days of reactor operation. This 1.5 % decline in DPAO abundance coincided with a 1.3 % increase of DGAO abundance and this overall facilitated a bacterial community that enabled complete removal of N, P and C from the influent wastewater.

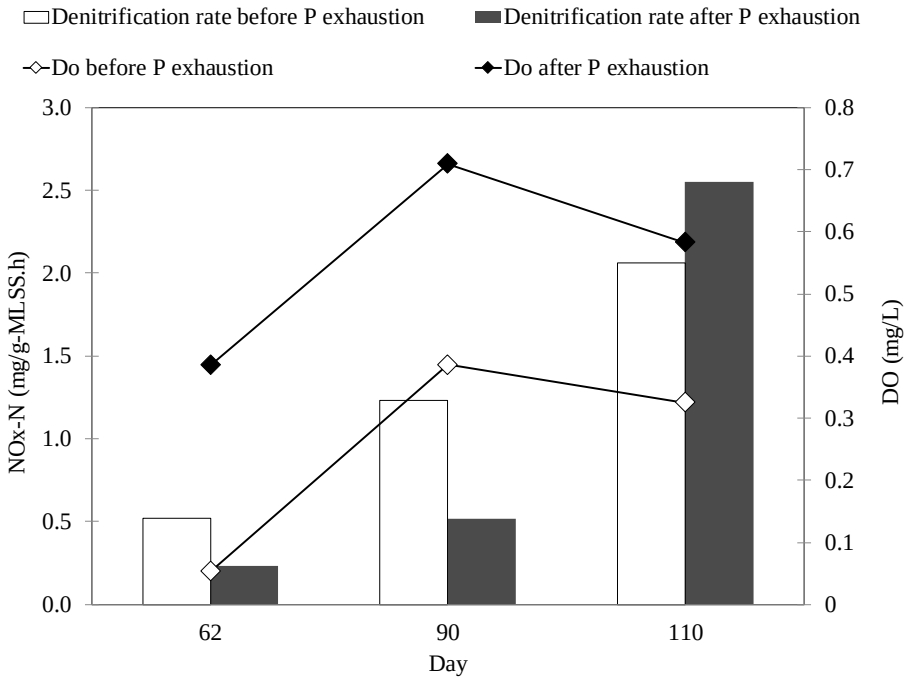

Figure 5.3. Denitrification rates in the presence and absence of P

5.4 *IMPLICATIONS OF THE FINDINGS*

The present study demonstrates that with a fine balance of key microbial communities and a careful operation of the reactor (i.e. DO management) it is feasible to achieve a PO_4^{3-}-P concentration as high as 100 mg/L at the end of the anaerobic phase of a SNDPR reactor cycle. A PO_4^{3-}-P concentration of 100 mg/L enables economies of scale to recover P specifically as struvite (Adnan et al., 2003a). Furthermore, this is the first study to demonstrate opportunities to recover P with a COD concentration that is typical of municipal wastewater. In addition to facilitating a concentrated stream of PO_4^{3-}-P for P recovery, the SNDPR successfully facilitated removal of nutrients and carbon from final effluent discharged from the reactor.

5.5 *CONCLUSIONS*

This study examined whether a SNDPR process could facilitate recovery of P as a concentrated liquor at the end of the anaerobic phase of the reactor cycle. Based on the results the following can be concluded.

- A PO_4^{3-}-P concentration as high as 100 mg/L is achievable at the end of the anaerobic phase of the reactor cycle. This concentrated stream of PO_4^{3-}-P enabled recovery of influent PO_4^{3-}-P (10 mg/L) in a very small volume as a concentrated liquor.
- A COD concentration of 200 mg/L is adequate to create a concentrated stream of PO_4^{3-}-P suitable for P recovery at the end of the anaerobic phase of the reactor cycle. The use of COD to create such a high concentrated stream of P did not hinder effective removal of NOx-N. Specifically, C was not found to be a limiting factor for denitrification.
- Maintenance of a DO concentration of below 0.5 mg/L was critical to achieve a balanced microbial community in the granules of the SNDPR process.
- PAOs appear to have a higher affinity towards oxygen than nitrifiers, and an early completion of P uptake and a similar rate of nitrification and denitrification appear to be important to achieve good nutrient removal.
- DGAOs played a major role in the SNDPR process, specifically to remove NOx-N

Table 5.1. A comparison of SNDPR studies

Research	Operational Parameters													Wastewater Characteristics				Performance			
	Working vol (L)	Cycle length (h)	Anaerobic phase	Aerobic phase	Settling time	Decant vol (L)	MLSS (g/L)	DO (mg/L)	Temperature (°C)	Carbon source	HRT (h)	SRT (day)	pH	COD (mg/L)	NH_4^+-N (mg/L)	PO_4^{3-}-P (mg/L)	COD/N	COD/P	Max P release	SND efficiency	P removal	NO_x-N in the
(Lu et al., 2016)	8	6.0	2.2	2.7	60	2.0	2.2-3.5	0.8-1.6	20	NaAc	24	8	7.5	800	50	36	16	22	75	57	100	3
(Zeng et al., 2003a)	4	4.8	1.0	3.0	43	2.0	3.3	0.5-0.6	18-22	NaAc	9.6	15	7-7.5	400	40	15	10	27	75	98	100	0
(Lemaire et al., 2006)	5	6.0	1.5	3.6	40	3.0	3.9-4.6	0.4-0.5	20-22	NaAc	10	20	7-7.5	230	23	10	10	23	110		100	0
(Jia et al., 2013a)	5	6.0	1.5	3.0	70	3.0	3.0-3.5	0.4-0.8	25	Glucose/NaAc	10	20		400	40	4.2 10 14	10	95 40 28	12 17 30	81 80 86	86 87 89	7.6 7.9 5.7
(Wang et al., 2016a)	8	6.0	1.5	3.5	45	4.0	3.2-3.5	0.4-0.7	25 10	NaAc	12	16	7-7.5	400	40	15	10	26.7	23 25	53 84	90 91	9.2 5.4
(Li et al., 2008)	4	8.0	2.0	3.0	60	1.8	3.5	0.2-0.5	21	NaAc Propionate/NaAc: 1/1 Propionate/NaAc: 2/1	16	22	7.3-8	300	35	12	9	25	68 60 58	53 63 79	81 94 97	8 6.5 3.5
(Meyer et al., 2005)	5	6.0	1.95	3.1	43	3.0		0.4-0.5		NaAc	10	15	7-7.5	136	18	11	8	13			100	3.5
(Jia et al., 2013b)	15	6.0	1.5	3.0	70	7.5	3.0-3.3	0.4-0.8	25	Glucose/NaAc	12	20	7-7.5	350	50	5	7	70	12	92	82	4
(Wang et al., 2015)	8	6.0	3.0	2.5	20	3.0	3.0	1.0		NaAc	14.6	10.9	7.2-8	254	65	6	4	42	26	49	94	10
This Study	4	6.0	2.0	4.0	20	2.8	3.0	0.5	22	NaAc			7-8	200	40	10	5	20	100	100	100	1.2

6 CONCLUSION AND FINAL COMMENTS

Overall this PhD thesis reveals novel strategies for facilitating P recovery from main and side streams of municipal wastewater. The mainstream strategy developed, focused on making use of influent carbon to recover P from wastewater. The side stream strategies focused on using external carbon to not only recover P but also to remove N from wastewater. The thesis provides new knowledge and process options to cost effectively recover P from municipal wastewater.

This chapter summarises the main findings of this thesis and discusses limitations, opportunities, and potential applications of the proposed strategies. Suggestions for future research are also provided.

6.1 *MAIN FINDINGS OF THE STUDY*

In this thesis, four experimental chapters examined three different strategies to recover influent P as a concentrated P liquor. Since influent P is low in concentration (8 – 10 mg-P/L), for P recovery to be economically viable, the influent P needs to be captured as a concentrated liquor (~ 100 mg-P/L). All strategies reported in this thesis, directly or indirectly relied on polyphosphate accumulating organisms (PAOs) to generate the concentrated P streams. Extending the learnings of Wong et al. (2013), the creation of a higher volume exchange ratio (i.e. a higher volumetric ratio between the wastewater and the recovery stream) was considered essential to create a recovery stream that is highly concentrated in P. A close examination of main stream configurations revealed a natural occurrence of such a higher volumetric ratio in the return activated sludge (RAS) line of conventional plug flow activated sludge plants.

6.1.1 PHOSTRIP TECHNOLOGY CAN BE REENGINEERED TO FACILITATE P RECOVERY FROM RAS LINES OF NON-EBPR PLANTS

Phostrip, a technology established in the 1970s for removing (stripping off) P from the RAS line, was explored for the first time to facilitate P recovery from treatment plants that are not engineered for enhanced biological phosphorus removal (EBPR) (Chapter 2). Although Phostrip was invented before EBPR, at present Phostrip is rarely used in full-scale treatment plants. The rationale of Chapter 2 is worthwhile because not only

removal of P from wastewater could be achieved, but also recovery of P as a concentrated liquor. Chapter 2 also demonstrated that increasing of P concentration in the recovery stream decreased the P release ability of RAS, suggesting a possible product inhibition caused by the P concentration in the recovery stream. Irrespective of this inhibition, the study revealed a strong positive linear correlation between the amount of acetate up taken and the amount of released P. This finding confirmed a minimum wastage and a cost-effective utilisation of the acetate to recover P from municipal wastewater.

Chapter 2 further examined the P release kinetics of RAS to understand the aforementioned product inhibition on P release. The kinetic data had a good fit to a modified Michaelis-Menton model and using this model it was possible to derive important parameters such as the maximum specific P release rate (V_{max}, which was found to be 17.94 mg-P/g-MLSS.h) and the PO_4^{3-}-P concentration at which V_{max} is halved (K_i, which was found to be 83.5 mg/L). This information facilitated a design of a novel side stream Phostrip P recovery process. Interestingly, the study revealed that recovering P using this strategy is more economical than using a chemical precipitant such as alum to remove P from wastewater. This method of P recovery is also postulated to enhance nitrogen removal enabling treatment plants to capitalise from external carbon addition to remove both N and P from municipal wastewater.

6.1.2 GRANULAR BIOMASS ENABLED A HIGH VOLUMETRIC REDUCTION FACILITATING P RECOVERY AT HIGH CONCENTRATION

Achieving a wastewater/recovery stream volumetric ratio higher than that achieved with RAS is difficult using activated sludge systems. This is largely a result of a poor biomass compaction observed with activated sludge. A significant compaction of biomass on the other hand is well known with granular biomass. However, facilitating a wastewater/recovery stream volumetric ratio of 5 with activated sludge or with biofilm media (e.g. with Kaldnes media used by Wong et al. (2013)) is practically impossible. Hence, Chapter 3 examined opportunities to create a highly concentrated P recovery stream by increasing the wastewater/recovery stream volumetric ratio using granular biomass. Without volume reduction, the P concentration during anaerobic P release was 100 mg-P/L, which is within a range typically observed for

EBPR. Upon the implementation of a wastewater/recovery stream volumetric ratio of 5, a P concentration of 457 mg-P/L was achieved in the recovery stream. Such a high concentration is extremely promising from a P recovery perspective, and it was largely attributed to the superior compaction properties of granular biomass enabling the creation of a high (here 5-fold) wastewater/recovery stream volumetric ratio.

6.1.3 A POSSIBILITY TO SELECTIVELY RETAIN SPECIFIC GRANULAR BIOMASS FRACTION FOR PROCESS OPTIMISATION

Since not all the enriched granular biomass were of similar density and size, the sedimentation properties of the granules were expected to be varied. Therefore, the P release efficiencies of different granular biomass fractions, which were collected based on the rate of sedimentation were also examined in Chapter 3. The study revealed that all granules were not equally capable of releasing P into the recovery stream. The granules with the highest rate of sedimentation failed to show a higher release of P and it was the granules with a medium rate of sedimentation (Fraction 3) that showed the highest release of P. Accordingly, there is a necessity to selectively retain these granules that have a higher ability to release P. Selectively retaining such granules not only would enable achievement of higher P concentrations in the recovery stream it also will reduce the contact time required with the recovery stream. A strategy to selectively retain such granules (by strategically wasting the other granules), however was not examined in this thesis and should be a focus of future research.

6.1.4 A NOVEL P RECOVERY POST-DENITRIFICATION PROCESS BASED ON GRANULAR BIOMASS WITH PROMISING STABILITY AND LONGEVITY

Application of granular biomass for carbon and nutrient removal from wastewater has been well reported. Granular biomass has also been used to facilitate post denitrification. A post denitrifying granular biomass, however, is yet to be utilised to recover P from wastewater, specifically using volume reduction as a strategy to create a P-enriched recovery stream. The long-term performance of a post denitrifying granular biomass reactor to remove N and P from wastewater and to recover the removed P as a concentrated liquor by applying volume reduction was examined in

Chapter 4. This study is of significance for two reasons. It is the first to demonstrate the potential of a granular post-denitrification configuration to recover P from secondary effluent. It is also the first to examine the long-term impact of volume reduction on the stability of anoxic granular biomass. The study revealed that operational conditions that facilitate granulation do not always enable selection of the right microbial diversity required for post denitrification and P recovery. It also highlighted the importance of maintaining an approximate F/M ratio of ~0.124 g-COD/g-MLSS to achieve good uptake and release of P.

Unlike the post denitrification configuration of Wong et al. (2013), the granular post denitrification configuration reported in Chapter 4 was able to facilitate a P concentration of 100 mg-P/L in the recovery stream with a single cycle of anaerobic P release. Achieving such a high concentration was feasible because of the higher secondary effluent/recovery stream volumetric ratio (4 times) and application of such a higher ratio was possible because of the anoxic granular biomass. Overall the thesis comprehensively demonstrates the beneficial aspects of using granular biomass to recover P from wastewater as a concentrated liquor.

6.1.5 SIMULTANEOUS NITRIFICATION DENITRIFICATION AND PHOSPHORUS REMOVAL (SNDPR) FOR MAINSTREAM P RECOVERY WITHOUT THE NEED TO DOSE EXTERNAL CARBON

Studies in Chapters 2, 3 and 4 of this thesis relied on an external source of carbon to facilitate recovery of P from municipal wastewater. In the study in Chapter 4 however, it was shown how the use of carbon by removing N in addition to recovering P could be maximised. The newly proposed Phostrip strategy (reported in Chapter 2), which requires an external source of carbon is also anticipated to enhance N removal, increasing the value proposition of the proposed P recovery technology. Municipal wastewater, however, has only a limited but a sufficient quantity of soluble carbon to remove/recover N/P respectively from wastewater. To date, no studies have examined mainstream recovery of P utilising this influent carbon. Hence, in Chapter 5 the mainstream recovery of P utilising a simultaneous nitrification denitrification and phosphorus removal (SNDPR) process was examined. SNDPR is a well-established main stream process and one of the beneficial features of this process is that it is able

to prevent wasteful oxidation of the naturally occurring carbon in wastewater. Hence, an SNDPR process is highly effective at facilitating removal of nutrients (N & P) from wastewater.

The prospective use of SNDPR to facilitate P recovery, however, is not evident from past literature. Hence, in Chapter 5 operational parameters that would enable SNDPR to facilitate mainstream P recovery were specifically examined. The study demonstrated the implications of dissolved oxygen (DO) concentration to facilitate creation of a high P concentration end of an anaerobic period of a reactor cycle, used to recover influent P as a concentrated liquor (e.g. 100 mg-P/L). The study emphasised the need to maintain a balance of key microorganisms in the granular biomass, perhaps with a careful control of DO in the reactor. Utilising DO (0.5 mg/L) as a strategy and a low influent chemical oxygen demand (COD) of 200 mg/L, this study for the first time demonstrated that mainstream P recovery is feasible by using only the influent carbon.

6.2 *KEY IMPLICATION OF THE THESIS*

In summary, this thesis provides several new lines of research and reveals P recovery strategies for both main and side stream. A summary of all strategies that are reported in this thesis (Chapters 2 to 5) is provided in Table 6.1. As shown, the $P_{release}/C_{uptake}$ ratio in all granular biomass reactors were approximately 0.3 and interestingly this ratio was found to be much higher (0.5) in activated sludge obtained from a non EBPR full-scale plant. While this finding is intriguing, this observation confirms the prevalence of PAOs in non-EBPR plants. The higher wastewater/recovery stream volumetric ratios and the higher P concentrations that could be achieved with granular biomass are also evident and this finding further increases the value proposition of granular biomass to treat municipal wastewater. When comparing the average P release rates, there appear to be phenotypic differences among bacteria in aerobic and anoxic granules. The phenotypic differences among aerobic and anoxic granules were not examined in this thesis and should be a focus of future research. In addition to phenotypic differences there also could be other factors such the energy gain (which is dependent on electron acceptor), that could be contributory towards a low P release rate in anoxic granules. A deeper understanding of all factors that is contributory towards a reduced P release

rate could potentially assist the development of new strategies and configurations to overcome these limitations.

Table 6.1. A comparison between different strategies reported in this thesis

Study	Process	Sludge	Operational mode	MLSS concentration in the recovery stream	Volume reduction ratio	P concentration in the recovery stream	P release rate	P/C
				mg/L		mg/L	mg/L.MLSS.h	mol/mol
Chapter 2	Phostrip	Activated sludge	-	9.2	2.6	100	14.34	0.52
Chapter 3	Aerobic granules	Granular sludge	Anaerobic/ Aerobic	20	5	500	17.71	0.31
Chapter 4	Post-denitrification	Granular sludge	Anaerobic/ Anoxic	7.2	4	115	5.29	0.25
Chapter 5	SNDPR	Granular sludge	Anaerobic/ Aerobic low DO	4	1	100	20.72	0.35

6.3 *SUGGESTIONS FOR FUTURE RESEARCH*

This thesis demonstrated in lab-scale both main and side stream technologies to biologically recover P from municipal wastewater. Opportunities to recover P using a novel Phostrip configuration using a non-EBPR sludge were demonstrated in Chapter 2. Although results in Chapter 2 are convincing, there is a necessity to further validate these findings using activated sludge of other non-EBPR plants. In Chapter 2 it was also hypothesized that there would be an enhancement of N removal and P recovery with the introduction of the Phostrip P recovery process to a non-EBPR plant. This hypothesis need further validation with systematic lab-, pilot- and full-scale studies. The new Phostrip configuration proposed also need validation. Further, separation of the concentrated P liquor from the RAS after the Phostrip process is challenging because RAS has already been dewatered after secondary sedimentation. Hence, to

effectively harvest the P-enriched liquid from the Phostrip tank, methods other than gravity sedimentation need to be developed. Accordingly, hydrocyclones and centrifugation could be considered in future studies. It is also important to note that there are capital and operational costs associated with each of these methods. Thus, the selection of a suitable method requires a proper cost benefit analysis.

This thesis clearly demonstrates the benefit of using a granular post denitrification configuration to remove N and recover P from secondary effluent. The thesis proposes and successfully demonstrated the application of volume exchange to create a concentrated P recovery stream. The long-term implication of operating such a granular post denitrification reactor with volume reduction was also successfully demonstrated in this thesis. Nevertheless, the implications were only assessed in terms of reactor performance, and long-term implications of volume reduction on granular structure and the microbial diversity were not assessed. Hence, this should be a focus in a future study.

P recovery using granular post denitrification and SNDPR was examined using synthetic wastewater. However, it was extremely difficult to replicate municipal wastewater in lab-scale using synthetic chemicals. Hence, validating the lab-scale findings of Chapters 3 to 5 with real municipal wastewater is highly desirable and should be considered in future studies.

Finally, a techno-economic analysis for all of the new technologies proposed in this thesis is paramount prior to full-scale application of these technologies.

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