DEVELOPMENT OF LOW ENERGY AERATION SYSTEM FOR ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL (EBPR)

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Abstract

In a world that is witnessing an everlasting growth and accelerating increase in its population, an increase in the amount of wastewater produced is inevitable. In order to recycle this wastewater back to the environment, all nutrients should be removed. Unfortunately, removing the nutrients from wastewater is expensive due to the oxygen and chemicals requirement.

Phosphorus removal is an important part of wastewater treatment process; Enhanced Biological Phosphorus Removal (EBPR) is one of the main processes responsible for phosphorus removal in wastewater treatment plants. EBPR consist of two major phases: anaerobic phase and aerobic phase. Aeration costs in the aerobic phase are relatively high in EBPR system. Finding a new approach for decreasing the amount of aeration needed for EBPR systems recently has grown in importance. Most of the research done on EBPR process was focusing on continued aeration, the effect of intermittent aeration is not widely researched. Thus, this research aims to overcome the previously mentioned challenges towards achieving stable EBPR process through different optimization techniques. To achieve this goal, a new aeration strategy has been developed to stepwise decrease the dissolved oxygen (DO) to reach very low DO conditions for EBPR. The new strategy depends on using intermittent aeration as a method of providing DO to the system. The SBR was operated over the span of 140 days under very low DO concentrations ranged from 0.5-1.0 mg/L, and achieved stable nutrients removal with removal efficiencies of: phosphorus removal efficiency (99%), ammonia removal efficiency (99%), COD removal Efficiency (100%).

In addition, the effect of acetate to propionate ratio as a carbon source for EBPR systems under low DO concentrations have been studied, to investigate the effect of carbon source on the competition between Glycogen Accumulating Organism (GAO) and Polyphosphate Accumulating Organism (PAO) in EBPR systems. Propionate was found to be the best carbon source for EBPR process, after different compositions of COD were used as a carbon source for the EBPR process. The combination of low DO concentrations and propionate as a carbon source has been found to be a successful approach in controlling the competition between GAO and PAO in EBPR systems.

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Finally I would like to dedicate this work to my family back home especially my grandfather Abdel atty Serry, Father, Mother, and dear two brothers, Marwan and Moataz, for their constant support and Love.

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Acronyms

ANAMMOX	Anaerobic Ammonium-Oxidizing Bacteria
AOB	Ammonium-Oxidizing Bacteria
BNR	Biological Nitrogen Removal
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tanks Reactors
DO	Dissolved Oxygen
DPAO	Denitrifying Polyphosphate Accumulating organism
EBPR	Enhanced Biological Phosphorus Removal
FBBR	Fluidized Bed Bioreactor
FISH	Fluorescence in situ hybridization
FNA	Free Nitrous Acid
GAO	Glycogen Accumulating Organism
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor

MBR	Membrane bioreactor
NLR	Nitrogen Loading Rate
NOB	Nitrite-oxidizing Bacteria
P	Phosphorus
PAO	Polyphosphate Accumulating organism
SBNR	Shortcut Biological Nitrogen Removal
SBR	Sequential Batch Reactors
SHARON	Single reactor for high activity ammonia removal over nitrite
SRT	Solids Retention Time
WWTPs	Wastewater treatment plants

Chapter 1
Introduction

1.1 Background

Wastewater disposing is a great threat to our fresh water sources especially that the amount of fresh water to salt water is fixed and not increasing. The presence of nutrients in the effluents of wastewater treatment plants has been a major problem to the environment ecosystem. The presence of nutrients in wastewater is usually attributed to human activities, such as: industry, agriculture, and domestic use, these nutrients are held responsible for the increase of the levels of eutrophication in the world water bodies such as rivers, lakes and seas (de-Bashan and Bashan 2004).

Biological nutrients removal was introduced as a cheap and reliable way of removing both nutrients, one of the earliest description of an nutrients removal activated sludge process was a three stages sludge treatment system for the removal of the carbon sources in the waste water (Burdick et al. 1982).

Nutrients such as nitrogen and phosphorus are the main source of nourishment for wild life. The increase of the amounts of phosphorus and nitrogen leads to an increase in eutrophication. Eutrophication stems from the Greek word “Eutrophos” which means “well nourished” and it describes the biological reactions of aquatic systems to nutrient enrichment (Michigan Department of Environmental Quality and Operator Training and Certification Unit 2004).

The level of eutrophication in lakes, nitrogen and phosphorus levels should be controlled. Phosphorus can be considered as the main contributor to eutrophication since it plays a major role in the growth of algae and most of photosynthesis organisms (Mainstone and Parr 2002; Oehmen et al. 2007a; Seviour et al. 2003). Recently, Enhanced Biological Phosphorus Removal (EBPR) has been broadly adopted due to the increase in regulations on the amount of phosphorus allowed to be discharged to the environment. EBPR is more economical and environmental friendly compared to physical and chemical phosphorus removal due to the addition of large amount of metal salts and energy requirements for the chemical and physical processes, respectively (Seguí et al. 2017).

1.2 Problem Statement

EBPR process requires two subsequent biological reaction phases: anaerobic and aerobic phases. During the aerobic phases, dissolve oxygen (DO) is required to activate the second stage of the biological reaction and use the produce energy from the anaerobic phase to remove the phosphorus from wastewater. This amount of aeration increases the expenses of the process. Therefore, reducing the DO requirement for EBPR is important to decrease the running costs of WWTP (Pan et al. 2015).

Decreasing the DO concentration for EBPR process has been suggested as an effective way of improving EBPR operation (Beun et al. 2001, de Kreuk et al. 2005). However, determining the lowest DO parameter that can achieve successful phosphorus removal with no setback in performance remains a challenge hindering the process performance. Successful intermittent aeration can be a key solution for overcoming the aforementioned challenge and reduce energy consumption and the total running costs by almost 50 % (Lochmatter et al. 2013) (Guadie et al. 2014).

However, the effect of intermittent aeration on the EBPR process is not extensively researched, as continuous aeration is the most common aeration strategy in EBPR systems (Maltais-Landry et al. 2009). Most of the studies investigating intermittent aeration were performed on subsurface wastewater or fluidized bioreactors, no deep investigation was performed on the application of intermittent aeration on SBR systems (Wang et al. 2015, Pan et al. 2015, Guadie et al. 2014,Lochmatter et al. 2013).

One of the main problems facing the adoption of intermittent aeration in EBPR systems in the Waste Water Treatment Plants (WWTP), is the re-release of phosphorus under the reintroduced anaerobic condition. PAO releases phosphorus under anaerobic conditions and then retake phosphorus under aerobic conditions (Mino et al. 1998). While intermittent aeration is formed of subsequent and consecutive pulses of on and off aeration slots, the slots of no aeration usually triggers the anaerobic phosphorus release phenomena of the PAO. This leads to re-release of the phosphorus back into the system. Hence, the goal of this study is to optimize the intermittent aeration process to be able to suppress the release of the phosphorus into the system under anaerobic conditions using different patterns of intermittent aeration and its effect on the EBPR process.

1.3 Objectives

In this research, the development of Full EBPR process were undertaken. The specific objectives of this research are:

- Developing a successful SBR system for EBPR process with high phosphorus, COD, and ammonia removal efficiency.
- Overcoming the reported problem of destruction of stable phosphorus removal at low DO concentrations.
- Developing new aeration strategies while maintaining DO concentrations at the required range while avoiding any biomass decay.

- Evaluating the factors affecting stable Enhanced Biological Phosphorus Removal performance.
- Identifying the most influential COD source for EBPR process.

  Evaluating the effect of anoxic conditions on PAO activity.

1.4 Thesis Layout

This thesis comprises five chapters.

Chapter 1: Introduction, Chapter 2 a comprehensive literature review including the compounds and elements found in wastewater, characteristics of the EBPR process, PAO microbiology, and different phosphorus removal techniques.

In Chapter 3, the detailed description of the materials and methodology of the experimental SBR used to achieve high efficiency complete EBPR process under different aeration strategies. Additionally, the SBR performance results are presented and the different parameters affecting EBPR performance are evaluated.

Chapter 4 focuses on the optimum COD source for EBPR system, the detailed description of the materials, methodology, and SBR performance results are presented and discussed

Finally, chapter 5 compiles the major findings of this study.

1.5 Thesis Contribution

This study provides an insight into enhance biological phosphorus removal optimization techniques, which will help to reduce the oxygen and organic carbon sources for EBPR systems. This study aimed at reaching a stable biological phosphorus using DO limitation conditions and optimized aeration strategies. A new technique for aeration strategy has been developed in a SBR to decrease the aeration requirements for the EBPR process.

The new aeration strategy utilizes intermittent aeration as the aeration method for the whole operation of the aerobic period. Moreover, an aeration strategy depending on a stepwise decrease in DO concentrations has been applied to allow the biomass to adapt gradually to each DO concentration and enhance the performance. Furthermore, different compositions of COD sources have been tested to figure the optimum COD source for EBPR system.

Chapter 2

Literature Review

2.1 Introduction

Biological nutrients removal process (BNR) is a term used to define a biological process used to treat wastewater from nutrients such as nitrogen and phosphorus. The presence of such nutrients in wastewater effluents especially phosphorus can have a strong impact on environment, due to recent increase globally in the levels of eutrophication, nutrients removal has gained increased attention to reverse the negative effect of excess releasing of nutrients to the environment, nutrients can be removed chemically, physically, and biologically. Our main focus will be on the biological process as it is the best treatment process in terms of economy, environmental benefits, and simplicity.

Moreover, different bioreactors technologies has been used to perform EBPR process in wastewater treatment plants, every process had certain advantages and disadvantages. These systems are discussed and illustrated in this Literature review.

Thus, this literature review aims to deeply discuss the background of wastewater treatment process specifically focusing on biological treatment.

2.2 Phosphorus

Phosphorus is a major contributor in agriculture and industrial sectors. Although phosphorus is an important element in nature life cycle, however, the excessive release of phosphorus to surface water bodies causes tremendous problems to the echo system. Phosphorus is one the main causes of eutrophication in surface water bodies, most of recent studies indicates that phosphorus could be the main source of eutrophication not the nitrogen as previously thought (Zou et al. 2014), phosphorus is one of the main contributing factors for the growth of harmful algae and different photosynthetic microorganisms such as toxic cyanobacteria (blue-green algae) (Oehmen et al. 2007a).

Phosphorus can be found in nature in different forms such as:

Organic phosphorus: a soluble or a particulate form of complex organic compounds that decomposes to Ortho-P.

Polyphosphate (condensed phosphate): soluble chained molecules that is a result of home, industrial detergents potable water treatment, also decomposes to Ortho-P.

Orthophosphate: Simple soluble Phosphate (PO_4), household cleaning agents, industrial cleaners; phosphoric acid, conversion of organic and poly phosphate are the main sources for the Orthophosphate.

2.2.1 Phosphorus removal

Phosphorus removal from waste water can be achieved through applying: chemical, physical and biological treatment methods. A combination between two or three methods can be applied as illustrated in Figure 2-1.

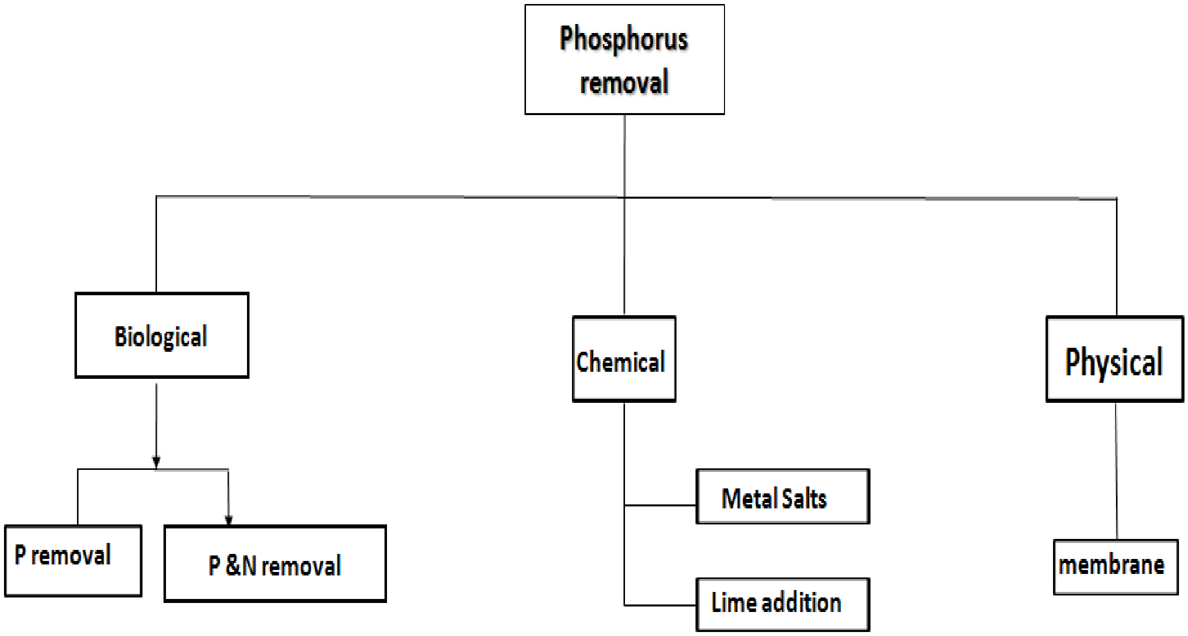


Figure 2-1: Phosphorus removal pass way

2.2.2 Chemical phosphorus removal

Chemical phosphorus removal has been one of the major treatment methods used worldwide for phosphorus removal in wastewater treatment plants, chemical phosphorus removal can be achieved by the addition of chemicals such as: ferric ion salts, ferrous iron salts and lime. The chemicals added helps the phosphorus to coagulate and then precipitate at the bottom of the reactor, to eventually be removed among the sludge (Metcalf and Eddy 2003). Chemical phosphorus removal can only achieve 80-90 % of phosphorus removal (Fytianos et al. 1998). with new restriction on effluent phosphorus discharge of 1 mg/L, a secondary filtration is needed to decrease the effluent concentration discharged to water bodies (Fytianos et al. 1998). The chemical reaction between phosphorus and metals is a very long and complex reaction which needs extended time and enormous amount of chemicals. The high amount of chemicals needed for the process makes the chemical phosphorus removal a very expensive process. Nonetheless, one of the major concerns for the chemical treatment method is the increased amount of sludge formed from lime addition (Deng et al. 2017).

2.2.3 Crystallization technologies

Crystallization technology was first introduced in 1970 in Netherlands (Metcalf and Eddy 2003), in crystallization technique caustic soda or milk lime is added under certain conditions to enhance crystals formation, calcium phosphate is crystallized in to grain sized particles. To avoid any setbacks in performance grains are regularly removed from the system for better fluidizing characteristics, since crystallization technology is mostly used in fluidized reactors systems, this technique requires highly skilled WWTP operators and regular monitoring of the system. One of the main advantages of crystallization is that it can be fitted in most waste treatment systems with no increase in sludge production. However the cost chemicals added is a main disadvantage (Morse et al. 1998).

2.2.4 Alum based technology

Alum based technologies uses aluminum based compounds, such as Aluminum hydroxide $Al(OH)_3$, Aluminum hydroxide is a very powerful coagulant for both orthophosphates and polyphosphates, as it reacts easily with phosphorus, the reaction occurs instantaneously under certain parameters, most importantly low pH of around 3.6 (de-Bashan and Bashan 2004). A system formed of a column filled with limestone, silica sand, and activated aluminum oxide removed over 99% of phosphorus present in the system in an experiment done by (Baker et al. 1998). Adsorption characteristics of aluminum sulfate can be enhanced to improve the system efficiency by adding organic polyelectrolytes, such as tannin, synthetic, clay and anionic polyelectrolyte.

2.2.5 Iron based technology

Blast furnace slag a byproduct of the steel industry was found to have great phosphorus adsorption characteristics. Iron-based compounds release metal cations (Mg, Ca, and Fe) to the system. The produced cations and their hydroxides act as coagulants for phosphorus removal (Deng et al. 2017), Iron/calcium oxides, produced from the steel industry, and the fine-grained activated aluminum oxide outperformed other oxides in performance. It has been reported that those compounds were able to remove more than 99% of the phosphate from wastewater effluent within one hour (de-Bashan and Bashan 2004). The iron salt compounds are also effective in inhibiting phosphate release and sulfide production, it was found that iron salt compounds helps the enrichment of iron reducing bacteria and inhibiting sulfate-reducing bacteria which helps in inhibiting the phosphorus release (Ivanov et al. 2005). However, these compounds have a negative side effect as they increase the phosphorus content of the sludge produced.

2.2.6 Calcium based technology

Due to its low costs and ease of operation calcium based compounds are one the most common technologies used for chemical phosphorus removal, calcium reacts with phosphorus to form compounds that easily precipitate such as calcium phosphate $Ca_3(PO_4)_2$ (Yi and Lo 2003), calcium based technology can only work in systems rich in calcium and phosphorus, in which phosphorus concentration should be more than 50mg/L, while the calcium concentration should be more than 100mg/L, this is considered to be a major setback, since most of WWTP has a phosphorus concentration less than 20mg/L , that's why calcium based technology is mainly used in industrial wastewater treatment not domestic EBPR (Carlsson et al. 1997).

2.2.7 Physical phosphorus removal

Filtration of the particulate phosphorus can achieve high phosphorus removal efficiency, by adopting different types of filters, most commonly the conventional sand filters, or through the appliance of membrane technology. Due to its higher removal efficiency, membrane technology got more attention in the last couple of years, as it is able to remove both soluble and particulate forms of phosphorus. Membrane bioreactor combines biological and physical technologies through suspended growth technique. One of the main disadvantages of physical is the high energy requirements, as high pressure is applied to separate phosphorus from the wastewater, this requires extensive amount of energy to perform the process.

2.3 Enhanced biological phosphorus removal

Biological phosphorus treatment is one of the oldest methods of phosphorus removal; it has been adopted in many WWTP worldwide, as it achieves phosphorus removal without the need for chemical precipitation. Enhanced biological phosphorus removal is achieved through activated sludge process by circulating sludge through anaerobic and aerobic stages as shown in Figure 2-2 (Barnard, 1975).

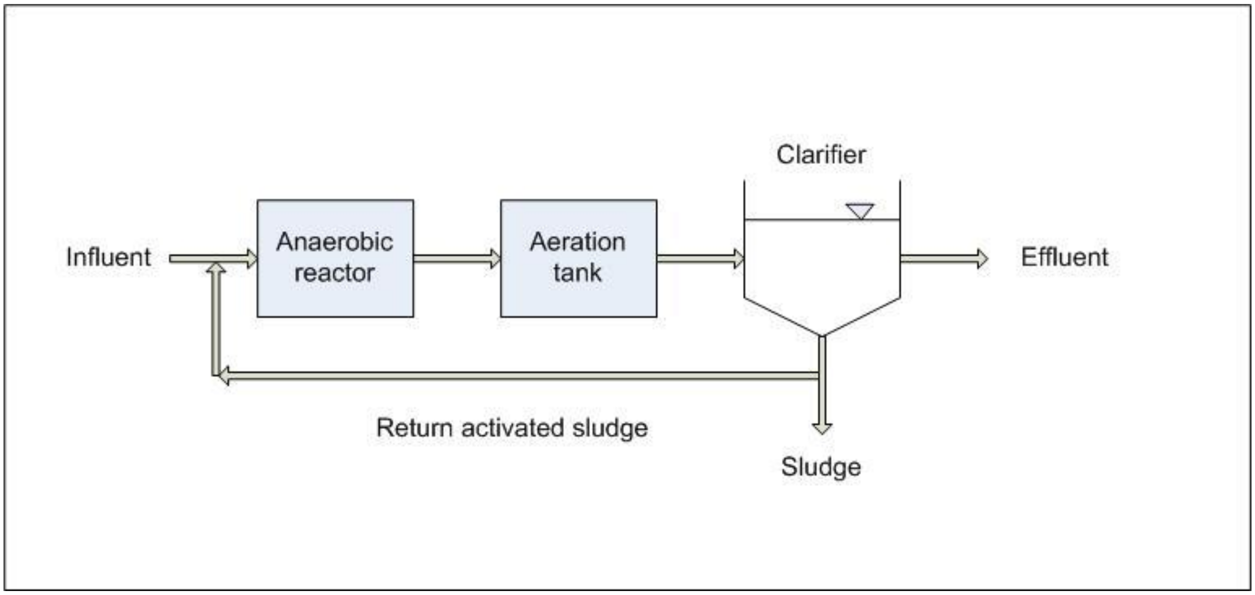


Figure 2-2: EBPR system (Metcalf and Eddy 2003)

EBPR is environmentally sustainable and inexpensive option for Phosphorus removal in comparison to chemical and physical phosphorus removal. However, EBPR have many challenges, many of WWTP witnesses fluctuation in performance and process upsets (Blackall et al. 2002). In many cases, external factors such as extra nitrate dosage to the anaerobic reactor or high rain water flow, or nutrient limitation can be the reasons for these process upsets.

2.3.1 Polyphosphate Accumulating Organisms (PAO)

Polyphosphate accumulating organisms (PAOs) are the main group of bio-organism responsible for biological phosphorus removal. PAO store phosphate intracellularly in the form of polyphosphate, therefore, phosphorus removal is performed by PAO cell removal from the liquid phase in the activated sludge (Zeng et al. 2003). PAO is different than other types of microorganisms, as PAOs can take up volatile fatty acids (VFAs) as a carbon source under anaerobic conditions as illustrated in Figure 2-4, and then store them intracellularly in the form of carbon polymers (poly-b-hydroxyalkanoates (PHAs)) (Oehmen et al. 2007a). The energy needed for the biotransformation process is taken from the release of phosphate from the PAO cells. Reduction of power is needed for PHA formation, that's why it is produced in great values through the glycolysis of internally stored glycogen (Mino et al. 1998).

In the aerobic stage PAO utilize the PHA stored in their cells for cells growth, glycogen replenishment, and polyphosphate storage and phosphorus uptake as illustrated in Figure 2-3. Net phosphorus removal out of wastewater is done by removing the high concentrated polyphosphate sludge out from the EBPR systems (Henze et al. 1999).

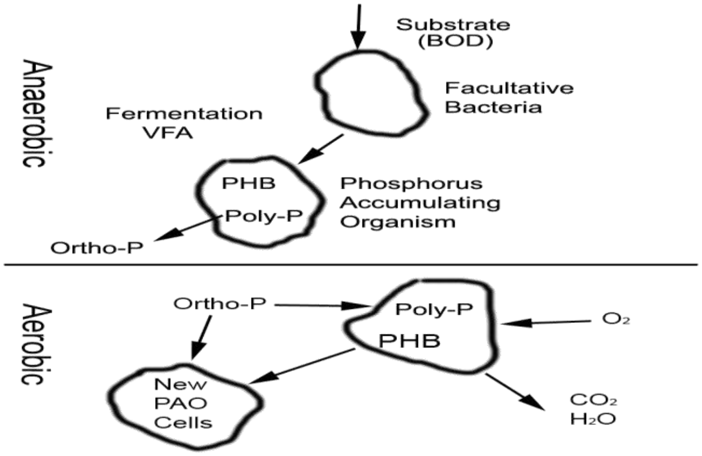


Figure 2-3: PAO metabolism (Lin et al. 2015)

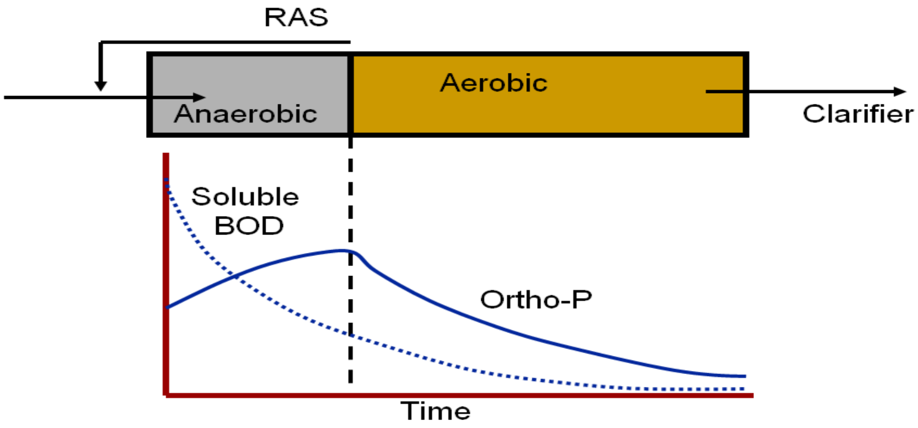


Figure 2-4: EBPR cycle (Lin et al. 2015)

2.3.2 (PAO) Microbiology

One of the first morphological description of the PAO was made by Fuhs and Chen in 1975 after observing activated sludge enriched with PAO, the microorganisms were described as a non-motile rods or cocci as illustrated in Figure 2-5, that exist in the form of clusters that contain Neisser positive granules (Henze et al 2008). Another group of bacteria named Microlunatus phosphovorus were described by Fuhs and Chen and were hypothesized to achieve phosphorus removal as well (Fuhs and Chen 1975), however, recent studies challenged this hypothesis, due to the lack of evidence of PHA circulation in their cells on the contrary to PAO (Oehmen et al. 2007b). Other groups of bacteria such as Lampropedia spp, and Tetrasphaera spp, were isolated from EBPR systems, however, did not show PAO characteristics.

A successful method of PAO isolation has been proposed by (Seviour in 2003), it is done through micro-manipulation techniques for the enriched cultures of the microbial organisms (Lu et al. 2006), From a metabolic prospective PAO are mainly able to take organic substances under anaerobic conditions then use the hydrolysis of their stored poly phosphate to utilize energy with no need for external electron acceptor (Van Loosdrecht et al 1998).

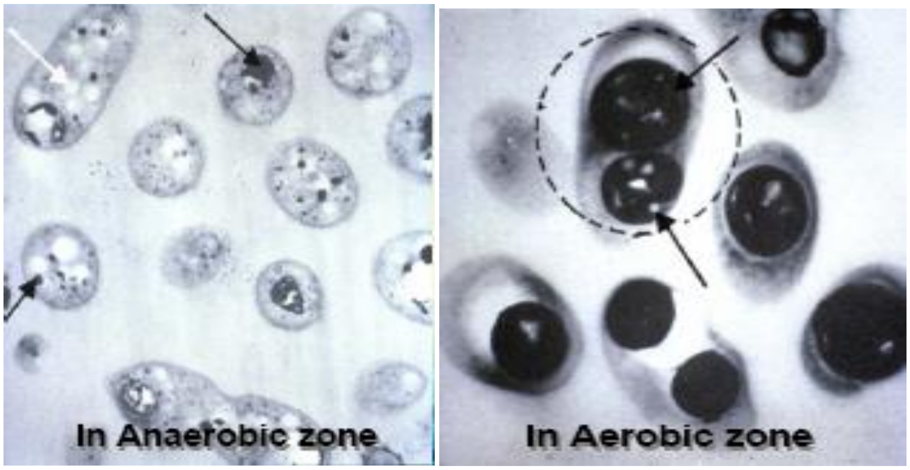


Figure 2-5: PAO in anaerobic, aerobic zones (Lin et al. 2015)

Despite of the challenges facing researchers in the isolation of PAOs, recently the use of molecular techniques, gave a better insight on the nature of PAO. Bond et al. 1995 observed variation in the biological structure among non-phosphate and phosphate-removing sludges, it was determined through phylogenetic analysis of 16S rRNA clone libraries, particularly there was a great abundance of Rhodocyclus group from subclass 2 of the Betaproteobacteria in the phosphate-removing sludge. This conclusion is supported by Bond et al. (1999a) observation of Rhodocyclus bacteria through FISH analysis in different sludges with good EBPR performance.

The subclass 2 Betaproteobacteria related to Rhodocyclus, known as ‘‘Candidatus Accumulibacter phosphatis’’ were discovered by Hesselmann et al. (1999), they are usually abbreviated to Accumulibacter, and sometimes referred to as Rhodocyclus-related bacteria. Hesselmann et al. (1999) and Crocetti et al. studies demonstrated that Accumulibacter resemble the characteristics of PAO phenotype of anaerobic/aerobic cycling of PHA and poly-P, the discovery was achieved through chemical staining. Zilles et al. (2002a). An analysis of the sensitivity and specificity of the probes for Accumulibacter Table 2-1 has been illustrated by Saunders (2005). It was found out that a combination of the Crocetti et al. (2000) probes (PAO651, PAO462, and PAO846) is the most specific and sensitive for Accumulibacter from any of the available probes for these bacteria.

Different surveys were conducted to assess the presence of Accumulibacter in full-scale plants, a wide research conducted by (Zilles et al., 2002a; Saunders et al., 2003; Kong et al., 2004; Gu et al., 2005; He et al., 2005; Wong et al., 2005) in ten different countries across four continents, Accumulibacter was found to be present in large abundance (4–22%) of all bacteria analyzed. A study conducted in Australia observed a strong correlation between the percentage of Accumulibacter present in the sludge and the performance of EBPR (Saunders et al., 2003). A study was performed to detect PHA and polyphosphate cycling using Sudan Black B and Methylene blue chemical staining, however, cells displaying the Accumulibacter and PAO phenotype were not found.

Kong et al. (2004) combined microautoradiography (FISH–MAR) with FISH analysis, discovered that the metabolism of Accumulibacter in three different EBPR plants in Denmark Was strongly correlated with the PAO phenotype as described by the proposed biochemical models for PAOs. However, many studies have mentioned that it was not observed in some of the cells binding the FISH probes for Accumulibacter to store polyphosphate (Zilles et al., 2002a; Wong et al., 2005), even though poly-P granules have been Monitored in other cells that were not Accumulibacter (Zilles et al., 2002a; He et al., 2005; Wong et al., 2005). This discovery suggests that it is possible for other PAOs to be present in these sludges.

Wong et al. (2005) combined chemical staining with FISH in sludges that displays such effect, and with specific -group probes that showed most of the bacteria belongs to the Betaproteobacteria, while substantial percentages were still Gammaproteobacteria, Alphaproteobacteria and Actinobacteria as shown in Table 2-1. More studies focused on identifying other strains of PAOs, the development of FISH probes to target such organisms, and assessing them as appropriate organisms in large scale EBPR plants is much recommended.

Table 2-1: Probes used for PAO detection

Probe	**Sequence 5’-3’**	**Specificity**	**Reference**
Probes designed for (potential) PAOs			
PAO462	CCGTCATCTACWCAGGGTATTAAC	Most Accumulibacter	(Lanham et al. 2012)
PAO651	CCCTCTGCCAAACTCCAG	Most Accumulibacter	(Lanham et al. 2012)
PAO846	GTTAGCTACGGCACTAAAAGG	Most Accumulibacter	(Lanham et al. 2012)
RHC439	CNATTTCTTCCCCGCCGA	Rhodocyclus/Accumulibacter	(Lanham et al. 2012)
RHC175	TGCTCACAGAATATGCGG	Most Rhodocyclaceae	(Lanham et al. 2012)
PAO462b	CCGTCATCTRCWCAGGGTATTAAC	Most Rhodocyclaceae	(Lanham et al. 2012)
PAO846b	GTTAGCTACGGYACTAAAAGG	Most Rhodocyclaceae	(Lanham et al. 2012)
Actino-221a	CGCAGGTCCATCCCAGAC	Actinobacteria—potential PAOs	(Lanham et al. 2012)

2.3.3 Denitrifying Polyphosphate Accumulating Organisms (DPAO)

An anoxic phase can be introduced to EBPR system to enrich DPAO culture, this has an extra advantage of removing nitrite and nitrate from EBPR systems, and reducing amount of oxygen needed as electron acceptor (Kishida et al. 2006). Denitrifying phosphorus accumulating bacteria (DPAO) are a group of PAO capable of utilizing both oxygen and nitrite as an electron acceptor (Chang et al. 2000), DPAO are able to accumulate phosphorus up to 20 to 30 g P g−1 TSS (Frison et al. 2016a). DPAO consume short chain fatty acids at the anaerobic phase and store them as polyhydroxyalknoates (PHA) to provide the needed ATP for the cells (Ahn et al. 2002), the reduction is supplied by the glycogen through the glycolytic metabolic pathway. In the later anoxic phase the DPAO use the PHA stored during the anaerobic phase to take up the released phosphorus of the anaerobic period with nitrite or nitrate being an electron acceptor (Seviour et al. 2003). DPAO gives the advantage of decreasing amount of nitrogenous compounds in wastewater effluents while decreasing amount of oxygen needed during aerobic phase which leads to a total decrease in aeration costs.

2.4 Nitrogen

Nitrogen is a chemical element found abundantly in nature in many forms such as ammonium, nitric acid, organic nitrates, and nitrites. The nitrogen compounds are a result of human activity and nature cycle, such as runoff, rainfall, and agriculture and farming runoff, industrial and domestic wastes (Reeves 1972). The most common form of nitrogen in wastewater effluents is ammonium (NH_4^+), it has been reported that typical domestic wastewater effluent contains approximately between (18 – 22) mg/1 as(N), or (1.1 - 1.6) billion lb. /yr. (Dutta 2015). The enormous amount of nitrogenous compounds discharged to water bodies, contributes to the growing worldwide problem of eutrophication, reducing the amount of nitrogenous compounds is a must to reduce the severe damage resulting from the excessive nitrogen discharge.

Nitrogen can removed through: chemical, physical and biological methods, the biological removal method is the most common method due to its cheap running cost and high efficiency (Soliman and Eldyasti 2016).

2.4.1 Conventional biological nitrogen removal

The conventional biological nitrogen removal process is done through two steps: nitrification and denitrification, first ammonia is oxidized to nitrate, and then nitrate is reduced to nitrogen gas, while nitrite being an inter-mediate product as shown in Figure 2-6.

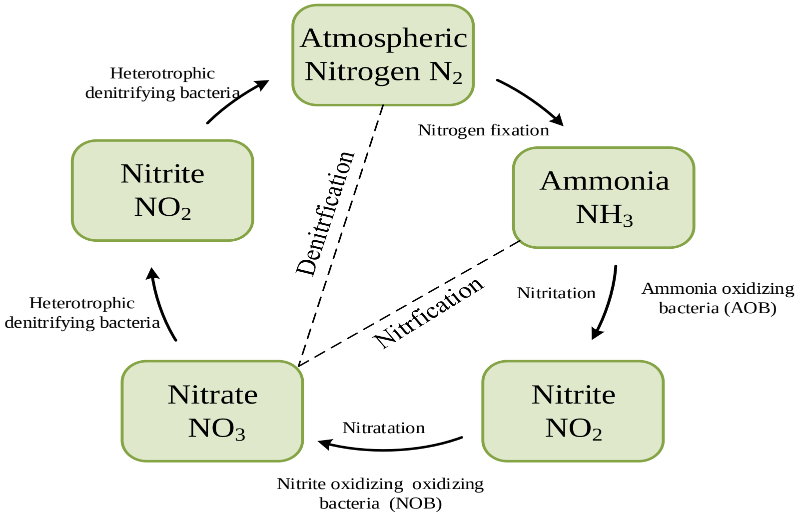


Figure 2-6: Nitrogen cycle (Soliman and Eldyasti 2016)

2.4.2 Nitrification

Nitrification step starts with ammonia oxidation, through the microbial conversion of ammonia, it is done through one of three types of bacteria: aerobic ammonia oxidizing bacteria (AerAOB), anaerobic ammonia oxidizing bacteria (AnAOB), ammonia oxidizing archaea (AOA) (Dutta 2015). Recent studies show that ammonia oxidizing archaea (AOA) is the most abundant type of ammonia oxidation bacteria found in environment; however more research is needed to fully understand the characteristics of the (AOA). Ammonia oxidizing bacteria (AerAOB) utilizes CO_2 as a carbon source (Liu and Capdeville 1994), while the conversion of ammonia is the primary source of energy, where 25 mol of ammonia is oxidized for each mole of carbon utilized, oxygen acts as an electron acceptor for the process this can be expressed by the following reaction equation 2.1. the optimal pH range for the reaction is in the range of (6.5 - 8.8) (Agrawal et al 2015).

$$\mathbf{NH_4^+ + 1.5\ O_2 \longrightarrow NO_2^- + H_2O + 2H^+} \qquad (2.1)$$

2.4.3 Ammonia Oxidizing Bacteria (AOB)

AOB are a group of bacteria responsible for the first step of the nitrification process, through oxidizing the Ammonia (NH_3) in to Nitrite (No_2), AOBs were first discovered in the early 20th century (Agrawal et al 2015). Since its discovery five groups of the AOB family were discovered (Koops and Pommerening-Röser 2001), they are classified according to their: shape, cell size, the structure of their flagyls (flagellation), and structure and formation of the intracytoplasmic membranes (Soliman and Eldyasti 2016). The AOB are members of Betaproteobacteria and Gammaproteobacteria class of bacteria, both groups being Gram-negative bacteria which include the species of the genera Nitrosomonas, Nitrosococcus, Nitrobacter and Nitrococcus (Rocco L. Mancinelli 1996).

2.4.4 Nitratation

The second step of the nitrification process is the oxidation of nitrite and it is called nitratation, this process is carried out by Nitrite oxidizing bacteria (NOB).

2.4.5 Nitrite Oxidizing Bacteria (NOB)

Nitrite Oxidizing Bacteria (NOB) are chemolithotrophic organisms responsible for oxidizing nitrite into nitrate as shown in equation 2.2. Many organisms are included under the nitrifying bacteria family and can achieve the nitrification process such as: Nitrospira , Nitrobacter , Nitrococcus , Nitrospina , and Nitrotoga (Mancinelli 1996). the optimum temperature for NOB ranges from 30 to 40 degrees , and optimum pH between 7.8 and 8.5 (Agrawal et al 2015). Other factors can affect NOB activity such as free ammonia concentration, free hydroxylamine toxicity, free nitrous acid.

$$\mathbf{NO_2^- + 0.473\ O_2 + 0.005\ NH_4^+ + 0.020\ CO_2 + 0.005HCO_3 + 0.005\ H_2O \longrightarrow NO_3^- + 0.002\ C_5H_7O_2} \qquad (2.2)$$

2.4.6 Denitrification

Unlike nitrifying bacteria in Denitrification process, denitrifying bacteria utilize carbon as an organic source of growth, and nitrate as an electron acceptor rather than oxygen as illustrated in equation 2.3. The optimum conditions for the process are: pH between 7 and 8, temperature in the range of (20-30)°C, it showed be mentioned that low oxygen concentration has been found to inhibit Denitrification (Metcalf and Eddy 2003).

$$\mathbf{NO_3 \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2} \qquad (2.3)$$

2.4.7 Shortcut nitrogen removal

Shortcut biological nitrogen removal is a nonconventional method for wastewater nitrogen removal through applying two processes: nitrite shunt and deammonification (Soliman and Eldyasti 2016, Dutta 2015). In shortcut nitrogen removal process nitrite shunt process denitrifies nitrites directly to nitrogen gas, while in deammonification process half of the ammonia is oxidized to nitrite and the remaining half of the ammonia content is oxidized by Anammox reaction using nitrite as an electron acceptor (Cao et al. 2016).

Shortcut biological nitrogen removal has many advantages in comparison to the conventional biological nitrogen removal, aeration requirements in shortcut process are lower than conventional process, also carbon requirements are reduced, as there no need for an extra carbon source (Kim,et al 2011). In deammonification there is a reduction in aeration and alkalinity needs, and Anammox bacteria does not require carbon as an organic source, carbon in the influent can be solid processed and used.

Shortcut biological nitrogen removal has some disadvantages, the redirection of carbon may affect other treatment processes, such as enhanced biological phosphorus removal, EBPR needs certain amount of organics to function properly, also EBPR needs high dissolved oxygen concentration which contradict with low oxygen requirement of shortcut process, nitrite shunt process my generate greenhouse gases specifically nitrogen oxide and nitrous oxide (Agrawal et al 2015)

.

The changing of the environmental conditions can achieve different results in the shortcut process, specifically pH and dissolved oxygen. Although it should be mentioned that in many cases it is not possible to completely control the biochemical reactions, and different measures are needed to achieve lowers limits for compounds such as ammonia. The treatment goal is converting the concentration of the total nitrogen in to nitrogen gas, total nitrogen content in municipal waste water ranges from (30 - 100) mg N/L, of which (65 - 75) % is in the form ammonium, while (25 - 35) % is in form of organic nitrogen.

Shortcut nitrogen removal process can be affected by several parameters such as:

-Maximum specific growth rate of AerAOB, AnAOB, ordinary heterotrophic bacteria (OHO), and NOB.

-Decay constants for NOB, AerAOB, and ANAOB.

- Temperature coefficients for kinetic parameters.

-Dissolved oxygen half saturation coefficient for NOB and AerAOB.

-Substrate half saturation coefficients for AnAOB, AerAOB, and NOB (this parameter may slow the growth rate for AnAOB, AerAOB, and NOB).

-Dissolved oxygen half saturation coefficient for OHO (the optimum dissolved oxygen concentration ranges between 0.3 to 0.7 mg/L).

-Hydrolysis parameters.

2.4.8 Simultaneous Nitrification and Denitrification (SND)

Simultaneous Nitrification and Denitrification (SND) is a term used to refer to an instance in the biological treatment where Nitrogen is removed in the same biological reactor. Oxygen is used to oxidize Ammonia in (SND): NH_4 is converted to NO_3, and then NO_3 is denitrified using organic carbon to nitrogen gas. SND has been observed in both methods of fixed film systems and suspended growth.

Different physical and biological factors may affect the SND process:

Macroenvironment conditions- zones with macroscopic anoxic/oxic presence are commonly formed due to nonhomogeneous aeration and mixing, especially in reactors with surface aerators.

Microenvironment conditions- Microscopic anoxic/oxic zones can be found within the sludge flocs, different levels of oxygen concentration within the reactor creates flocs that are denitrifying in the inner core while being nitrified in the outer layer.

Bulk oxygen concentration-dissolved oxygen level in the reactor should be satisfactory for the needs of both nitrification and denitrification processes.

Organic carbon availability- one of the most important factors to accomplish denitrification process is the availability of organic carbon, organic carbon is used as an electron donor.

2.5 Glycogen Accumulating Organisms (GAO)

The microbial competition between PAOs and other groups of microorganisms, such as the glycogen accumulating organisms (GAOs) is considered to be one of the main reasons for process upsets and degradation in EBPR systems worldwide (Crocetti et al. 2002; Erdal et al. 2003).

GAOs are a group of bacteria similar to PAO, as they need both anaerobic and aerobic conditions to perform their activity. However, GAO doesn't contribute in anaerobic Phosphorus release or aerobic phosphorus uptake, so no phosphorus removal is done by the GAO (sarvanamantu 2009). GAOs use glycogen as their main energy source for anaerobic VFA uptake and PHA formation, where PHA is aerobically oxidized, resulting in biomass growth and glycogen replenishment (A.Sathasivajn 2008).

Due to GAOs consumption of VFAs with no contribution to phosphorus removal, GAOs is a Group of undesirable organisms in EBPR systems. it has been found that the presence of GAO in EBPR systems leads to an increase in the aeration demands and increase in the total running costs for the WWTP (Burow et al. 2007).

2.5.1 Isolation techniques

Many factors may affect the competition between the GAO and PAO, manipulating the EBPR conditions such as: pH, carbon source, temperature, DO concentration, can be a strong tool in inhibiting GAO performance and give an advantage for the PAO (Winkler et al. 2011).

2.5.2 pH

Higher ambient pH has been proven to be an effective method to increase anaerobic phosphorus release (Filipe et al. 2001a). In one research conducted by (Smolders et al. 1994) the ratio of anaerobic phosphorus release to acetate uptake rate varied linearly between 0.25 to 0.75 P-mol/C-mol as the pH increased from 5.5 to 8.5. In this study the internal pH of the cells was unchanged contrary to the increase of the pH gradient, and the increase in the electrical potential difference through the cell membrane at a high pH. This showed that the increase in pH can result in an increase in the energy needed for acetate transport through the cell membrane. The increased energy is a result from the increase in polyphosphate degradation. nonetheless, the PHA accumulation, acetate uptake, and glycogen degradation rates of the PAO under patch test were independent of pH over the range 6.5–8.0 (Filipe et al. 2001a), this showed that the higher energy requirements to consume acetate does not negatively affect the ability to metabolize VFA. Under aerobic conditions, patch test showed that PHA utilization, phosphorus uptake, and biomass growth are inhibited by a low pH (6.5), and it was found that higher aerobic pH (7–7.5) would be more beneficial for PAOs over GAO (Filipe et al. 2001a).

On the other hand the GAO uptake of acetate was shown to be decreasing with the increase of pH, with increase in in anaerobic glycogen consumption and PHV accumulation (Filipe et al. 2001b). This means that higher pH does not only positively affect the energy demand for acetate uptake, but also have negative impact on the GAOs uptake of acetate, this can be an effective controlling strategy for the GAO inhibition, to give more favorable conditions for the PAO. This can be attributed to the PAO utilizing of poly-P as an extra energy while GAO cannot. It has been mentioned that the pH of 7.25 at anaerobic conditions is a critical point, since GAOs are able to anaerobically consume VFA faster than PAOs at a pH level less than 7.25, and PAOs take up acetate faster above this pH value. Other studies have found out that increasing the pH from 7 to a level between 7.5 and 8.5 during the anaerobic and/or aerobic have increased the phosphorus removal (Bond et al. 1999). The improvement in performance can be attributed to the shift in turns in the competition between the microbial performances between both of the GAO and PAO, the analysis of microbial population proved this assumption.

A deterioration in the phosphorus removal was found to be accompanied with the change in pH from 7.0 to 6.5, plus a visible shift in the structure of the microbial culture after being examined by 16S rRNA clone libraries developed at different pH levels. At pH 7.0 the clone library mainly consisted of Alphaproteobacteria, Betaproteobacteria, (including Defluviicoccusrelated clones), Actinobacteria and Bacteriodetes/Chlorobi. While At a pH of 6.5, the clone library consisted primarily of Alphaproteobacteria, Gammaproteobacteria, (including Sphingomonas-related organisms), Bacteriodetes/Chlorobi and Betaproteobacteria. (Zhang et al. 2005) Also , in another study two lab-scale reactors, one fed with propionate and one with acetate, the acetate reactor was dominated by Competibacter and the propionate reactor was dominated by Alphaproteobacteria GAOs, that's when the pH was maintained at 7 (Oehmen et al. 2005a), an increase in the ratio of Accumulibacter PAOs to GAOs was monitored in both of the two systems after the pH was increased to a level of 8, even though the phosphorus removal increased in both reactors but the GAOs were not completely inhibited in both reactors at high pH, even though the propionate feed reactor showed superior phosphorus removal compared to the feed acetate reactor.

To sum up, the literature clearly showed that the pH is an important factor controlling the competition between the PAO and GAO, increasing the pH was found to be more favorable towards the PAO performance over the GAO, this can increase the performance of the system in terms of phosphorus removal, this can be a key point in understanding and controlling the EBPR process, more importantly it should be noted that there a limit of how much pH can be useful to the system, it was reported that pH with level of more than 8 inhibited the phosphorus removal with a decrease in the VFA uptake (Schuler and Jenkins 2002).

2.5.3 Temperature

Temperature also is a big factor in controlling the performance of the phosphorus removal in the EBPR systems. (Brdjanovic, et al 1998) found out that the decrease is in temperature is strongly related to the decrease in system performance, the lower the temperature the lower the performance (Brdjanovic et al. 1998), it has been suggested that low temperatures affected the biochemical transformations. other research observed successful phosphorus removal at a temperature of 5°C (Panswad et al. 2003), Li et al. 2010 observed an increase in the phosphorus release while a decrease in the phosphorus uptake when the temperature increased from 20°C to 35°C, this can be attributed in the increase of the GAO portion of the microorganisms present in the system, with a decrease in the PAO portion (Li et al. 2010),(whang and park 2002) also showed similar results where the performance of the EBPR with better at a temperature of 20°C rather than 30°C (Whang and Park 2002). Low temperature was also found to be more favorable for the PAO over GAO (Erdal et al. 2003), PAO dominated the EBPR system at a temperature of 5°C as the PAO are able to alter their metabolic action while the GAO cannot, it should be noted that (Brdjanovic et al. 1998) found different results when decreasing the temperature, as performance increased by the decrease in temperature. It can be stated that low temperatures are more favorable to the PAO activity over the GAO, the optimum range for temperature is (5- 25) °C.

2.5.4 Carbon source

The type of carbon source VFA, non VFA, has a strong effluence on the PAO-GAO competition. The most common carbon source in EBPR systems is Acetate, propionate is also used but on smaller scale (Oehmen et al. 2005a). Valerate, Butyrate, and other sources of VFA are also being used as carbon sources, but not as common as Acetate and propionate. Other Non-VFA organic substrates such as amino acids and sugars are also being utilized as a carbon source in few wastewater treatment plants. Most previous of the studies focusing on EBPR technology, used acetate as the main carbon source. However, recently, the impact of propionate and other substrates on EBPR performance is being deeply studied. Even though the use of acetate as a sole carbon source in EBPR has been seen to achieve good and stable phosphorus removal efficiency, however, many reports documented phosphorus removal deterioration due to the microbial competition of GAOs with PAOs (Pijuan et al. 2004).

Recent studies found out that propionate could be a better source of substrate than acetate for better EBPR performance (Li et al. 2008, Lu et al. 2006). On many occasions, better phosphorus removal performance was reported with propionate used as a sole carbon source compared to Acetate during long-term laboratory scale enrichment studies (Oehmen et al. 2005a, Shen et al. 2017, Xie et al. 2017). These studies found out that systems feed with propionate had an advantage to PAOs over GAOs, this has been supported by examining the biomass by FISH analysis. In the lab scale reactors that were fed with propionate as the sole carbon source, Accumulibacter were observed and dominate the bacterial community, while Competibacter were absent from the biomass culture. Moreover, Accumulibacter were able to easily switch between propionate and acetate uptake at a similar rate. (Oehmen et al. 2007b). While Competibacter fed with Acetate as a sole carbon source consumed propionate at a level less than 5% of the Acetate uptake rate. Even though propionate has been documented to be more effective in selecting against Competibacter, different groups of GAOs, the Alphaproteobacteria, were found able to take up propionate at high rates (Lanham et al. 2012). These observations raised questions about the Viability of propionate as carbon source in providing PAOs with advantage over GAOs. Nonetheless, most recent studies reported far more improved phosphorus removal performance with a propionate carbon source.

2.5.5 Sludge age

Sludge age was found to be one of the main factors affecting the performance of the EBPR (Rodrigo et al. 1999; Whang and Park 2002), it was observed that as the sludge age increased the system performance decreased, in an experiment done by (Crocetti et al. 2002) different samples of sludge were taken from an SBR system that been operating over a long period of time, it was found that older sludge contained higher percentage of GAO, this indicates that low sludge age is more favorable for the PAO performance.

2.5.6 Dissolved Oxygen

DO is a major factor affecting the performance of the EBPR and controlling the competition between the PAO and GAO (Griffiths et al. 2002b). Many resources has indicated that the optimum DO concentration for EBPR for the PAO to outcompete the GAO was between (2.5–3.0 mg/L), while higher DO between (4.5–5.0 mg/L) was found to be more favorable for the GAO performance (Dutta 2015; Lemaire et al. 2006; Mino et al. 1998). This can be a challenge to most waste water treatment plants as the growing trend is to reduce the DO concentration in the treatment process to reach a level between (0.5–1.5 mg/L) to cut down running costs.

2.6 Technology approaches

There are many different types of reactor configuration used to run EBPR systems, most commonly: SBR, upflow granular sludge reactors, and attached growth biofilm. All of the three technologies aim to accomplish three main objectives:

Biomass retention: Biomass should be concentrated in the reactor despite their growth.

Sequential aerobic/anaerobic environment: the reactor must provide enough time and space that would support the ecological environment for PAO to achieve phosphorus removal.

Out selection of GAO: the process should consist of provision to counter the growth of the GAO.

SBR and upflow granular sludge reactors rely on gravimetric selection, the retention of biomass is done by the granulation of sludge which is retained because it’s high settling velocity.

2.6.1 Sequencing batch reactors

SBR technology applies four operation stages: filling, reaction, settling, and decant. The SBR process for EBPR systems combines miniature cycles of aeration, feeding, and mixed –unaerated conditions as illustrated in Figure 2-7 with pH aeration/anaerobic control principle (Dutta 2015). The cycles are repeated many times until the reaction is complete, SBR cycle ranges from 6 to 12 hours depending on the Nutrients concentration in the influent, if the suspended solids loaded to the reactor are higher than normal, hydrocyclone will operated for longer time (Cao et al 2016). Shortcut SBR process is a low energy nutrient removal SBR process, usually there are 3 cycles each day consisting of 8 hours , divided into feeding stage, aeration stage, and anaerobic stage. GAO activity is to put to an end by controlling temperature, carbon source, and dissolved oxygen conditions.

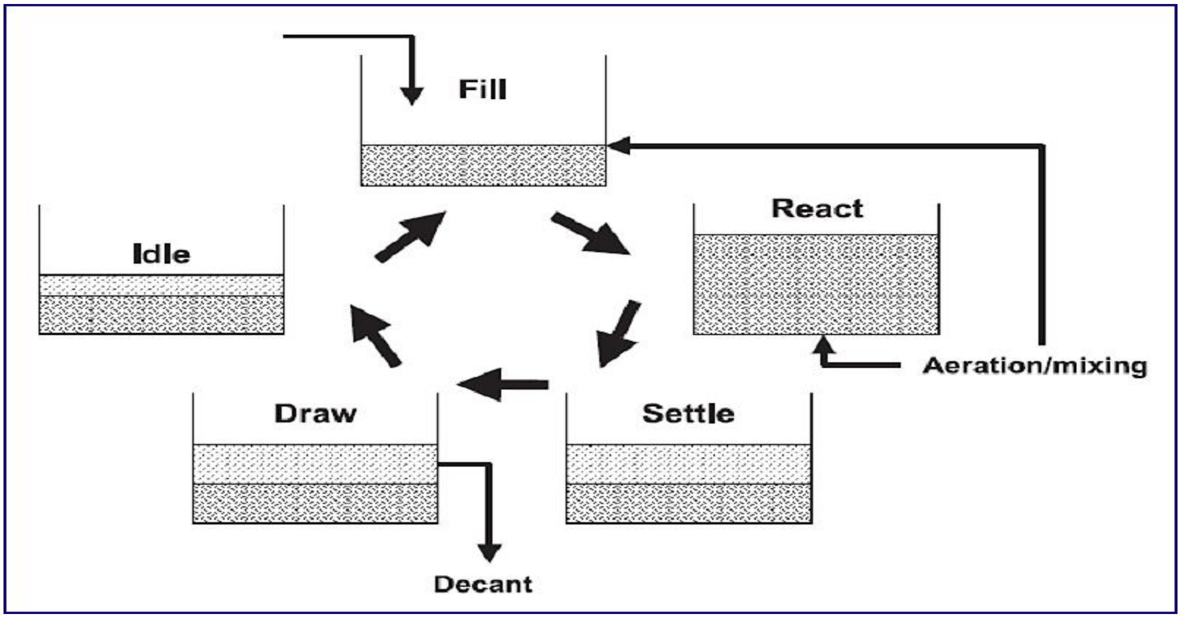


Figure 2-7: SBR system (Dutta 2015)

2.6.2 Attached growth biofilm reactors

Attached growth biofilm reactors are used for the treatment of high strength nutrient rich streams, the biofilm reactors are continuous flowing system with continuous aeration strategy as illustrated in Figure 2-8. In attached growth PAO biomass is attached to a moving carrier media with a protected large surface area, while sieves are used to retain the PAO biomass.

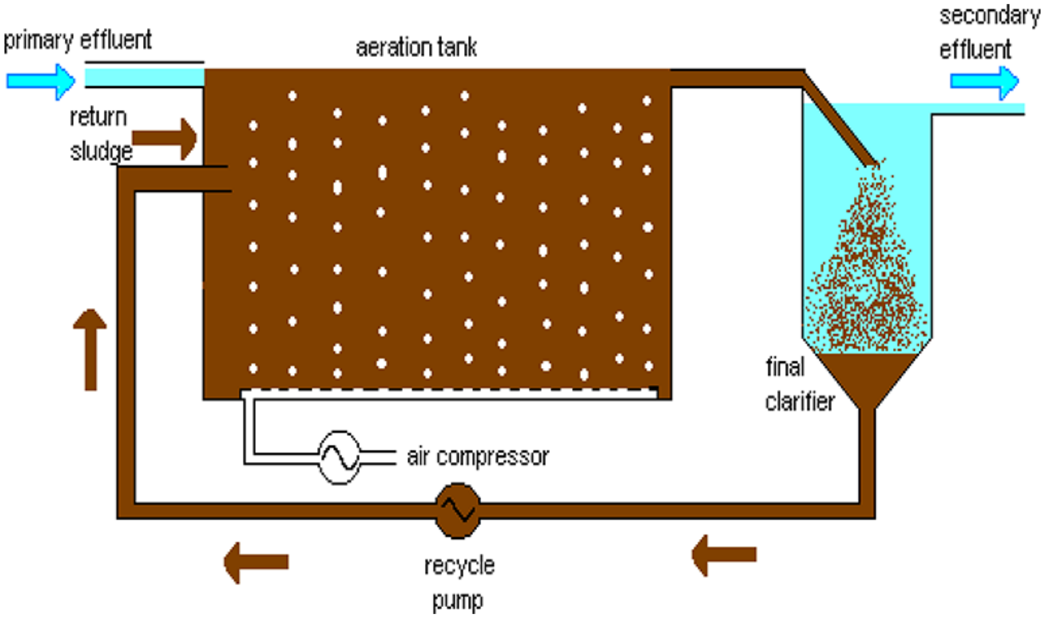


Figure 2-8: Attached growth biofilm reactor (Morse et al. 1998)

2.6.3 Upflow granular sludge reactors

Granular sludge reactors are characterized by high volumetric efficiency, it is a single stage reactor containing plate settlers, the plates are placed inside a new or already build reactor which is designed to retain higher gravity particles while allowing particles with smaller specific gravity particles to wash out of the reactor as illustrated in Figure 2-9. The granulated sludge is retained in the reactor while the treated water is flown out through the settlers leaving the biomass within the reactor.

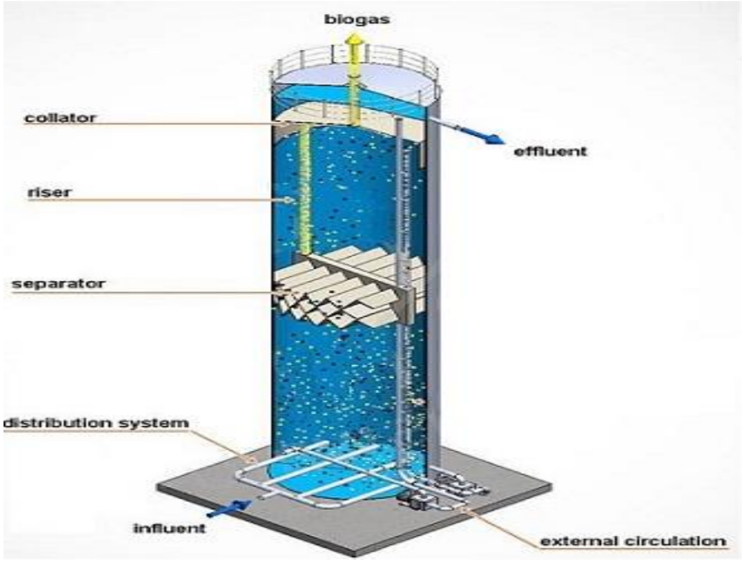


Figure 2-9: Granular sludge reactor (Morse et al. 1998)

2.6.4 Fluidization and Fluidized Bed Reactor (FBR)

The main concept of fluidized bed reactor and fluidization evolves around passing wastewater through a static bed of solid particles with an external velocity enough to keep the particles suspended and allowing to behave as if they were fluid not particles (Bello et al. 2017). As the wastewater is introduced to the fluidized bed at low velocity, it runs through the voids of the solid particles, however, the bed remains fixed. The increase in velocity, expands the bed expands, until the particles become suspended, then the buoyancy force achieve a balance between the drag force and the gravitational forces as illustrated in Figure 2-10. Fluidization technique has many advantages such as, uniform temperature distribution excellent particle mixing and high mass transfer rate. Initially was developed for gas generation by Fritz Winkler in 1920s. Fluidized bed reactor has many applications in chemical and biochemical industries such as powder technology, coal gasification, catalytic cracking, metal refining, food processing and many other applications (Tavoulareas, 1991).

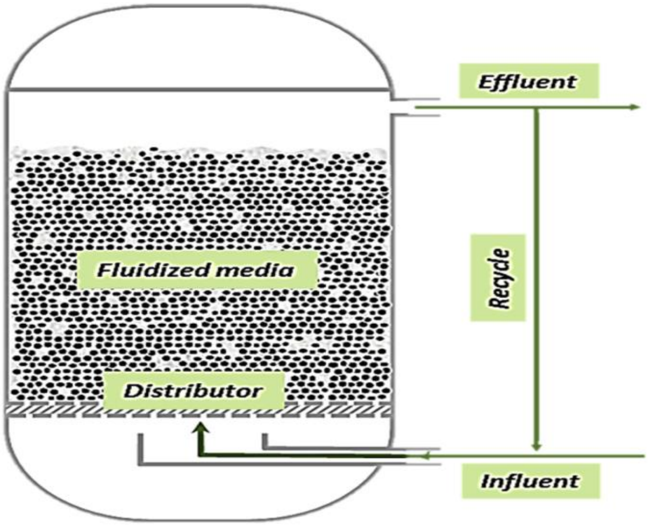


Figure 2-10: Fluidized bed reactor (Bello et al. 2017)

However, in the early 70s fluidized bed reactor was investigated as a possible reactor for biological wastewater treatment. By 1984, a full-scale FBBR was developed and installed. Fluidized bed reactor has gained a lot of interest as it has more effectiveness in wastewater treatment compared to other wastewater treatment technologies such as activated sludge and fixed-bed column (Burghate and Ingole, 2013). Efficient mixing, high rates of mass transfer, and low sludge production are among the main features that make fluidized bed reactor an affective technology (Andalib et al., 2014).

Chapter 3

Effect of intermittent aeration patterns on EBPR performance

3.1 Introduction

Enhanced Biological Phosphorus Removal (EBPR) is one of the most common methods for phosphorus removal in Wastewater Treatment Plants (WWTP) worldwide (Oehmen et al. 2007b). EBPR is considered to be a more environmentally friendly and a better economical option for phosphorus removal compared to chemical and physical treatment. However, aeration costs for EBPR process is one of the main challenges facing WWTP, cutting down aeration requirements through implementing new aeration strategies for EBPR can have a strong impact on wastewater treatment process (Bond et al. 1999). Different aeration strategies and optimized techniques for providing DO for EBPR process were investigated in the Lab to reach maximum efficiency of P-removal with the lowest possible DO level.

3.2 Materials and Methods

3.2.1 Reactor design and operation

A laboratory-scale sequencing batch reactor (SBR) with a working volume of 5L was used to perform enhanced biological phosphorus removal as shown in Figure 3-1. The reactor had an internal diameter of 16 cm, a height of 29 cm. The effluent was discharged from the reactor through a tube adjusted at a height of 11 cm from the top of the reactor to prevent biomass washout during the decanting phase. The system had ambient temperature (22 ± 1 °C), while the pH was not controlled varied between 7.5 and 7.8. DO set points were controlled according to the defined aeration strategies, using a DO probe connected to a control box for better handling of the aeration strategy.

The system was seeded with fresh activated sludge (MLVSS = 5000 mg/L) from Humber Wastewater Treatment Plant, Ontario, Canada. The hydraulic retention time (HRT) was controlled at 12 h during all different system configurations whereas the solid retention time (SRT) was not controlled but calculated and found to be approximately 25 days.

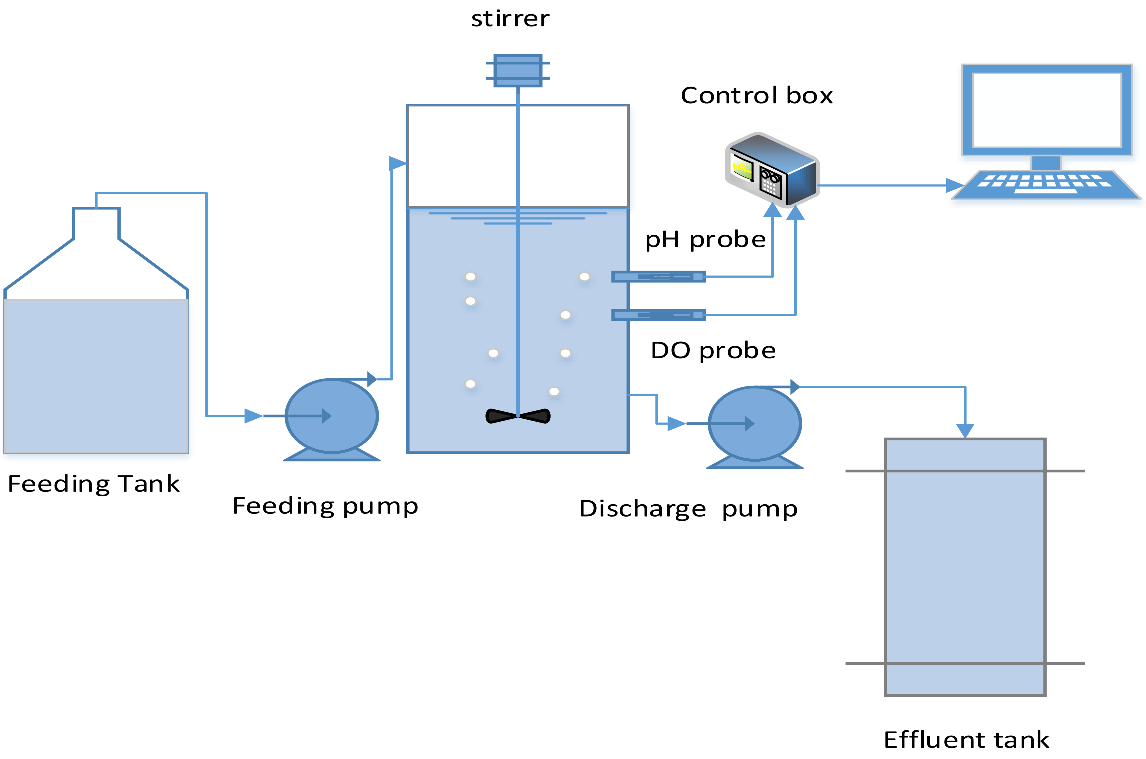


Figure 3-1: Schematic diagram of the SBR used in this experiment

3.2.2 Synthetic wastewater

The reactor was fed using synthetic wastewater containing per (1L) the following:

260 mg CH_3CH_2COONa (300 mg chemical oxygen demand (COD)), 32.9 mg KH_2PO_4 (7.5 mg P); 42 mg K_2HPO_4 (7.5 mg P); 38.2 mg NH_4Cl; 85.0 mg $MgSO_4.7H_2O$; 10.0 mg $CaCl_2$. This formula lead to a volatile fatty acid (VFA, i.e., propionate) to P ratio of 6.4 mg C/mg P.

The propionate was used rather than acetate as a sole carbon source for the VFA, to give an advantage for PAO over GAO, as it has been reported that propionate is more favorable carbon source for the PAO activity (Oehmen et al. 2005a,Whang and Park 2002,Oehmen et al. 2005c). Trace elements solution (1 mL/L) was used, the formula of the used trace element is shown in Table 3-1:

Table 3-1: Trace elements

Chemical	**mg/L**
EDTA	15000
$ZnSo_4.7H_2O$	430
$MnCl_2.4H_2O$	990
$FeSo_4.7H_2O$	500
$CuSo_4.5H_2O$	250
$CoCl_2.6H_2O$	240
$Na_2MoO_4.2H_2O$	220
$NiCl_2.6H_2O$	190
Na_2SeO_4	210
$H_3BO_4.7H_2O$	14

3.3 Analytical methods

Solid and liquid phase analyses of COD, (PO^{3-}_4 -P), (NH_4^+-N), (NO_3^--N), (NO_2^--N), , MLSS and MLVSS were performed by the standard method, samples were collected 2-3 times per week at six stages of operation: influent, after 1 hour of anaerobic reaction, after 2 hours of anaerobic reaction, end of anaerobic stage, after 1 hour of aerobic reaction, effluent. Also samples of influent and effluent were collected on daily bases to monitor the daily performance of the system only phosphorus and COD were measured in this procedure, on the contrary COD, ($PO^{3-}{}_4$-P), (NH_4^+-N), (NO_3^--N), (NO_2^--N) were measured for all six stages. Approximately 20 ml of each sample was filtered through 0.45-µm membrane filter and was stored in a cold room with temperature less than 5°C.

Phosphate ($PO^{3-}{}_4$-P), Ammonia (NH_4^+-N), COD, Nitrate (NO_3^--N), Nitrite (NO_2^--N), Total suspended solids (TSS), Volatile suspended solids, were all measured following the procedure described at (Rice, et al 2014).

3.4 Process methods

The SBR system kept operating for 170 days to achieve a stable phosphorus removal performance, different intermittent aeration strategies were implemented through the operation period, the operation period was divided into 6 phases. In phase one (1-30 days) the SBR system was operated with 4 cycles a day, 6 h for each cycle, each cycle consisted of 15 min feeding, 180 min anaerobic phase, 120 min intermittent aeration as shown in Figure 3-2.

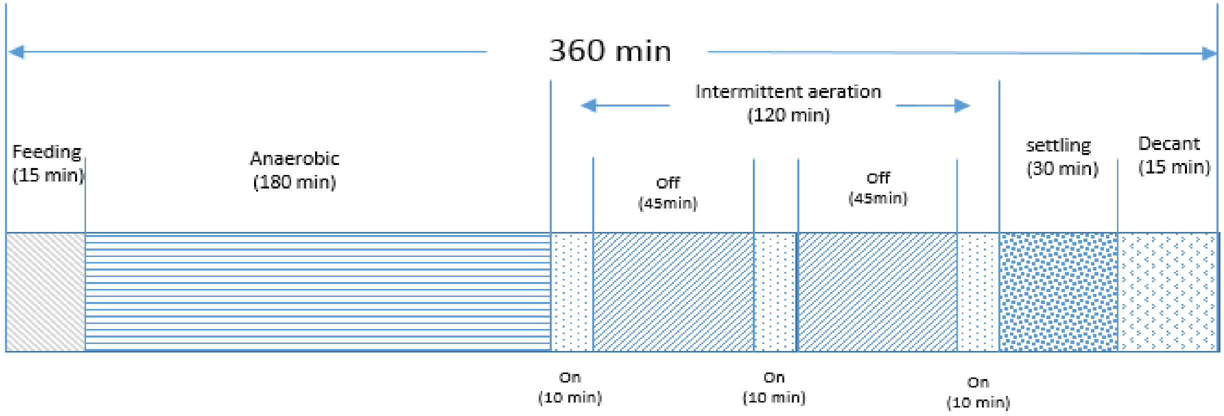


Figure 3-2: System configuration phase one

Similarly, SBR cycle duration in phase two (30-60 days) was 6 h, however, the anaerobic reaction phase was decreased to 60 min, and an anoxic phase has been introduced after the anaerobic phase as shown in Figure 3-3. The anoxic phase was introduced to promote the development of DPAO culture, DPAO are a group of PAO capable utilizing nitrate or nitrite as an electron acceptor during anoxic conditions. Nitrite was chosen to be our electron acceptor as the nitrite anoxic phosphorus uptake rate is higher than the uptake of the nitrate (Wang et al. 2015b). A solution of KNO_2 was added to the system at the beginning of the anoxic phase giving an initial concentration of 33 mg/L NO_2-N, the NO_2-N concentration was gradually increased over the period of phase two to reach a final concentration of 66 mg/L. The same aeration strategy of phase I with 120min of intermittent aeration was used, whereas the idle time was eliminated and the settling time was increased to 30 min to improve biomass settling.

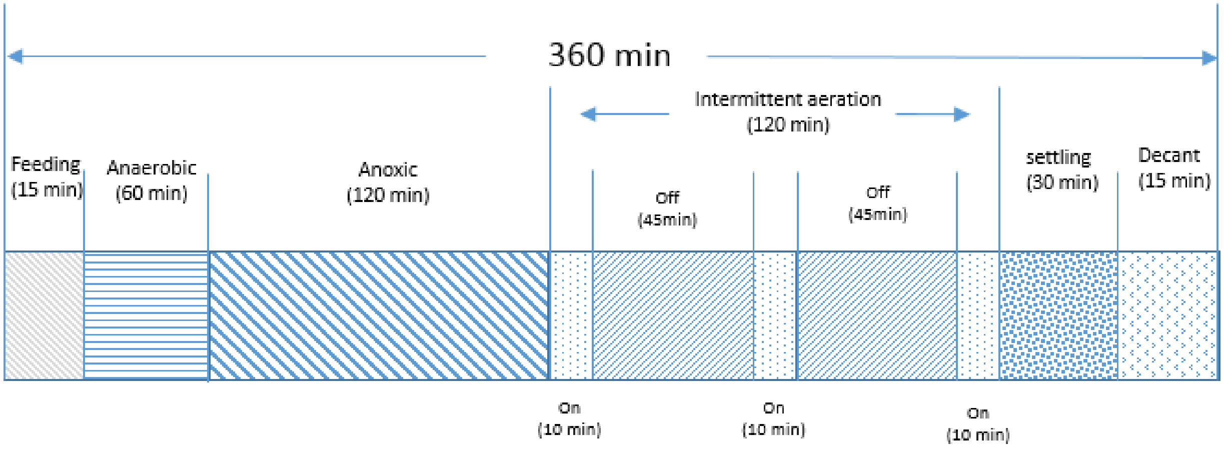


Figure 3-3: System configuration phase two

In phase three (60-100 days) the system operated with 4 cycles a day, 6 h for each cycle, it started with 180 min anaerobic phase, followed by 120 min of continues aeration, 30 min settling, 15 min decanting, the anoxic phase was removed in this phase. Intermittent aeration was re-introduced in Phase four (100-130 days), each cycle consisted of 15 min feeding, 180 min anaerobic reaction, 120 min intermittent aeration, 30 min settling, 15 min decanting. The cycle configuration in Phase five and six (130-170 days) was same as the cycle configuration of phase four only the aeration strategy of the interment aeration was changed.

3.4.1 Aeration strategy

Different aeration strategies were used in this experiment to evaluate the effect of different aeration patterns on the phosphorus removal, the aeration strategy was implemented and controlled by a control box to improve the efficiency and the accuracy of the process, the DO measurements were done using two different DO probes and a correlation between the readings of both probes was done.

There have been a wide range of DO values reported in the literature. (Lemaire et al. 2006) mentioned that DO concentration of 2.5-3.0 mg/L seemed to be a favorable condition for the EBPR operation (Lemaire et al. 2006), as it gives the advantage for the PAO over the GAO, while the DO of 4.5 -5.0 mg/L was more favorable for the GAO. However, high rates of phosphorus removal at DO level of 1.0-1.5 mg/L have been reported by (Frison et al. 2016b, Zhang et al. 2016). The aerobic phase in the majority of the EBPR systems lasts for long period of time to assure total removal of phosphorus, where the aerobic phase is longer than the anaerobic phase. Y.wang et al.201 achieved phosphorus removal at an aeration period of 30 min (Wang et al. 2015b)

An intermittent aeration strategy was implemented in phase one and two, the intermittent aeration phase was set for 120 min, three pulses of DO were introduced to the system, each of the pulses lasted for 10 min followed by a non-aeration phase which lasted for 45 min as shown in Figure 3-4, the DO concentration of phase one was 1 mg/L.

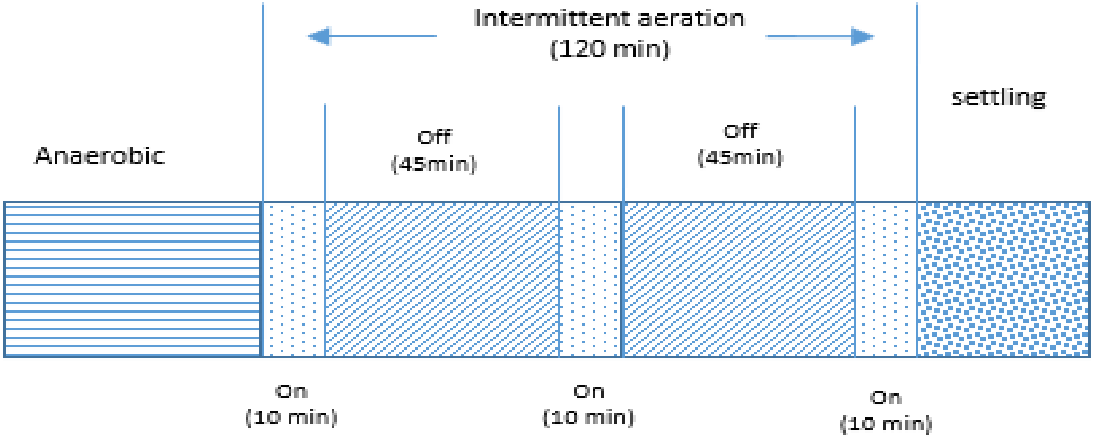


Figure 3-4: Aeration strategy phase 1 and 2

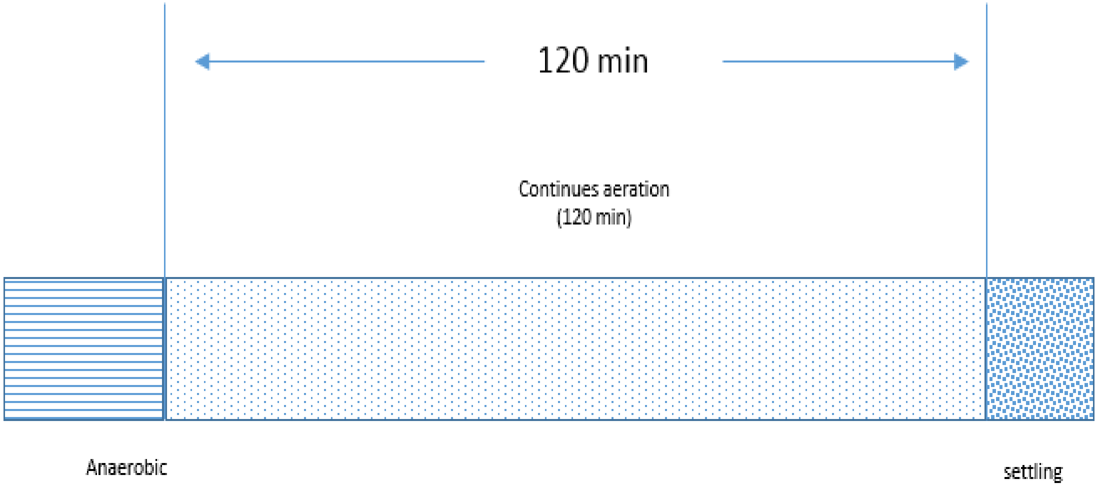


Figure 3-5: Aeration strategy phase 3

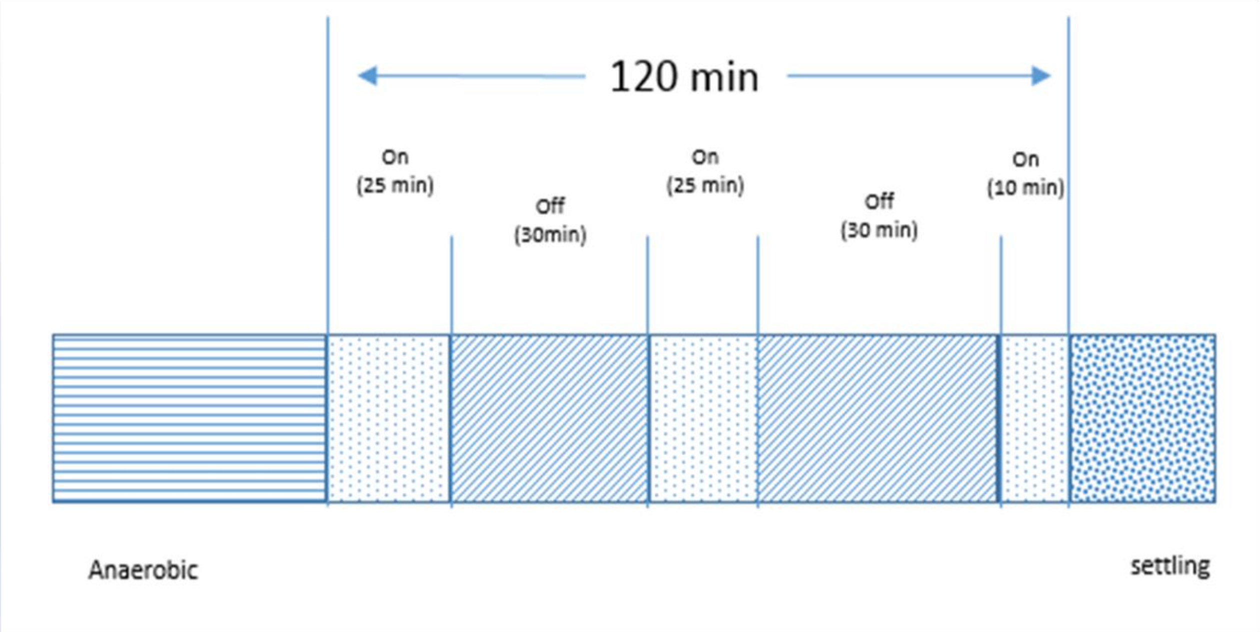


Figure 3-6: Aeration strategy phase 4

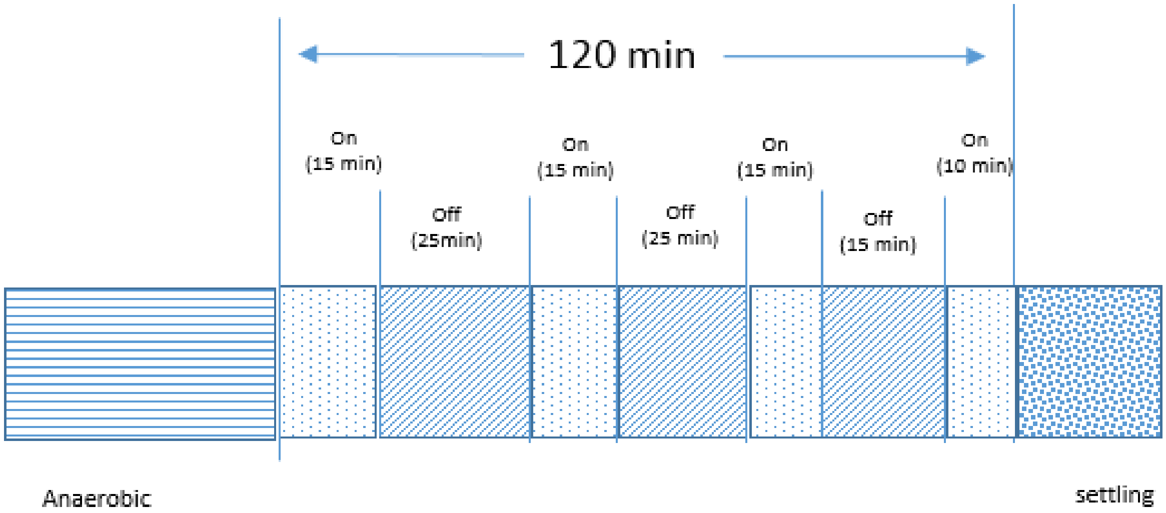


Figure 3-7: Aeration strategy phase 5

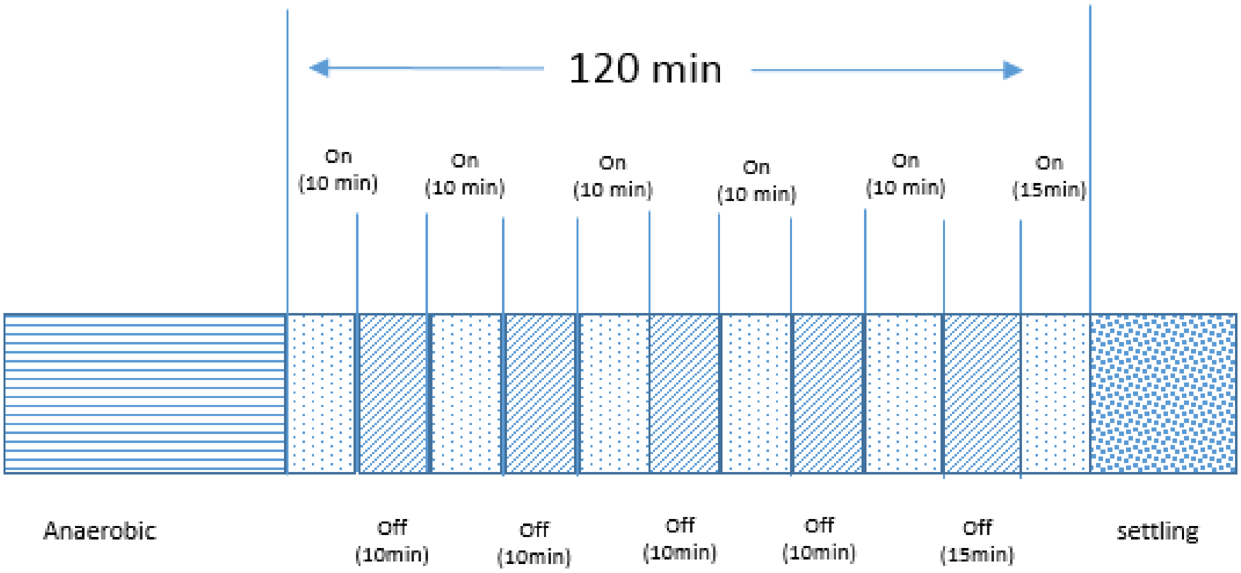


Figure 3-8: Aeration strategy phase 6

A continues aeration strategy was implemented in phase 3 as shown in Figure 3-5, it started with an uncontrolled DO, then gradually the DO concentration was decreased step wisely over the span of 1.5 month, the final DO reach was in the range between 1.8-2 mg/L, the aeration phase lasted for 120 min.

Intermittent aeration was reintroduced in phase four with different aeration pattern, the intermittent aeration phase was set for 120 min, it consisted of 3 pulses of aeration 10-25 min as shown in Figure 3-6, interrupted by two 30 min periods of no aeration, this contributed in a total aeration time of 60 min, and the DO was kept controlled between 1.5-1.8 mg/L. While in Phase five the intermittent aeration phase consisted of 4 aerated pulses 10-15 min of aeration as shown in Figure 3-7, interrupted by three 15-25 min periods of no aeration, this contributed in a total aeration time of 55 min, and the DO was kept controlled between 1.2-1.5 mg/L.

Phase six intermittent aeration pattern consisted of six aeration pulses of 10 min each except for the last pulse it was 15 min, the aeration pulses were interrupted by periods of 10 min as shown in Figure 3-8, and Figure 3-9, of no aeration except for the last period it was 15 min, the DO concentration was at 0.8 mg/L.

Table 3-2: Aeration patterns

	Air on	Air off	Air on	Air off	Air on	Air off	Air on	Air off	Air on	Air off	Air on
Phase1 and 2	**10**	**45**	**10**	**45**	**10**	**-**	**-**	**-**	**-**	**-**	**-**
Phase3	**120**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Phase4	**25**	**30**	**25**	**30**	**10**	**-**	**-**	**-**	**-**	**-**	**-**
Phase5	**15**	**25**	**15**	**25**	**15**	**15**	**10**	**-**	**-**	**-**	**-**
Phase6	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**15**	**15**

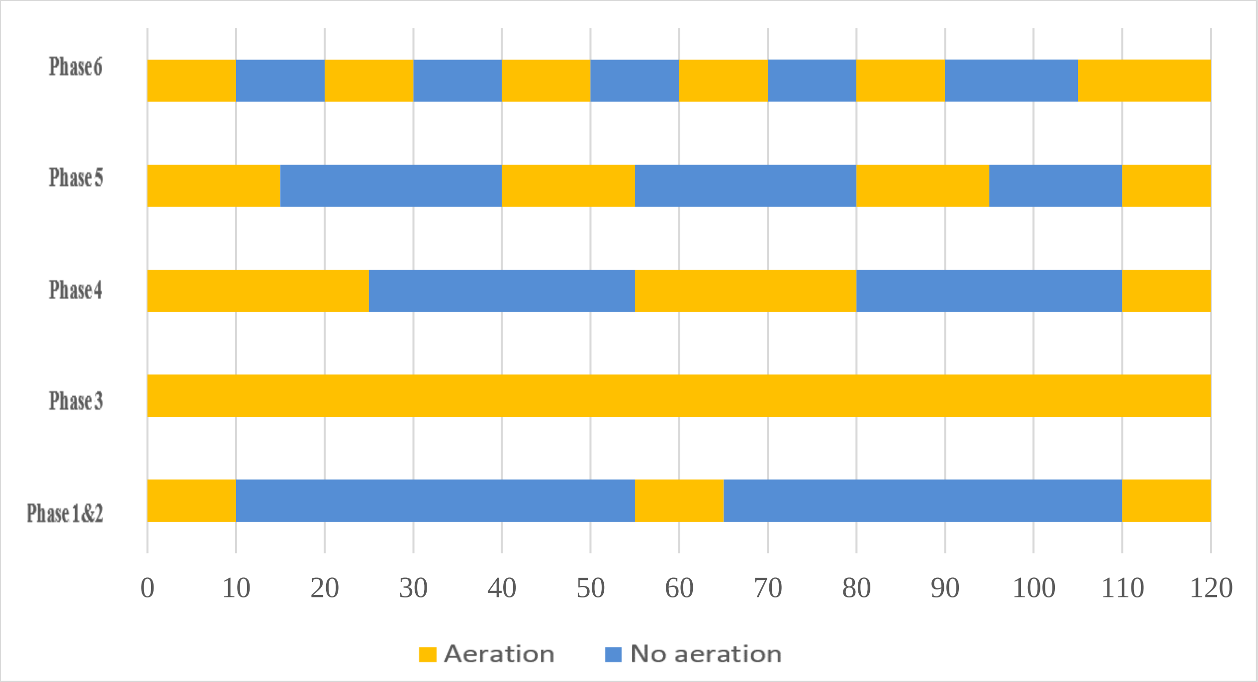


Figure 3-9: Aeration patterns (min)

3.5 Results and discussion

Phase one:

In phase one the system seemed to be suffering under the low DO conditions, at the first week of operation the system had COD a removal efficiency of 95±3%, then the efficiency started to decrease gradually to reach 81%. Through the whole operation of phase one no phosphorus removal has been achieved, the system had low phosphorus release and uptake rate as shown in Figure 3-14, this is attributed to the low DO concentrations, the ammonia removal rate varied between 19-72%.

Phase two

Due to the low removal efficiency of the system in phase one, an anoxic phase was introduced in phase two after the anaerobic phase to provide more electron acceptors in the form of nitrite to help improve the system performance. Initially phosphorus removal improved with a removal efficiency of 15% in the first week of operation, the removal efficiency did not increase with time and plummeted again to zero removal efficiency, both ammonia and COD removal also decreased gradually to reach COD removal efficiency of 57% and ammonia removal efficiency of 19%.

The introduction of nitrite to the system had a negative effect on the performance. The system started to lose biomass after, which can explain the drop in the COD removal efficiency as shown in Figure 3-12, this may have been attributed to the toxicity of the nitrous oxide for the biomass.

Phase three

The anoxic phase was eliminated from the system configuration in phase three, the new configuration consisted of 3 hours anaerobic phase, and 2 hours of aerobic phase with continues aeration pattern without intermittent aeration as shown in Figure 3-15.

The aim was to improve the system performance and rehabilitate the biomass culture after the stress conditions of phase one and two, at the beginning of phase 3 the DO concentration in the aeration phase was uncontrolled, it ranged between 3.5-4.2 mg/L, a gradual improvement in the system performance started to be monitored, the COD, phosphorus and ammonia removal efficiency started to increase gradually due the favorable conditions, at the end of phase 3 the COD removal efficiency reached 99.6% as shown in Figure 3-12, PO_4 removal efficiency reached 92% as shown in Figure 3-11, while the ammonia removal efficiency reached 73 % as shown in Figure 3-10. The MLVSS concentration in the system increased, at the end of phase 3 the MLVSS in the system was 3500 mg/L, the DO was gradually decreased over the span of 40 days to insure no drop in the system performance and that the bacteria was able to coup with graduate decrease in the DO concentration, the DO concentration finally reached a level of 1.8 mg/L.

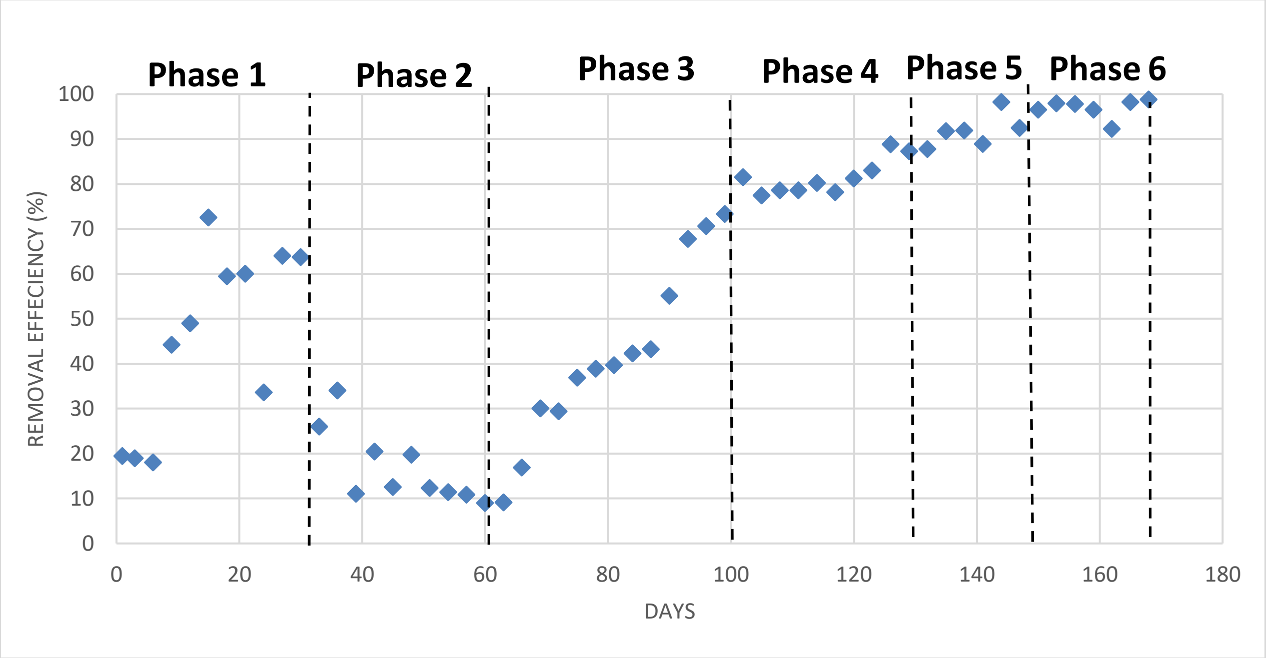


Figure 3-10: Ammonia removal efficiency

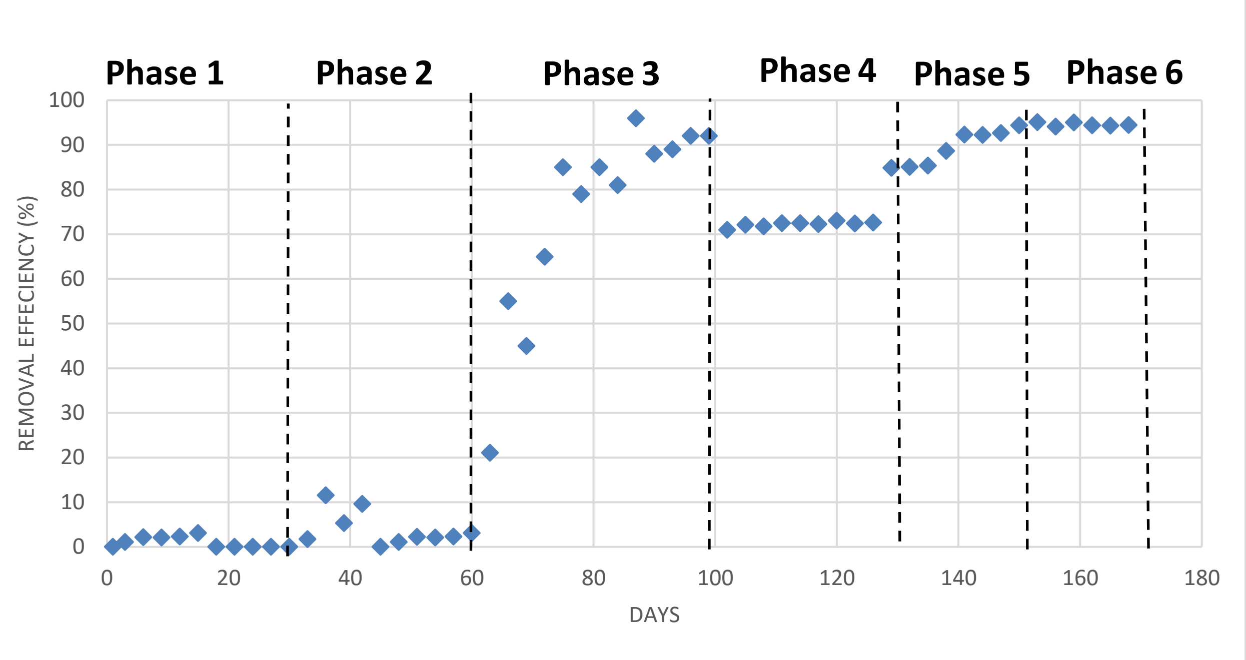


Figure 3-11: Phosphorus removal efficiency

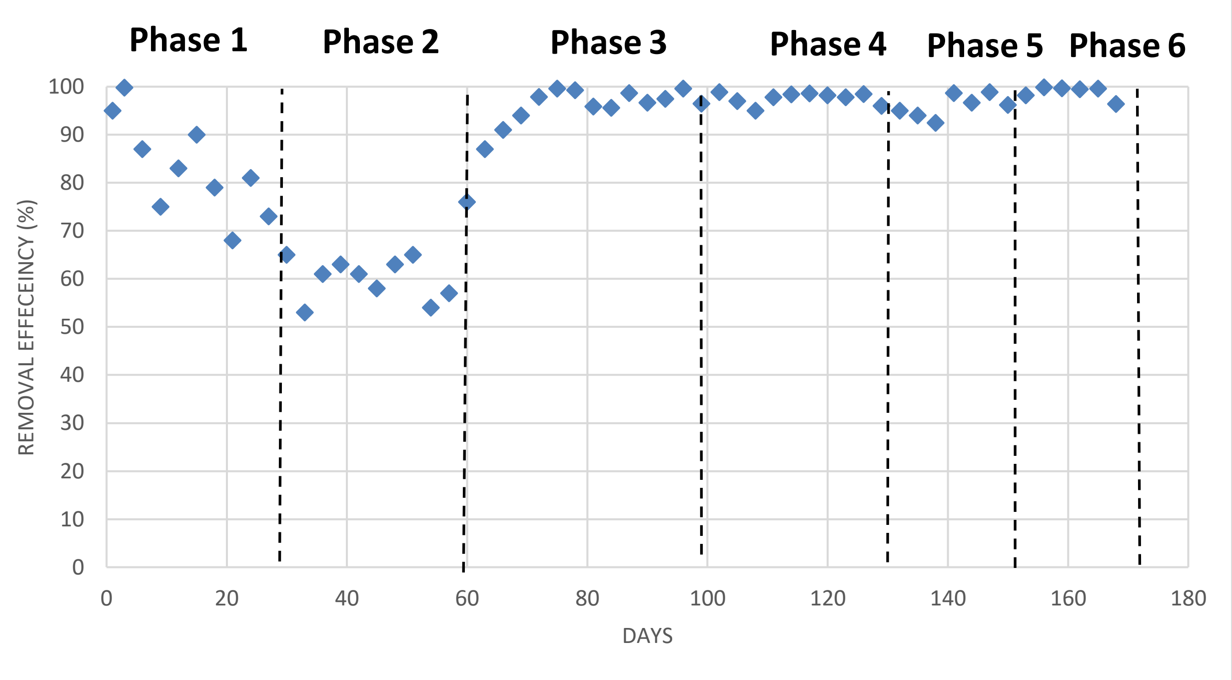


Figure 3-12: COD removal efficiency

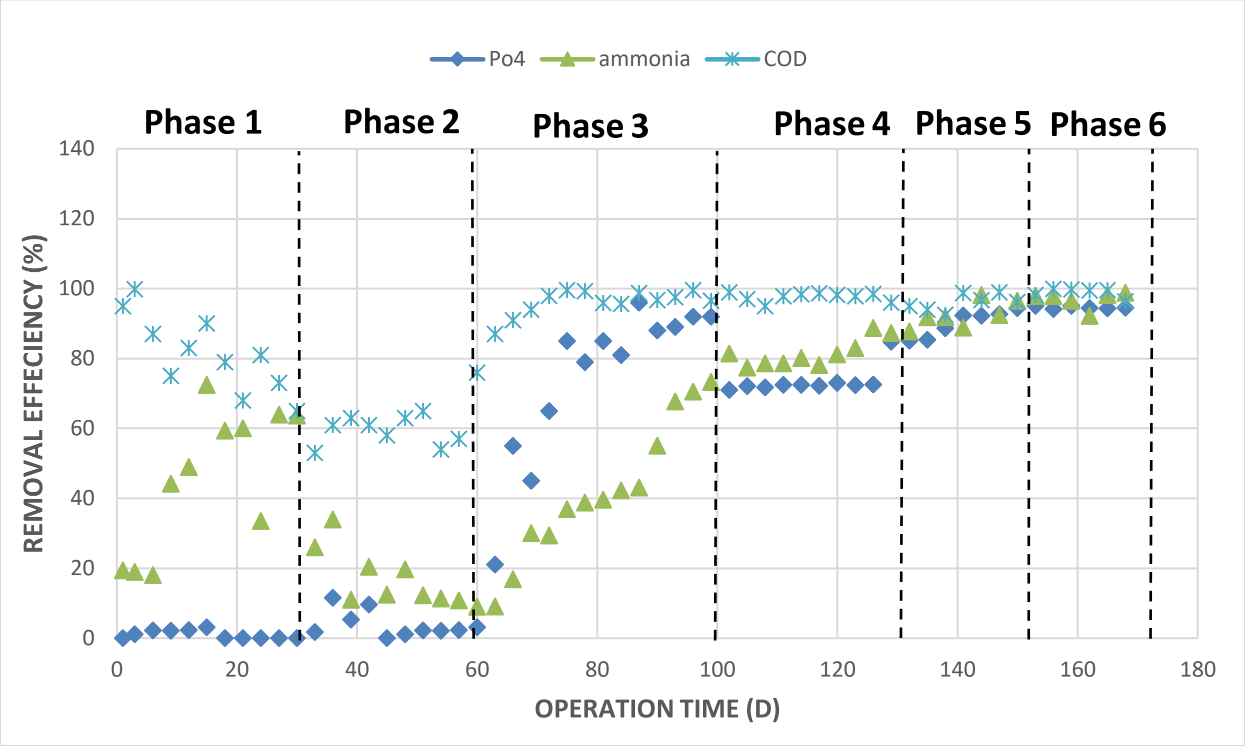


Figure 3-13: System performance

Phase Four

After the re-stabilization of the system in phase 3 Intermittent aeration was reintroduced in phase 4, the system performance witnessed variation in the performance after the reintroduction of the intermittent aeration pattern, the phosphorus removal dropped from 92% to 72 %, while ammonia removal efficiency improved, it increased from 73 % to 80 %, as illustrated in Figure 3- 13 the COD removal efficiency was 99%, the intermittent aeration pattern had a relatively long period of no aeration 30 min this could be the reason for the drop in the phosphorus removal, the long non-aerated period of the intermittent aeration triggered the anaerobic phosphorus release phenomena of the PAO, the phosphorus was initially removed at the aeration phase of the 25 min then started to be re-release again in the subsequent long 30 min of aeration shutoff as illustrated in Figure 3-16, this lead to a decrease in the performance. On the contrary the ammonia removal efficiency did not decrease, since it is performed by a different strain of bacteria different from the PAO, denitrifying bacteria doesn't release the ammonia under anaerobic conditions.

It was presumed according to the Data analysis, that the period of aeration shutoff can have more effect on the performance of the system more than the aerated period, to improve the system performance, the aeration shutoff period should be shortened to suppress the phosphorus re-release, this can be achieved by adding an extra slot of aeration, without increasing the total aeration time, the aeration period will remain the same but will be better if distributed more throughout the intermittent aeration process. Smaller but frequent aeration slots can be better than longer but smaller amount of aeration slots.

Phase Five

To avoid the phosphorus release under anaerobic condition due to the prolonged non-aerated period, the intermittent aeration pattern was changed, both aeration and non-aeration slots were shortened to give the chance for the introduction of an extra aerated slot in-between to cut the span of the anaerobic conditions. This led to an improvement in the performance of the system the phosphorus removal efficiency stepped up to 95%, ammonia removal performance reached 91 %, and COD removal efficiency was stable at 99% as illustrated in Figure 3-13.

It became clear that decreasing the anaerobic conditions in the intermittent aeration pattern can help improve the phosphorus removal performance even if the total period of the aeration conditions did not increase, as the total aeration period counted for 55 min as shown in Figure 3-17.

Phase Six

Reducing both the on and off slots of the aeration pattern helped to improve the system performance as shown in Figure 3-18, there was no re-release of phosphorus under anaerobic conditions, and the short aeration slot did not give enough time for the DO profile to increase in the system, this helped the system to achieve high phosphorus removal efficiency with very DO levels, the DO level at phase six did not exceed 0.4 mg/L, the phosphorus removal reached a level of 99% removal, 99% ammonia removal and 100 % COD removal as shown in Table 3-3.

Table 3-3: Removal efficiency

Phase	**DO(mg/L)**	**Phosphorus removal Efficiency (%)**	**Ammonia removal Efficiency (%)**	**COD removal Efficiency (%)**
1	1	0	72 ± 1.8	81 ± 1.19
2	1	15 ± 2.35	25 ± 2.75	57 ± 2.54
3	1.8-2	92 ± 1.64	73 ± 2.2	99.6 ± 1.5
4	1.5-1.8	72 ± 2.4	80 ± 3.68	99 ± 1.24
5	1.2-1.5	95 ± 1.8	97 ± 1.6	99 ± 3.36
6	0.4	99 ± 2.6	99 ± 1.24	100 ± 3.4

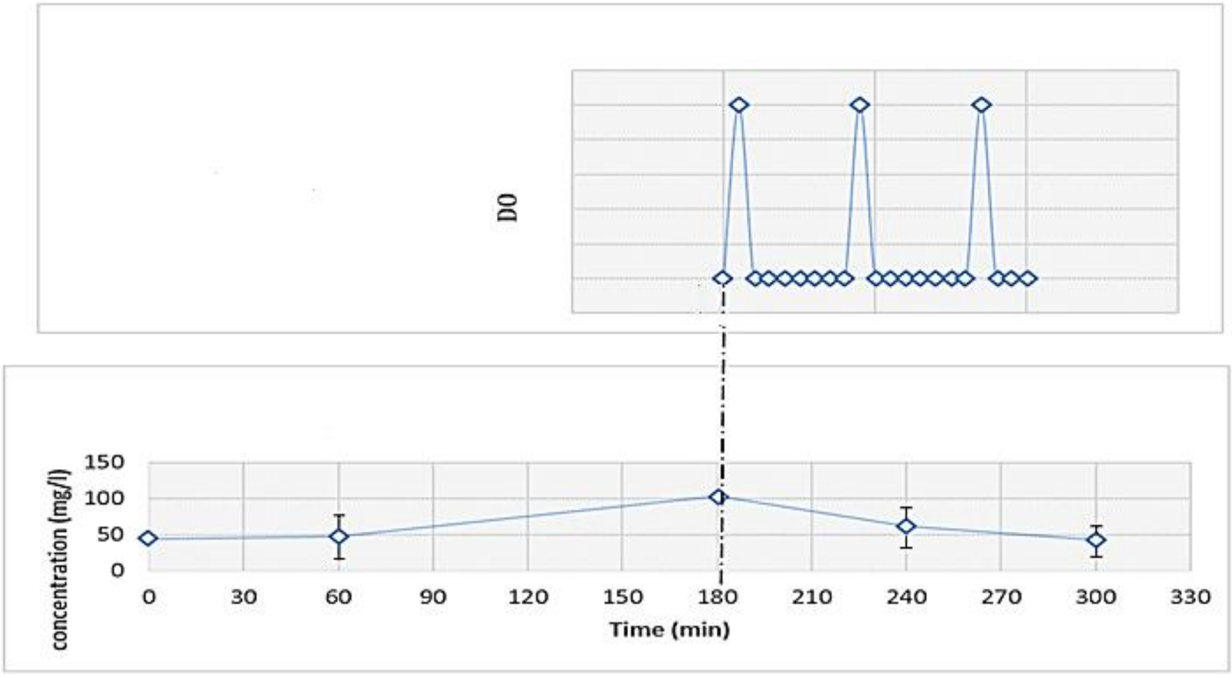


Figure 3-14: Phosphorus profile vs DO phase 1 and 2

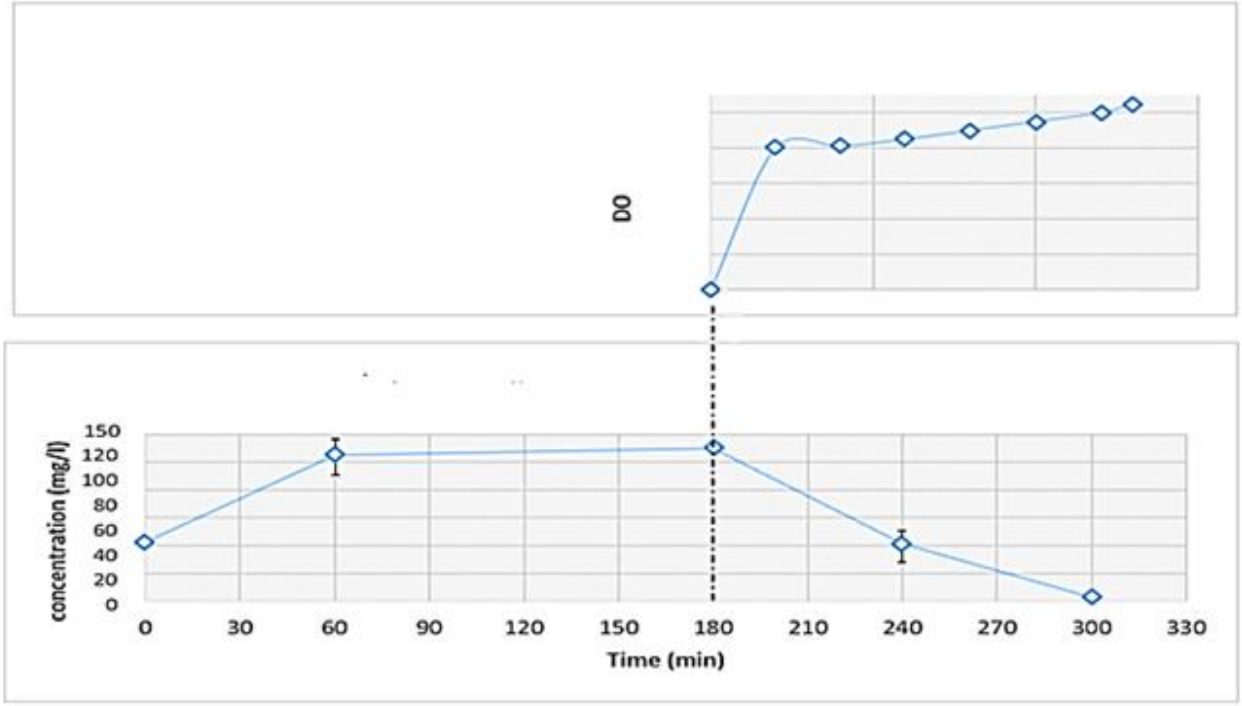


Figure 3-15: Phosphorus profile vs DO phase 3

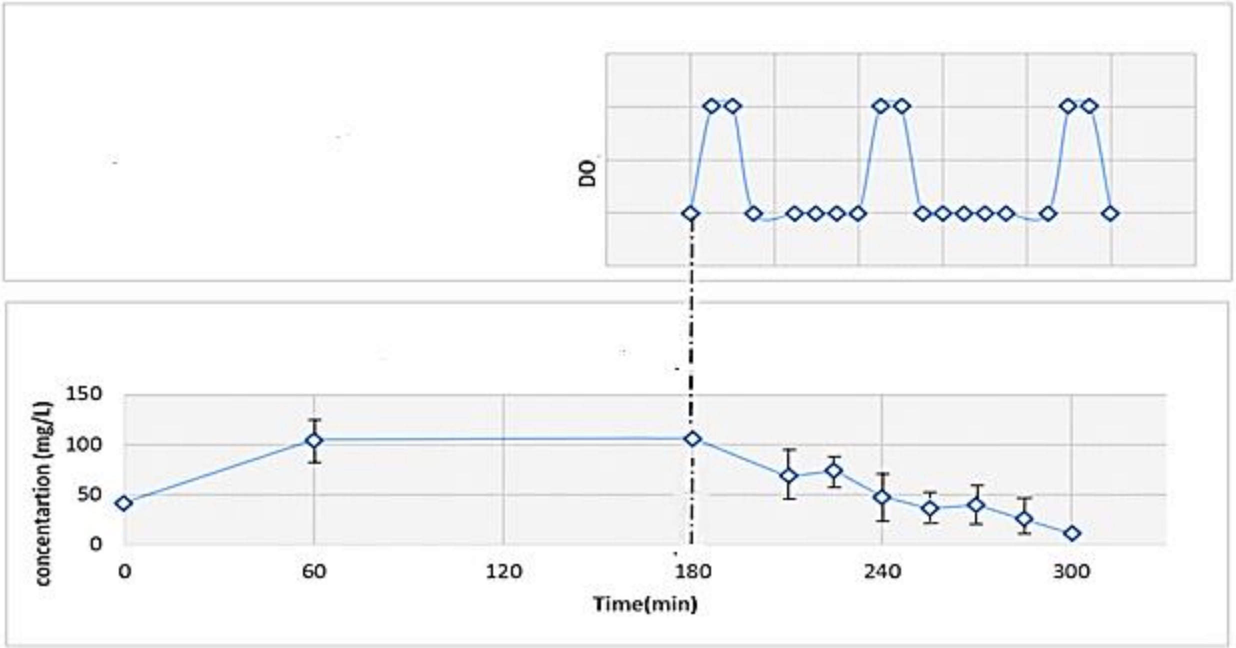


Figure 3-16: Phosphorus profile vs DO phase 4

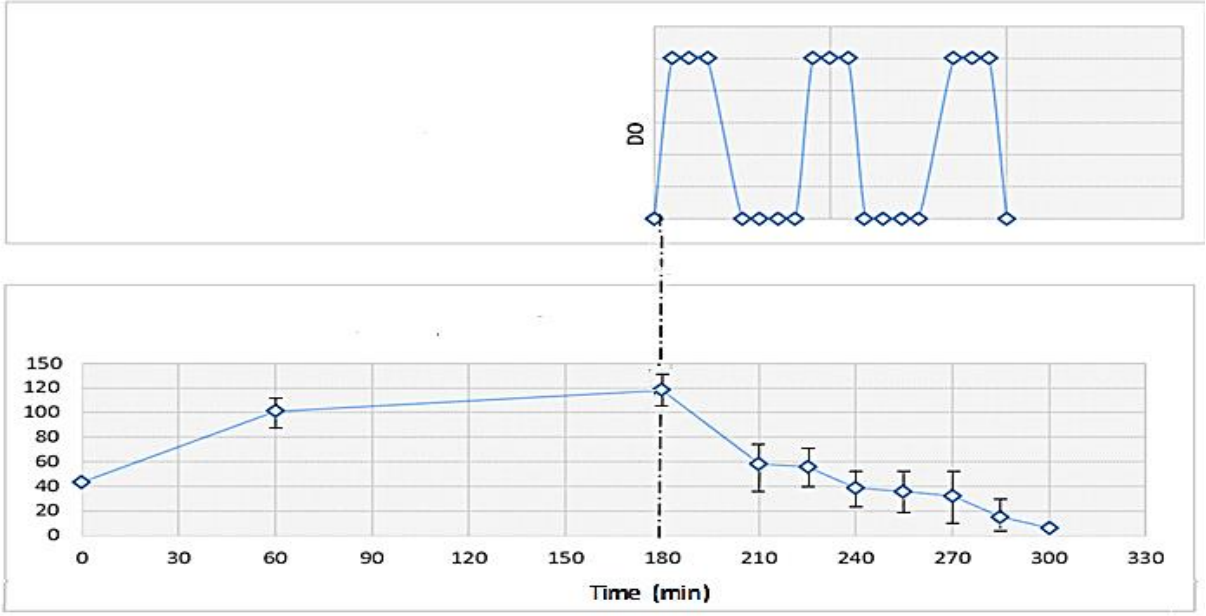


Figure 3-17: Phosphorus profile vs DO phase 5

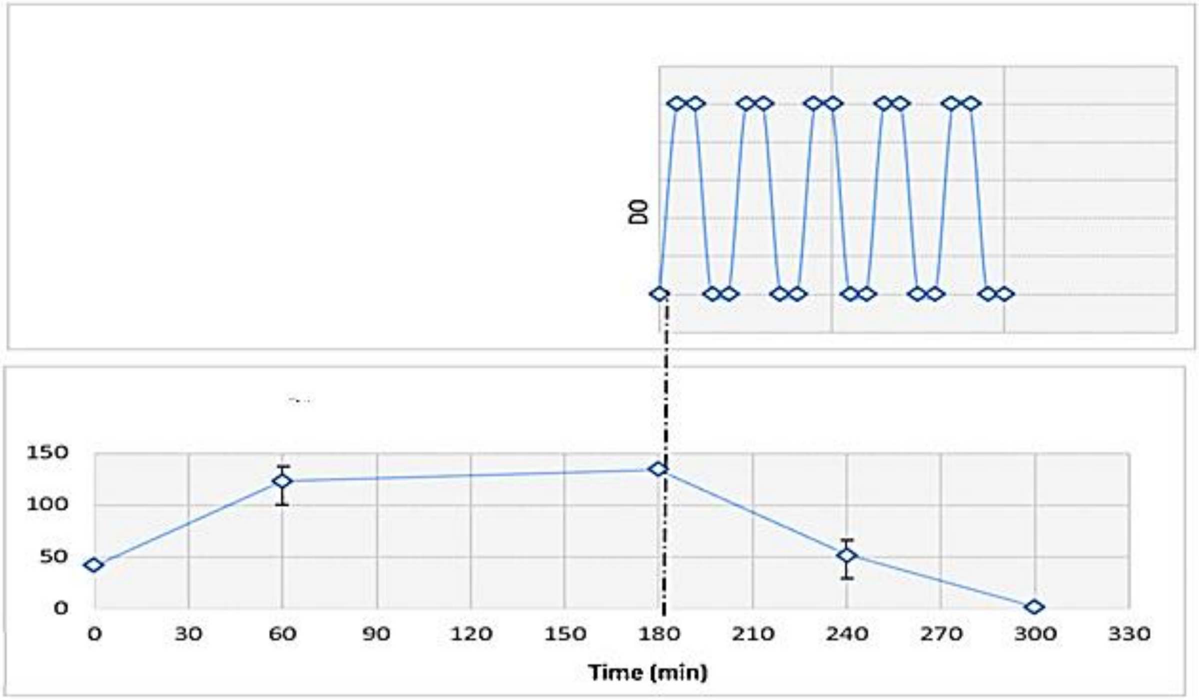


Figure 3-18: Phosphorus profile vs DO phase 6

3.5.1 Phosphorus profile

During the stable operation period of the system, the phosphorus removable efficiency was determined by the phosphorus in the concentration of the influent and effluent, while the activity of the PAO was monitored through the analysis of the phosphorus profile through each cycle.

The influent Po_4 content was 15 mg/L, the P content after the anaerobic stage varied from one phase to another it reached 100-130 mg/L as shown in Figure 3-19, the final P content phosphorus in the effluent also varied according to each phase, in the successful phases it was in the range of 0.2-3 mg/L as shown in Figure 3-19.

The analysis showed that oxygen requirements and intermittent aeration duration are one of the main factors affecting the phosphorus removal process, at phase one and two due to low DO concentration, and long anaerobic period during the intermittent aeration, the phosphorus was not being removed from the system. The continues and high DO concentration in phase three lead to great improvement in the phosphorus removal, the reintroduction of the intermittent aeration pattern lead to a decrease in the performance due to the large anaerobic period in the intermittent aeration process, through the consecutive phases, the anaerobic phases in the intermittent aeration were shortened to stop the PAO from rereleasing of the phosphorus again into the system, this improved the phosphorus removal performance of the system to reach a removal efficiency of (100 ± 2.4%).

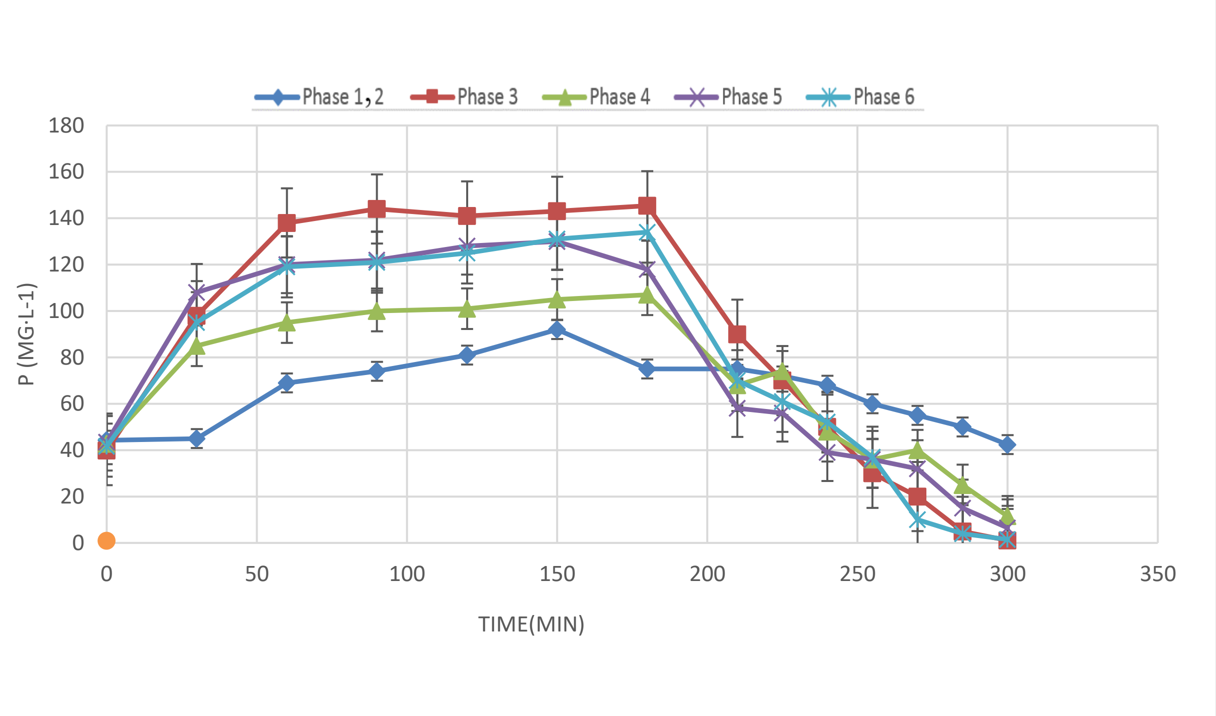


Figure 3-19: Phosphorus removal profiles

3.6 Conclusion

An EBPR system was successfully demonstrated for high level phosphorus removal, with low DO concentrations. Combined aeration strategies and optimization techniques enabled the system to overcome low aeration requirements challenges. The system was able to achieve high removal efficiency of Phosphorus 99 ± 3.24% and COD removal Efficiency 100 ± 2.4%. Intermittent aeration proved to be a successful technique for providing the aeration requirements for EBPR systems, shortening the anaerobic period of the intermittent aeration improved the phosphorus removal and inhibited phosphorus release during intermittent aeration phase due to long anaerobic conditions.

Chapter 4

Effect of acetate to propionate ratio on biological phosphorus removal under Low DO parameters

4.1 Introduction

Polyphosphate accumulation organism (PAO) are the main organisms responsible for the phosphorus removal in EBPR system, PAO consumes volatile fatty acids (VFAs) under anaerobic conditions and convert them to poly-bhydroxyalkanoates (PHAs) (Oehmen et al. 2005c), PAO drives its energy for the process from the hydrolysis of glycogen and polyphosphate, they are two polymers stored internally in PAO cells. Glycogen is glycolysised to Co_2 and PHA, while Polyphosphate breaks down to orthophosphate and released into water (Oehmen et al. 2005b), this results in an increase in phosphorus levels in wastewater. Under aerobic conditions PHA oxidation becomes the main source for energy gain and cell synthesis.

Performance setback and process upsets and deterioration has been frequently reported and documented in EBPR systems, the reasons for the system deterioration is not completely fully understood. However, in many cases the performance setback was attributed to source of COD used. Many studies have attributed the system deterioration to the presence of another group of microorganisms called glycogen-accumulating organisms (GAOs). GAO competes with PAO over the VFA sources during anaerobic condition, similar to PAO, GAOs take up VFAs under anaerobic conditions and convert them to PHAs, except that GAO hydrolyze glycogen as their sole source of energy rather than polyphosphate such as in the case of PAO (Erdal et al. 2003). GAOs do not achieve anaerobic phosphorus release or aerobic phosphorus uptake; therefore GAO do not achieve phosphorus removal in EBPR systems. Since the GAO are able to anaerobically consume VFA likewise the PAO, which leads to a competition on the limited amounts of VFA present in the influent, inhibition of the GAOs is essential to eliminate system setbacks and deterioration (Griffiths et al. 2002b).

While traditionally acetate has been the most common type of VFA used in EBPR systems, since it is usually the most common form of VFA found in the majority of wastewater influents (Zeng et al. 2004, Xie et al. 2017). However, recently propionate have gained more attention in many generated research, and is being seen as a useful factor for giving the advantage for PAO over the GAO (Pijuan et al. 2004), this can be found in the work done by (Crocetti et al. 2000,Chen et al. 2004,Cech and Hartman 1993,Jeon and Park 2000). In a research conducted by (Oehmen et al. 2005d) it was found that cultures of Competibacter were not able to metabolize propionate under anaerobic conditions, on the contrary Accumulibacter were able to metabolize both Acetate and propionate under anaerobic conditions, in another research conducted by oehman et al. 2005, two reactors with same operational conditions, one was fed with acetate the other was fed propionate, the propionate fed reactor showed more stable and consistent performance (Oehmen et al. 2005a).

Dissolved oxygen concentration can have a great effect on the competition between GAO and PAO (Griffiths et al. 2002a), it was found that high DO levels between(3.5-6)mg/L are more favorable for the GAO activity, while Low levels (1.5-2.5)mg/L, gave the advantage for the PAO over the GAO (Oehmen et al. 2007b).

In this experiment different ratios of propionate and acetate were used as carbon source used, coupled with low DO conditions, to investigate the effect of carbon source under low conditions on the EBPR system, to eliminate system fluctuations and setback.

4.2 Materials and Methods

4.2.1 Reactor design and operation

A laboratory-scale sequencing batch reactor (SBR) with a working volume of 5L was used to perform enhanced biological phosphorus removal (Fig 1). The system was kept at a room temperature (22 ± 1 °C) while the pH was not controlled and varied between 7.3 and 8. DO set points were controlled using a DO probe connected to a control box as shown in Figure 4-1. Electric stirrer was used to constantly keep mixing the reactor during the aerobic and anaerobic phase.

The system was seeded with fresh activated sludge; the MLVSS was 5000 mg/L. The hydraulic retention time (HRT) was 12 h, while the solid retention time (SRT) was not controlled but calculated and found to be approximately 22 days.

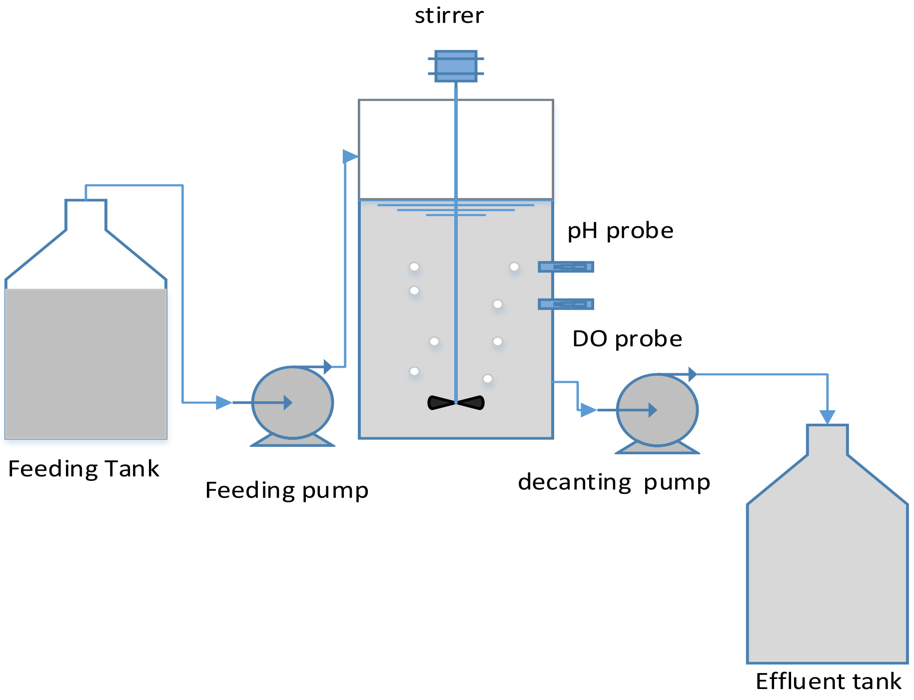


Figure 4-1: Schematic diagram of the SBR used in this experiment

4.2.2 Synthetic wastewater

For the SBR synthetic wastewater was used through all the experiment Containing an organic concentrated solution and mineral salt solution, the solution was diluted from the stock solutions of acetate and/or propionate and mineral salts. The synthetic wastewater contained per (1L) the following in Table 4-1:

Table 4-1: Synthetic wastewater composition

Chemical	**Equivalent (mg/L)**
CH_3CH_2COONa	300
CH_3COONa	300
KH_2PO_4	7.5
K_2HPO_4	7.5
NH_4Cl	10

4.3 Analytical methods

Solid and liquid phase analyses of soluble orthophosphate (PO^{3-}_4–P),COD, (NH_4^+-N), (MLSS), and (MLVSS) were performed by the standard method , samples were collected 3 times per week at different stages throughout the experiment operation: firstly a sample was collected at the influent, second sample after 1 hour of operation(anaerobic reaction), third sample after 2 hours of operation (anaerobic reaction), fourth sample at end of anaerobic stage, fifth sample after 1 hour of aerobic reaction, a last sample was collected from the effluent. Approximately 20 ml of each sample was filtered through 0.45-µm membrane filter and was stored in a cold room with temperature less than 5°C. Phosphate (PO^{3-}_4 –P), Ammonia (NH_4^+-N), COD, Total suspended solids (TSS), Volatile suspended solids, were all measured following the procedure described at (Rice, et al 2014).

4.4 Process methods

The SBR system was operated for 140 days, each day consisted of 4 cycles, 6 h for each cycle, and each cycle consisted of 15 min feeding, 180 min anaerobic phase, 120 min aerobic phase as shown in Figure 4-2.

Different compositions of COD substrate were used, in phase one acetate was used as a sole source of carbon, in phase two propionate was used as a sole source of COD, in phase three a composition of 75% propionate and 25% acetate was used, in phase four the composition was 50 %acetate and 50% propionate, in phase five 25% propionate and 75% acetate, finally in phase 6 acetate was reintroduced as a sole carbon source.

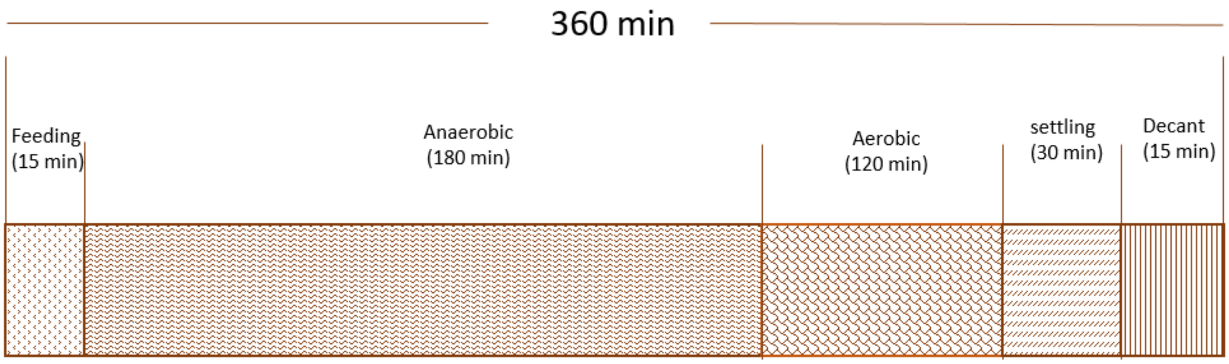


Figure 4-2: SBR system configuration

5. Results and discussion

Phosphorus removal performance

The SBR was operated for 140 days with no change in operational conditions except for the composition of COD used and DO concentration. DO levels were stepwise decreased throughout the operation to observe the effect of low DO concentrations with the change in the acetate to propionate ratio. After the initial period of system stabilization, measurements of phosphorus in the system started to be documented. The system operation was divided into five phases, phase one lasted for 30 days, the system witnessed fluctuation in the system performance in phase one the average phosphorus removal efficiency in phase one was around 65%, 96% being the highest removal efficiency and 45% being the lowest as shown in Figure 4-3.

The aeration pattern in phase one was continues uncontrolled aeration, the DO concentration was not controlled but measured, it varied between 4.2-4.8 mg/L which is considered to be a relatively high DO concentration for an EBPR system. The fluctuation in the system performance in phase one can be attributed to slight dominance of GAO in the system, the combination of acetate as a carbon source coupled with high DO concentrations of 4.0–6.0 mg/L, is considered to be a favorable conditions for the GAO, on contrary to the PAO becomes more dominant in systems that have a DO concentrations of around 1.5–2.5 mg/L (Oehmen et al. 2007b,Whang and Park 2002,Carvalheira et al. 2014).

In phase 2 propionate became the sole carbon source for the system, the initial DO concentration was 3.0mg/L and was systematically decrease throughout the phase to reach finally a DO level of 1.2 mg/L. the system performance improved and stabilized in phase two, the average phosphorus removal efficiency in phase two improved to reach 100 ± 3.24%, the average removal efficiency was 99.2 ± 2.24%, this removal efficiency became the highest removal efficiency among all phases.. The improved performance can be correlated with (Pijuan et al. 2004), where propionate was found to be more favorable for the PAO over the GAO, Also low DO concentration are more favorable conditions for the PAO.

In phase 3 a composition of 75% propionate and 25% acetate was used, the initial DO concentration was kept at 1.2 mg/L, the average phosphorus removal efficiency in phase three was around 95%, 98% being the highest removal efficiency and 93% being the lowest. In phase 4 the composition was 50 %acetate and 50% propionate, the DO concentration was kept at the same level of phase 2 and 3 with DO concentration of 1.2mg/L.

The phosphorus removal efficiency in phase4 decreased, the average phosphorus removal efficiency in phase 4 was around 93 ± 3.2%, in phase 5 a composition of 25% propionate and 75% acetate was used, the removal efficiency dropped again, the average removal efficiency was 91 ± 1.24%, a fluctuation in system performance started to be noticed, this can be attributed in increase in the acetate portion of the carbon source. Finally in phase 6 acetate was reintroduced as a sole carbon source, the turbulence in the system performance increased and became more visible, the average removal efficiency became 85 ± 2.24%, in both phases 1 and 6 acetate was the sole carbon source, the average removal efficiency in phase 1 was 65 ± 3.1% while in phase 6 was 85% as shown in Figure 4-6, the reason for the improved system efficiency in phase 6 can be attributed to the low DO concentration which gave the advantage for the PAO over the GAO.

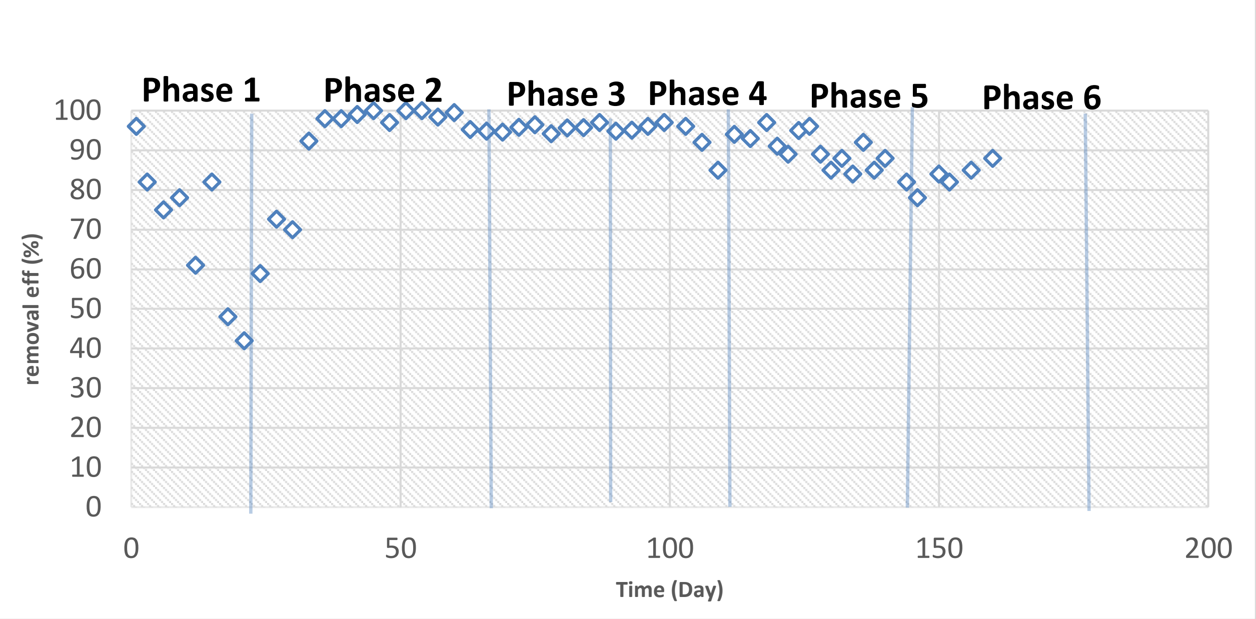


Figure 4-3: Phosphorus removal efficiency

COD removal performance

COD removal has been steady throughout all the process phases the average COD removal efficiency was 99%. The steady COD removal coupled with low phosphorus removal efficiency can be a strong indication on GAO domination in the system, which has been seen in phase 1. In the later phases both COD and phosphorus removal efficiency were high as illustrated in Figure 4-4, which is an indication on PAO domination in the system.

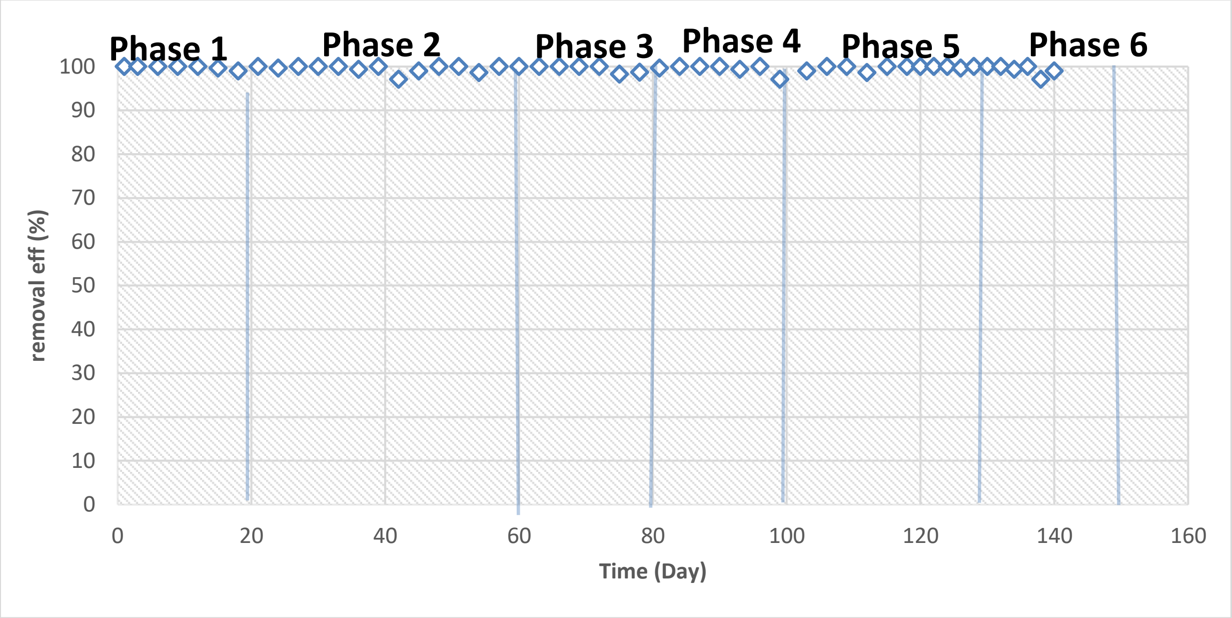


Figure 4-4: COD removal efficiency

Nitrogen removal efficiency

Ammonia removal efficiency steadily increased over the course of the experiment regardless which carbon source was used, however, the system showed consistent ammonia removal with no fluctuation in performance when propionate was used as a sole carbon source, the low ammonia removal efficiency in phase one can be attributed to the slow growth of nitrifying bacteria in the system as illustrated in Figure 4-5, as it took longer time for them to develop the needed bacterial culture.

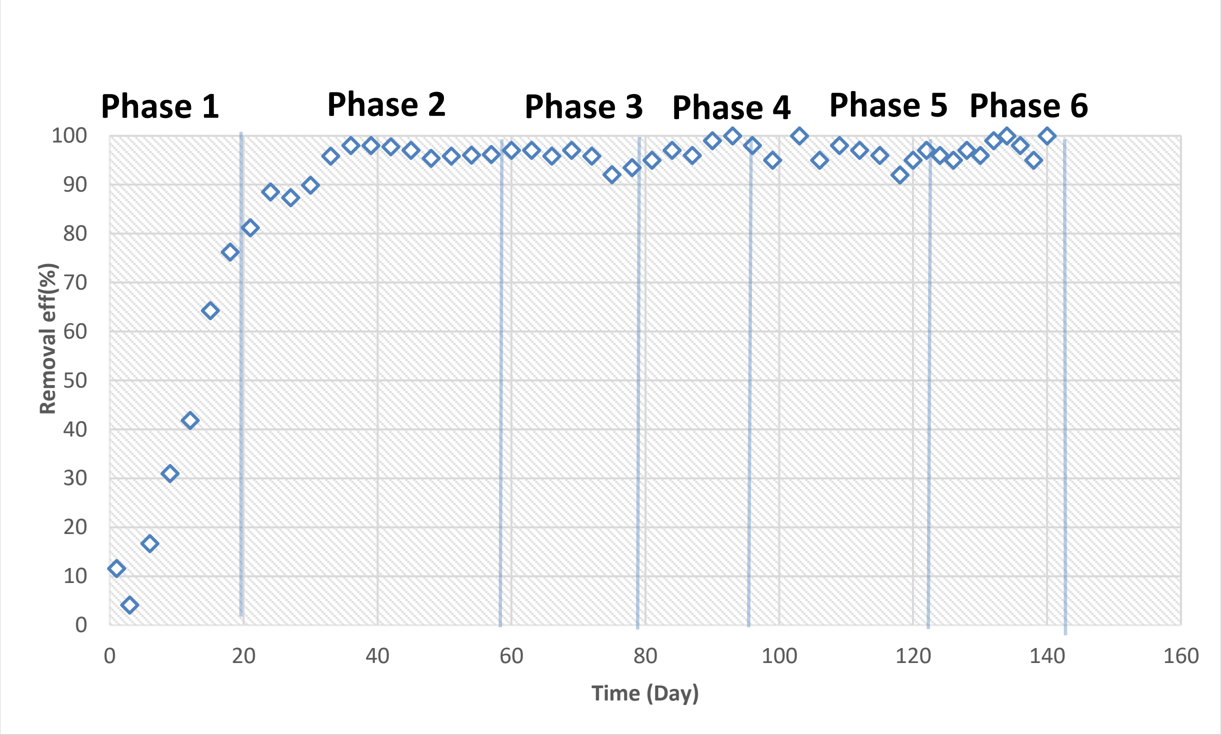


Figure 4-5: Ammonia removal efficiency

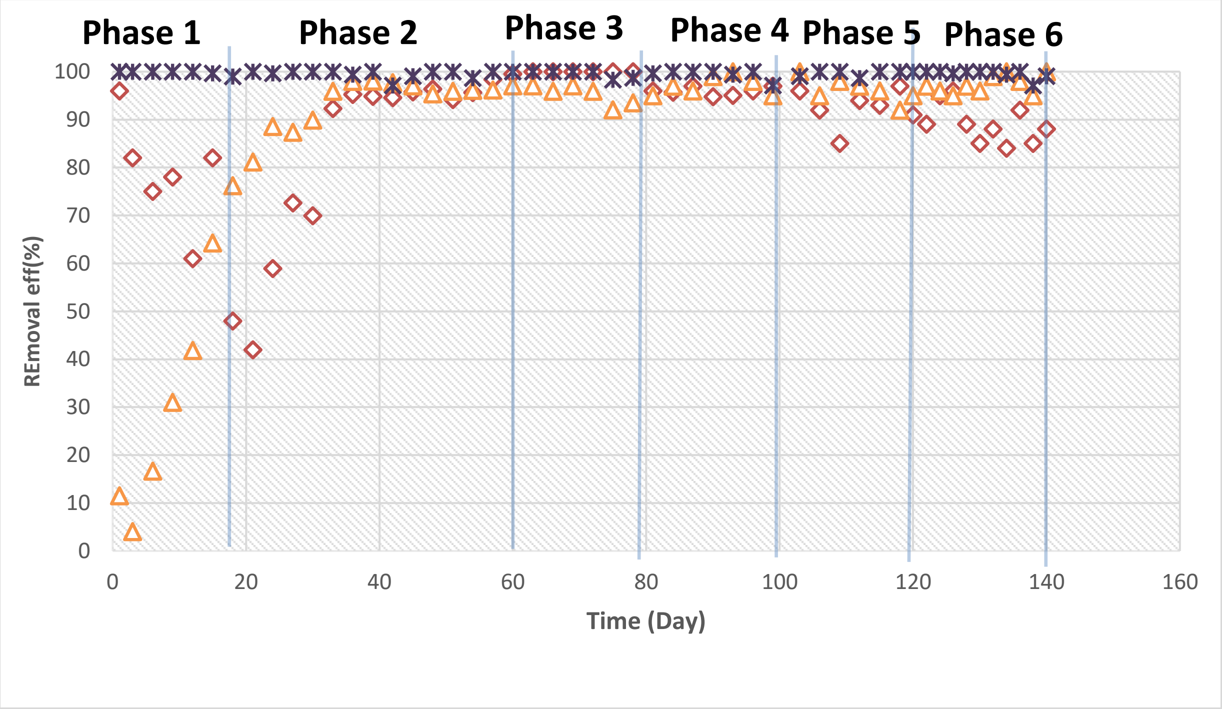
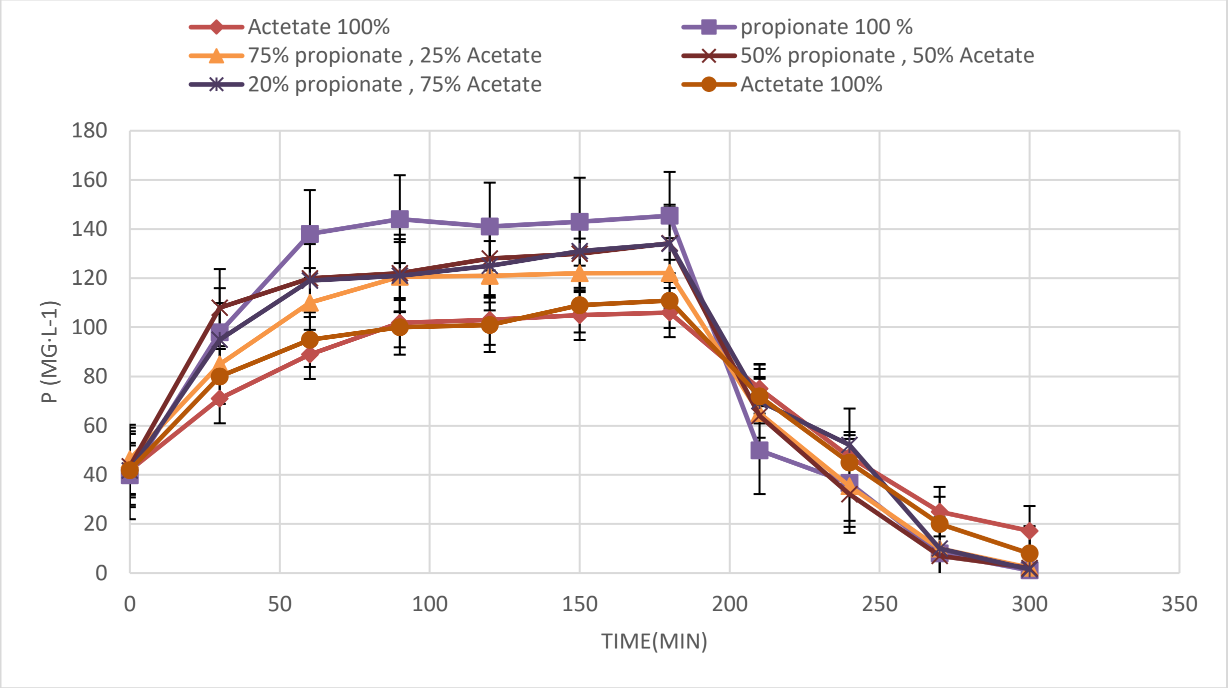


Figure 4-6: System performance

Changes of phosphorus, COD profile

Further analysis for the system performance was made, through investigating the biochemical transformation of COD and phosphorus. During the anaerobic phase COD was almost totally depleted regardless of the Carbon source used.

High levels of anaerobic phosphorus release were monitored in phase 3 where 75% propionate and 25% acetate was used, with a rate of 140.09 ± 4.4 mg/L, the lowest anaerobic phosphorus release was observed in phase 1 while acetate was the sole carbon source, the anaerobic phosphorus release seemed to decrease as the acetate ratio increased in the COD composition, except for the phase 3 where 75% propionate and 25% acetate had a higher phosphorus than phase 2 where propionate was the sole carbon source 130.09 ± 3.2 mg/L as shown in Figure 4-7.



. Figure 4-7: Phosphorus profile

6. Conclusion

In the experiment, the effect of different ratios of propionate to Acetate on the efficiency of phosphorus removal in EBPR systems was investigated under low DO levels. During the operation the increase in Acetate fraction lead to a decrease in the system performance. The average phosphorus removal efficiency was as follows: phase 1 (65 ± 3.1%), phase 2 (99.2± 1.2%), phase3 (95± 2.5%), phase 4 (93.3± 3.2%), phase 5 (91± 1.2%), phase 6 (85± 2.5%). These results suggested that propionate can be a more effective source of COD for EBPR systems more than Acetate, through providing PAO bacteria with a competitive advantage over GAO bacteria. Acetate fed systems frequently showed unstable phosphorus removal performance, mainly due to competition by GAOs for Acetate uptake. However, propionate fed systems showed a high and stable level of phosphorus removal and low effluent phosphorus concentrations.

Chapter 5

Conclusions and future work

5.1 Conclusion

The main objective of this thesis is to develop an efficient and sustainable system for Enhanced biological phosphorus removal, through applying different optimization techniques, to improve the system performance while decreasing the running costs of operation.

The main achievements were as follows:

- Stable biological phosphorus removal has been achieved in a SBR process for EBPR with removal efficiencies of: **phosphorus removal Efficiency (99%), COD removal Efficiency (100%).**
- High rates of phosphorus removal have been achieved under very low DO concentrations (0.4-0.8 mg/L).
- The developed DO control strategy using intermittent aeration technique has been proved to be an effective strategy for providing aeration for EBPR process.
- Propionate was found to be the best carbon source for EBPR process, after different compositions of COD were used as a carbon source for the EBPR process.

- The combination of low DO concentrations and propionate as a carbon source has been found to be a successful approach in controlling the competition between GAO and PAO in EBPR systems.

5.2 Future work

Considering the great potential of the Enhanced Biological Phosphorus removal process, several research points can be suggested for future work to further enhance the EBPR process and reduce the energy need of WWTPs:

- Developing a system to achieve both Enhanced Biological Phosphorus removal and partial nitrification processes in the same reactor.
- Studying the effect of stepwise increase in the nitrite concentration in EBPR systems and its effect on the DPAO bacteria
- Preforming batch test to figure out optimum anaerobic reaction time.
- Studying the viability of shortcut phosphorus removal as a novel process that aims to improve the EBPR performance.

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