Universitat Autònoma de Barcelona

# INTEGRATING ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL (EBPR) IN A RESOURCE RECOVERY SCENARIO

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## Doctoral thesis

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CERTIFICAMOS:

Que el ingeniero químico CARLOS ROBERTO CHAN PACHECO ha realizado bajo nuestra dirección, el Trabajo que con título "INTEGRATING ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL (EBPR) IN A RESOURCE RECOVERY SCENARIO", se presenta en esta memoria, y que constituye su Tesis para optar al Grado de Doctor por la Universitat Autònoma de Barcelona dentro del Programa de doctorado en Ciencia y Tecnología Ambiental.

Y para que se tanga conocimiento y conste a los efectos oportunos, presentamos a la Escuela de Doctorado de la Universitat Autònoma de Barcelona la presente tesis, firmando el presente certificado en

Bellaterra, 5 de septiembre de 2018

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# RESUMEN

El agotamiento de muchos de los recursos naturales vitales para nuestra especie constituye uno de los principales problemas a los que se enfrenta el planeta, y ello ha llevado a la comunidad científica a la búsqueda de soluciones inmediatas para resolver este problema. Actualmente, para contrarrestar esta situación se está mirando hacia fuentes, que en décadas pasadas eran impensables, tales como las aguas residuales. Las aguas residuales son una fuente en potencia de diferentes recursos tales como energía, biopolímeros y nutrientes. Sin embargo, estos no son aprovechados ya que se pierden durante el tratamiento de estas aguas que, de un modo resumido, se centran en eliminar y degradar contaminantes

El fósforo (P) es un recurso que, de ser recuperado de las aguas residuales, podría reciclarse para su uso en la sociedad. Este elemento tiene un papel importante para la vida, ya que está implicado estructuralmente tanto en las membranas celulares como en los huesos. Además, es fundamental para el desarrollo de la agricultura ya que es uno de los principales constituyentes de los fertilizantes. En la actualidad, la principal fuente de P son las rocas fosfáticas. La explotación de esta fuente ha llevado a una disminución en la calidad de las reservas y según estimaciones se prevé que podrían llegar a suponer un problema de abastecimiento entre los próximos 50 y 100 años.

Si bien es cierto que el P es importante para la preservación de la vida, también es un potencial agente contaminante. El vertimiento de aguas ricas en P a ríos y lagos es el principal causante del fenómeno conocido como eutrofización, fenómeno que produce un deterioro en la calidad de estos cuerpos superficiales de aguas debido a un crecimiento excesivo de algas y plantas. Esto ha llevado a la creación de procesos de tratamiento para la eliminación del P de las aguas residuales, siendo la opción más empleada la precipitación química mediante la adición de sales inorgánicas. Sin embargo, este método presenta muchas desventajas entre las que se encuentran el elevado coste y la excesiva producción de fangos químicos sin ningún uso en la sociedad que simplemente se desechan. Por otro lado, el proceso de eliminación biológica de P (Enahanced Biological Phosphorus Removal, EBPR) está ganando terreno y está siendo implementado en estaciones depuradoras de aguas residuales (EDARs) de todo el mundo. El proceso EBPR se basa en la actividad metabólica de un grupo de bacterias llamados organismos acumuladores de P (Polyphosphate Accumulating Organisms, PAO) para eliminar este nutriente de las aguas residuales.

Aunque si bien el proceso EBPR se ha implementado de manera exitosa a escala real, puede no encajar con la perspectiva de las EDARs modernas, ya que éstas actualmente están siendo diseñadas para funcionar como biorrefinerías. Es decir, sistemas en donde se recuperen recursos y/o energía llevando implícito la utilización eficiente de recursos. Es por ello que la investigación desarrollada en esta tesis se ha enfocado principalmente en obtener sistemas SBR-EBPR más sustentables, caracterizados por un mínimo consumo de energía, producción de metano y recuperación de P. Bajo este enfoque, se estudió a largo plazo un sistema SBR-EBPR cuya configuración fue modificada con la finalidad de obtener un líquido altamente enriquecido en P, el cual pueda usarse en un proceso de recuperación en forma de una precipitación de un mineral con elevado interés para nuestra sociedad (por ejemplo, la estruvita, un fertilizante de liberación lenta) (capítulo 4). También se ha estudiado el comportamiento de la actividad EBPR usando tiempos de retención celular (Sludge Retention Time, SRT) cortos, comentadas en la bibliografía como uno de los factores que obstaculizan la integración del proceso EBPR a sistemas energéticamente más eficiente (por ejemplo, el A/B process) (capítulo 5 y 6). Por último, se estudió la influencia del PHA en la producción de metano a partir de diferentes biomasas obtenidas en diferentes periodos operacionales del sistema SBR-EBPR (diferentes SRTs) (capítulo 7).

En el capítulo 4, la configuración del sistema SBR-EBPR consistió en incluir una etapa de extracción de sobrenadante al final de la etapa anaeróbica (después de un período de sedimentación). El líquido extraído tiene la mayor concentración de P en el ciclo SBR, lo cual implica que la disponibilidad de este nutriente estaría limitada para los requerimientos metabólicos de los PAO, comprometiendo la eficiencia del proceso EBPR. Por ello, se evaluaron diferentes volúmenes de extracción. Los resultados obtenidos mostraron que extraer hasta un 10% del volumen total no afectó la capacidad de eliminación de P del sistema, logrando eficiencias de eliminación de hasta más del 90%. Pero al extraer un 15% del volumen total, condujo a una disminución de la actividad EBPR. Por otro lado, Se obtuvieron sobrenadantes con concentraciones de P entre 50-100 mg /L, los cuales son adecuados para ser usados en procesos de recuperación de P.

En los capítulos 5 y 6, se estudió la estabilidad del proceso EBPR a diferentes SRTs, esto con la finalidad de evaluar su posible integración a sistemas energéticamente eficientes. Con este fin, se operaron diferentes SBRs con una configuración convencional EBPR En el capítulo 5, se operaron tres diferentes SBRs a 25 ºC y a SRTs de entre 3-14 días.

Enfocándonos con los valores de SRT menores a 5 días, se observó que con el SRT de 3.6 días, la actividad PAO y la capacidad del sistema de eliminar P se mantuvieron. La pérdida de la actividad EBPR se observó con el SRT de 3 días. Así pues, un SRT de 4 días es recomendado para logar un proceso EBPR con un buen rendimiento. Sin embargo, este valor es mucho mayor en comparación con los SRTs que se usan en sistemas energéticamente eficientes, como en el A/B process. En el capítulo 6, la influencia de la temperatura y el SRT sobre el proceso EBPR fueron evaluadas, a corto y largo plazo, usando tres sistemas EBPR. Los resultados obtenidos, a partir de estos sistemas, demostraron que el proceso EBPR fue estable a 20 ºC y a 5 días de SRT. Bajo estas condiciones se logró eficiencias de eliminación de hasta el 90%. Sin embargo, a 10 ºC y 5 días de SRT la actividad EBPR se perdió progresivamente. Un comportamiento similar se observó con el SRT de 10 días, esto es, a 20 ºC se obtuvo un buen rendimiento del proceso EBPR (86% de eliminación de P), pero al disminuir la temperatura a 15 ºC la eficiencia de eliminación disminuyo (71% de eliminación de P) y se perdió a 10 ºC. Los coeficientes de temperatura obtenidos de los experimentos a corto y largo plazo mostraron que las velocidades de reacción involucrados en el proceso EBPR tienen un alto grado de dependencia de la temperatura a SRT de 3.5 días.

En el capítulo 7, la biomasa obtenida de los diferentes sistemas SBR-EBPR, estudiados en el capítulo 6 se sometió a pruebas de digestión anaerobia. Debido a que cada biomasa fue obtenida a diferentes SRT y a diferentes fases del ciclo EBPR (anaerobio y aerobio), estas contenían diferentes concentraciones de PHA. Según la bibliografía el contenido de PHA en la biomasa favorece la producción de metano. Los resultados obtenidos muestran que a menor SRT la concentración de PHA en la biomasa es mayor. Por ejemplo, el contenido de PHA de la biomasa obtenida a 5 días de SRT fue de un 18%, mientras que el contenido de PHA en la biomasa obtenida a 15 días de SRT fue de un 8%. También se observó que las biomasas con mayor contenido de PHA dieron mayor producción de metano. Por ejemplo, con la biomasa con un contenido de PHA del 18% se obtuvo una producción de metano de 401 mLCH4/g VSS, mientras que con la biomasa con un contenido de PHA del 8% se obtuvo una producción de metano de 329 mLCH4/g VSS.

# ABSTRACT

Exhaustion of many natural resources that are vital to our species constitutes one of the main challenges that our planet faces, which has lead the scientific community in the pursuit of immediate solutions for this problem. Currently, to counteract this situation, sources that long last were inconceivable, are being looked about, such as wastewater. Wastewater is potentially rich in resources as energy, biopolymers and nutrients; however, these are not leveraged and are lost during treatment, which in a summarized way, focus on the elimination and degradation of pollutants.

Phosphorus (P) is a resource that could be recovered from wastewater. This element has an important role for life, since it is structurally implied in both the cell membrane and the bones. Furthermore, it is essential for agriculture because it is one of the main components of fertilizers. Nowadays, exploitation of this resource has lead to a decrease in the quality of the reserves and according to estimations, it is anticipated that they could run out in the following 50 to 100 years.

Even if it is true that phosphorus is important for the preservation of life, it is also potentially a pollutant agent. Drainage of waters rich in phosphorus in rivers and lakes is the main cause of the phenomenon known as Eutrofization, which produces damage on the quality of these superficial bodies of water. This situation has lead to the creation of treatment processes to remove phosphorus from wastewater; being the chemical precipitation through addition of inorganic salts the most used one. However, this method has many disadvantages as its high cost and the excessive production of sludge. On the other side, the process of biological elimination of phosphorus (EBPR) is gaining popularity and it is being implemented in wastewater treatment plants worldwide (WTP). The EBPR process is based upon the metabolic activity of a group of bacteria called phosphorus accumulating organisms (PAO) to remove this nutrient from wastewater.

Although EBPR process has been implemented successfully at real scale, it does not fit in the perspective of modern WTP, since they are being designed to work as bio-refineries nowadays. This is, systems where components and/or energy are recovered counting on an efficient utilization of resources. That is why research in this thesis has focused mainly in the achievement of more sustainable systems SBR-EBPR, characterized by minimum energy

consumption, methane production and phosphorus recovery. With this approach, a SBR-EBPR system was studied in the long term by modifying its configuration in order to obtain an anaerobic supernatant enriched in phosphorus, which can be used in a recovery process as, for example, struvite (chapter 4). The behavior of the EBPR activity using short cell retention times (SRT) was also studied. This operational parameter is discussed in the literature as one of the factors that obstruct the integration of process BEP to systems more energetically efficient (chapters 5 and 6). Lastly, PHA influence was studied in methane production from biomasses obtained in different operational periods of system SBR-EBPR at different SRT (chapter 7).

In chapter 4, the configuration of system SBR-EBPR included one stage for the extraction of supernatant at the end of anaerobic stage (after a period of sedimentation). The extracted liquid has the largest concentration of P in the SBR cycle, which implies that the availability of this nutrient would be limited for the metabolic requirements of the PAO, compromising the efficiency of the process EBPR. For this reason, different extraction volumes were assessed. Results obtained showed that extracting up to 10% of the total volume the system's phosphorus removal capacity was not affected, achieving removal efficiency even higher than 90 %. Conversely, by extracting 15 % of total volume, the EBPR activity decreased. On the other side, liquids with P concentration between 50-100 mg/L were obtained, which are adequate to be used in processes of P recovery.

Stability of process EBPR was studied in chapters 5 and 6 in order to assess the possibility of its integration to energetically efficient systems. With this purpose, different SBR were operated with a conventional configuration. In chapter 5, three different SBR were operated at 25 °C and at 3-14 days SRT. It was observed that with the 3.6 days SRT the system sustained its EBPR activity. At a three-day SRT, EBPR activity was lost. A four-day SRT is recommended to achieve a good performance of the EBPR process. However, this value is much higher than SRT used in systems energetically efficient (for example, A/B process). In chapter 6, the influence of temperature and SRT on the EBPR process was assessed (in both the short and long terms) using three EBRP systems. Results obtained from these systems demonstrated that the EBPR process was stable at 20 °C and at a five-day SRT. Under these conditions, up to 90% removal efficiency was achieved. Nonetheless, at 10 °C and at a 5-day SRT, EBPR activity was lost progressively. A similar behavior was observed with a 10-day SRT at 20 °C, with an 86 % P removal, but when temperature was reduced to

15 °C the efficiency of removal decreased to 71 % and it was lost at 10 °C. The temperature coefficients obtained from the experiments in the short and the long terms showed that speeds of reaction involved in the EBPR process are highly dependable on temperature at 3.5-day SRT.

In chapter 7, biomasses obtained from systems SBR-EBPR were tested through anaerobic digestion. Since every biomass was obtained at different phases of the EBPR cycle (aerobic and anaerobic), these contained different concentrations of PHA. Results obtained showed that to a lesser SRT, PHA concentration in biomass increased. For example, the content of PHA in the biomass obtained at a 5-days SRT was 18 %, whilst, the PHA content in biomass obtained at a 15-days SRT was 8%. It was also observed that biomasses with a larger content of PHA registered a larger methane production. For example, with an 18 % PHA content biomass, a 401 mLCH4/g VSS methane production was obtained, whilst with an 8% PHA content biomass, a 329 mLCH4/g VSS was obtained.

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# CHAPTER 1

# Introduction

## 1.1. Water pollution

The deterioration of water quality is one of the biggest problems that the planet is facing and so, is a major concern for all countries. Nowadays, the ground and surface water at some places in the world are not suitable for human consumption due to the presence of pollutants. Water is mainly referred to as polluted when it is affected by anthropogenic contaminants and become unable to support a human use, such as drinking water.

Water pollutants can be classified on the basis of their nature into: biological pollutants including bacteria and virus; organic pollutants such as oxygen-demanding substances, fats and grease; and inorganic pollutants (as a result of human development) such as lead (Pb), nitrogen (N), phosphorus (P), Copper (Cu), Cadmium (Cd) and Zinc (Zn). These pollutants come from liquid wastes discharged from different municipal, agricultural or industrial activities.

## 1.2. Phosphorus sources in aquatic systems

Phosphorus (P) is one of the most abundant elements in the Earth (being the 11$^{th}$ element in order in abundance). It is present in nature as phosphates and it does not occur as elemental phosphorus (Evans and Johnston, 2004). In aquatic systems, phosphorus occurs in a wide variety of inorganic and organic forms, being the predominant aqueous species the orthophosphate (e.g. $HPO_4^{-2}$ and $H_2PO_4^-$) (Metcalf & Eddy, 2003). The intermediate transformations occurring in the aquatic phosphorus cycle are directly related to the microbial activity (e.g. the conversion of insoluble organic phosphate into dissolved inorganic P) and with interactions between the biomass in the water and in the bottom sediments. In natural conditions the phosphorus concentration in water is balanced (Rybicki 1997).

P load into aquatics systems may originate from (Che, 2011);

i) **Point source P**, which includes waste products discharged from a specific point, such as raw sewage of effluent from wastewater treatment plants or an industrial facility.

ii) **Non-point source (diffuse source) P**, which may come from a wide geographic area, such as from agricultural land (treated with manure or fertilizers), soil erosion, urban storm water, among others.

Most P in municipal wastewater come from human faeces and urine, the use of detergents and personal care products. It was calculated that on average 0.9 g-P/person/day is excreted in urine and 0.4 g-p/person/day is excreted in faeces (Jönsson et al., 2004). The total P concentration in municipal wastewater is commonly 6-8 mg /L but can be higher depending on the source (Parsons and Smith, 2008).

## 1.3. The importance of P removal from wastewater

In the last decades, P removal has become a concern topic among international researchers for two basic reasons:

i) It is a strong aquatic contaminant. If P concentrations in the receiving water bodies are higher than the concentrations assimilable by a population of living organisms, it will behave as a fertilizer that contributes to the growth of undesirable algae population (eutrophication) (Mainstone and Parr, 2002).

ii) It is a non-renewable resource necessary in modern agriculture to maintain high crop yields (Cordell et al., 2009). Food production requires application of fertilizers containing P. As a result of the increment of human activities, P is being depleted. Some statics show that availability of P reserves could be depleted within a few decades. Hence, it is necessary to find alternative sources to obtain this nutrient (Childers et al., 2011; Ulrich et al., 2014 ).

## 1.4. Phosphorus removal technologies: chemical phosphorus removal and biological phosphorus removal

The objective of P removal in wastewater treatment process is to reduce the concentration of phosphorus by converting soluble, colloidal and quasi-colloidal phosphorus into a particulate form that could be then removed from the wastewater.

The development of techniques for P removal has been in continuous transformation since the 1950s with the aim of developing processes that allow both the removal and

recovery of P in a sustainable manner. The current technologies to remove P include a wide range of options; i) chemical precipitation, in which P compounds are converted into a particulate form (by adding a metal salt); ii) biological P removal, which incorporate the P into the biomass; iii) the physico-chemical treatment, which is less widespread treatment alternative, and includes adsorption, ion exchange and deep-bed filtration (Zhou et al., 2008).

Of the aforementioned processes, the chemical removal of P is an attractive option due to its simplicity of operation (it is the most commonly used process in full-scale plants). The process can be performed for any P concentration and at any temperature (Che, 2011) at expenses of a cation dosage. This technology consists in the precipitation of P by the addition of salts of multivalent metal ions such as aluminium (III) (e.g. aluminium sulphate), iron salts (e.g. ferric chloride) or calcium hydroxide, into wastewaters (Metcalf and Eddy, 2003). The P reacts with the dissolved cations forming a precipitate which is removed as a sludge (Pratt et al., 2012). The typical P reaction with either aluminium or ferric chloride are shown in Equation 1 and 2 respectively (Metcalf and Eddy, 2003).

$$Al_2(SO_4)_3 \cdot 14.3H_2O + H_2PO_4^- \longrightarrow 2AlPO_{4(s)} + 3SO_4^{-2} + 14.3H_2O + 2H^+ \quad (1)$$

$$FeCl_3 + H_2PO_4^- \longrightarrow FePO_{4(s)} + 2H^+ + 3Cl^- \quad (2)$$

P removal by metal salts addition is commonly used in areas where effluent P requirements are extremely strict. By using chemical reactions, it is possible to achieve effluents with P lower than 0.1 mg P/L (Reddy, 1998). Wastewater treatment plants using this technology have reported P levels effluent of less than 0.02 mg/L (Takács et al., 2006; Newcombe et al., 2008).

Although the chemical precipitation remains the leading technology for P removal, it cannot be considered as a clean or sustainable method. One of the main negative points of this process is that due to the addition of chemical products, up to a 95% more of sludge is produced, which contains a large amount of unwanted chemical (e.g. iron phosphate) that make its final disposal a problem (Metcalf &Eddy, 2003). Moreover, if this sludge is subjected for anaerobic digestion, it will produce approximately 12% less biogas and 8% less methane when compared with the sludge that has not been chemically treated (Parsons and Smith 2008). Additionally, some other drawbacks can be listed for this process such as;

i) the operating cost of chemical P removal are much higher than biological P removal
ii) the negative impact of metal ions against the settling capacity, dewatering and thickening of the sludge and finally
iii) the formation of insoluble materials that may cause damage in pumps (Henze et al., 2002; Akpor & Muchie, 2010).

Because of the chemical treatment disadvantages mentioned above, researchers have focused on the development of more sustainable alternatives to remove P such as the enhanced biological phosphorus removal (EBPR) process. EBPR is a well-known process that has gained importance in the last few decades as it is considered as a sustainable technology for P-removal.

The first patented EBPR process was developed by Levin and Shapiro (1965). However, this system did not deplete the influent BOD (biochemical oxygen demand) (EPA 2010), it was not until 1970 when James Barnard (1975) created a system in which the use of the BOD was considered alongside P removal. He also demonstrated that it was necessary an anaerobic zone to obtain a more robust EBPR system. Starting in the mid-70s, different configurations of EBPR systems began to emerge such like the anaerobic-aerobic (A/O) process, the Anaerobic-Anoxic-Oxic ($A^2$/O) process and the University of Cape Town (UCT) process, among other configurations (EPA 2010). Since then, several investigations have been carried out to achieve a better understanding of the EBPR process, allowing the determination of the microbial populations present during the process (López-Vázquez et al., 2008; Mielczarek et al., 2013); the metabolic pathways of phosphorus accumulating organisms (PAO) (Lanham et al., 2013; Welles et al., 2016) and also the determination of important parameters affecting EBPR such as: the solid retention time (SRT) (Ge et al., 2015; Li et al., 2016 ), temperature (Brdjanovic et al., 1998; Erdal et al., 2006; N. Li et al., 2010), pH (Filipe et al., 1997), carbon sources (Pijuan et al., 2004; Li et al., 2008 ;Guerrero et al., 2012) etc.

High efficiency EBPR processes have been widely implemented in biological wastewater system at either lab-or full scale ( Park et al., 2006; Chen et al., 2016; Yang et al., 2016; Keene et al., 2017), demonstrating the feasibility of this process as an environmental-friendly alternative to capture P from wastewater for its further recovery.

## 1.5. Enhanced biological phosphorus removal (EBPR)

EBPR is widely used process to remove P in wastewater and is based on the P storage as poly-P inside microorganisms. This process takes advantage of the PAO metabolism to capture P because of its capacity to accumulate more P than their nutritional requirements. The ability of PAO to store P (0.38 mg P/mg TSS) is higher compared to that of other heterotrophic organisms (0.023 mg P/mg TSS) (Wentzel et al., 1989). To achieve P removal, PAO use three different internal storage compounds: polyphosphate, polyhydroxyalkanoates (PHA) and glycogen (which generate energy and/or reducing power) (Lanham et al., 2014). The PAO metabolism is activated by subjecting the PAO-enriched biomass through anaerobic phase followed by an aerobic and/or anoxic phase (Barnard, 1975; Metcalf & Eddy, 2003).

Anaerobically, organic substrate (typically Volatile fatty Acids, VFA) present in the liquid is captured and intracellularly stored it as PHA by PAO; PHA chemical composition depends on the carbon source assimilated. This transformation requires on one side energy, that is mainly supplied by hydrolysing stored poly-P and, on the other hand, a reducing power that is provided by the anaerobic degradation of the intracellularly stored glycogen (Mino et al., 1998). During P degradation, PAO release of P from its cells to the liquid phase (Reddy, 1998) reaching up to 90 mg/L, that are high in comparison with that of wastewater influent that are around 5 to 8 mg/L (Che, 2011).

In the subsequent aerobic/anoxic phase, the PAO take up P and stores it intracellularly as P. Therefore, the concentration of P in the bulk liquid is reduced and both, the P released during the anaerobic zone and the P initially present in the effluent, are removed during the aerobic phase and finally the excess sludge containing P is withdrawn (Mino et al., 1998; Oehmen et al., 2007). The energy required for phosphorus removal is obtained from the oxidation of the stored substrate as PHA using oxygen and/or nitrate/nitrite as electron acceptors (Che, 2011). Also, during this phase, new biomass is produced (PAO population increase) and the glycogen is replenished using PHA as both carbon and energy sources (figure: 1.1) (Metcalf & Eddy, 2003). As mentioned, it is also possible to perform EBPR process under anaerobic-anoxic conditions, due to the ability of specific PAO, i.e. denitrifying PAO (DPAO). DPAO can use nitrate or nitrite instead oxygen as electron acceptor and therefore, perform P uptake and denitrification simultaneously (Mino et al., 1998; Henze et al., 1999)

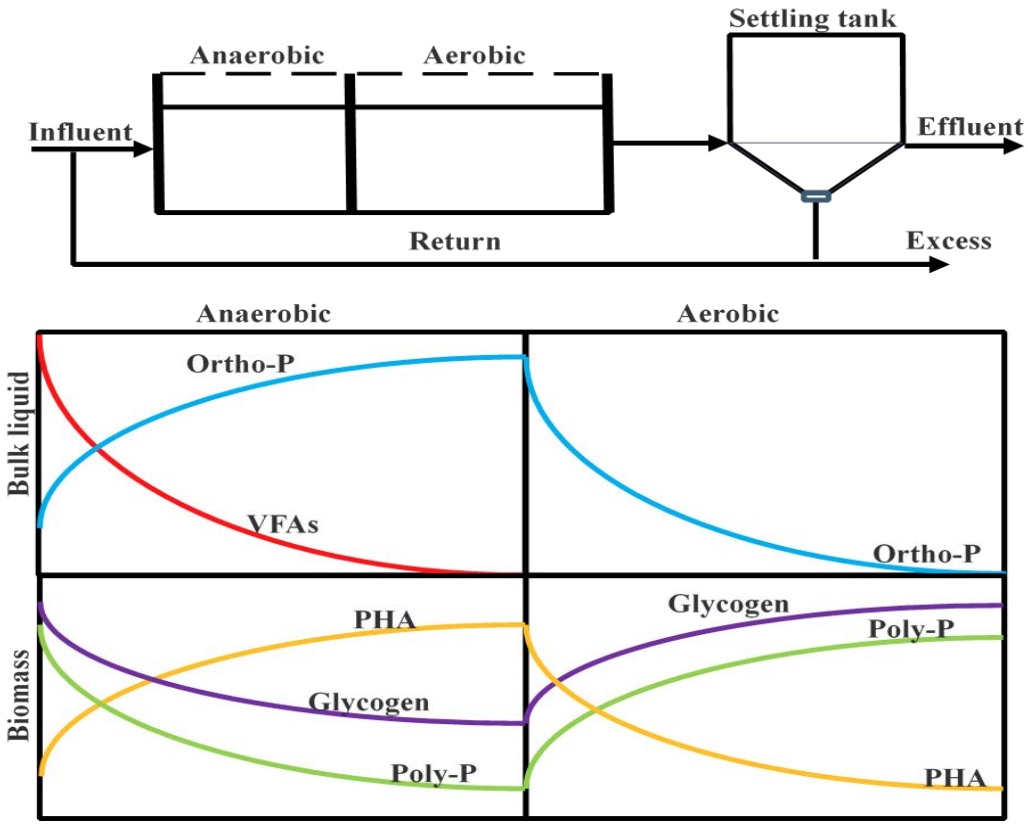


Figure 1.1. PHA, VFA, polyphosphate and orthophosphate behaviour during EBPR process of a two stage (anaerobic and aerobic).

## 1.6. Phosphorus accumulating organisms (PAO)

In general, it is currently accepted that PAO are the key microorganisms in the EBPR process. Although the ability to take up P is widespread among bacteria, only PAO are capable of accumulate it in high amount using $O_2$, $NO_3^-$, and $NO_2^-$ as final electron acceptors (up to 12% compared to 3% dry weight in non-PAO) (Nielsen et al., 2012). PAO remove most of the P form wastewater. Different biochemistry models have been proposed in order to understand the metabolism of PAO (see section 1.5). In summary, these models describe the ability of PAOs to store large amounts of P (Figure 1.2).

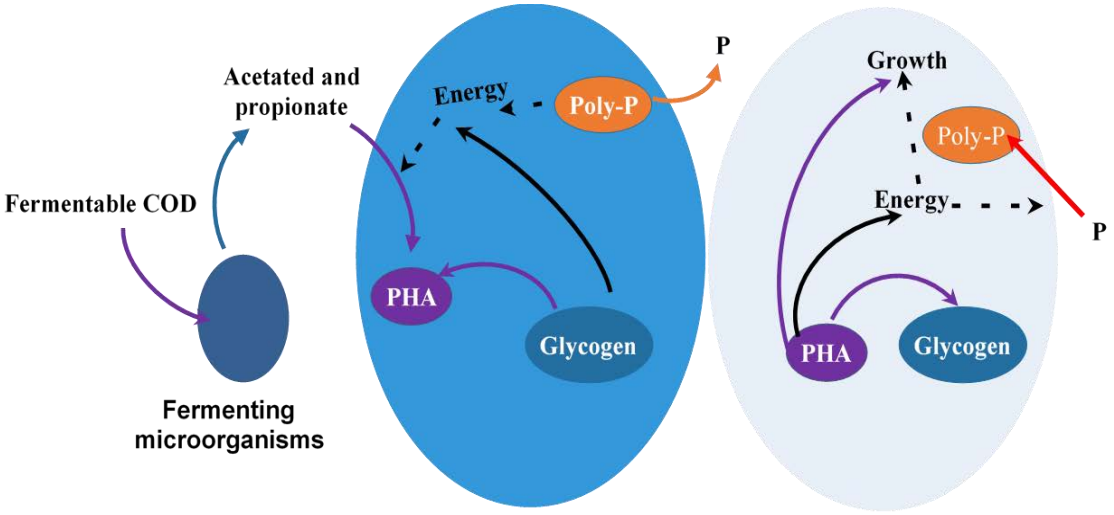


Figure 1.2. Simplified biochemical model for PAO metabolism: left, anaerobic phase; right, aerobic phase.

Since PAO are essential for understanding the EBPR process, many bacteria have been recognised as potential PAO, such as: *Acinetobacter spp, Microlunatus phophovorus, Lampropedia spp, and the Gram-positive Acinobacteria.* However, such microorganisms do not exhibit all of the characteristics described in the biochemistry models for the EBPR process or do not occur in significant numbers in EBPR systems (Kim et al., 2010). To date, the most studied PAO is *candidatus* accumulibacter phosphatis (accumulibacter). Accumulibacter can be classified into two main groups (groups I y II), and each group has been further classified into several clades (e.g. clade 1A-E) (He et al., 2007). It has been demonstrated that *Accumulibacter* clades have different ability to use $NO_3^-$ for P uptake. For example, all *Accumulibacter* clades are able to use $O_2$ as electron acceptor, but only clade IA is able to reduce $NO_3^-$ for P uptake (He et al., 2007). On the other hand, clade IIA has been showed to use $NO_2^-$ but not $NO_3^-$ (Flower et al., 2009). Besides *Accumulibacter* (clade IA), PAO capable of reducing $NO_3^-$ have also been identified. These potential DPAO include the genera *Aquaspirillum*, *Azoarcus*, *Thauera and Rhodocyclus* (Thomsen et al., 2007).

Recently, a range of modern molecular methods such as PCR-clone libraries, fluorescence *in situ hybridization* (FISH), microautoradiography (MAR), among others, have been used to study the microbial communities involved in EBPR process. These tools have demonstrated that *Rhodocyclus-related Candidatus* Accumulibacter phosphatis (hereafter Accumulibacter) is an important PAO in EBPR process (He and Mcmahon, 2011). Several investigations have demonstrated the abundance of *Accumulibacter* in lab-scale reactors fed with acetate or propionate (Nielsen et al., 2012). Nevertheless, its relevance in

full-scale EBPR has not been stablished. It has been estimated that the content of *Accumulibacter* in full-scale wastewater treatment plants (WWTPs) performing EBPR, represents between 13 and 18% of the total bacterial population (Zilles et al., 2002). However, the actinobacterial genus *Tetrasphaera* (claimed to be PAOs) is found in significant abundance in full-scale EBPR plants constituting up to 30 % of the total biomass content (Nguyen et al., 2011).

On the other hand, determining the substrate spectrum available to *Accumulibacter* is essential for a better understanding of the ecological niches of this PAO in wastewater treatment environments where different carbon sources are present. Kong et al. (2005) found that *Accumulibacter* is able to take up short-chain volatile fatty acids (VFAs) including acetate, propionate, pyruvate and glutamate, but no formate, ethanol, glucose and several amino acids including leucine, glycine and aspartate. In contrast, *Tetrasphaera* is capable to uptake certain complex carbon sources under anaerobic condition such as: amino acids and glucose, as well as acetate (Nguyen et al., 2011).

## 1.7. Glycogen accumulating organisms (GAO)

Glycogen Accumulating Organisms (GAO) have a metabolism that is very similar to that of PAO; they are able to store biodegradable organic matter (mainly VFAs) under anaerobic conditions as PHA and use it as a carbon and energy source under aerobic conditions (figure 1.3). However, unlike PAO, GAO do not exhibit the typical anaerobic P-release and subsequent aerobic P-uptake as they obtain the energy required for the VFA uptake solely from glycogen hydrolysis (Oehmen et al., 2005). EBPR performance is directly affected by the competition between PAO and GAO. Therefore, from the P removal perspective, GAO are considered as undesirable microorganisms since they compete with PAO in EBPR systems for anaerobic VFA uptake (Lopez-Vazquez et al., 2008).

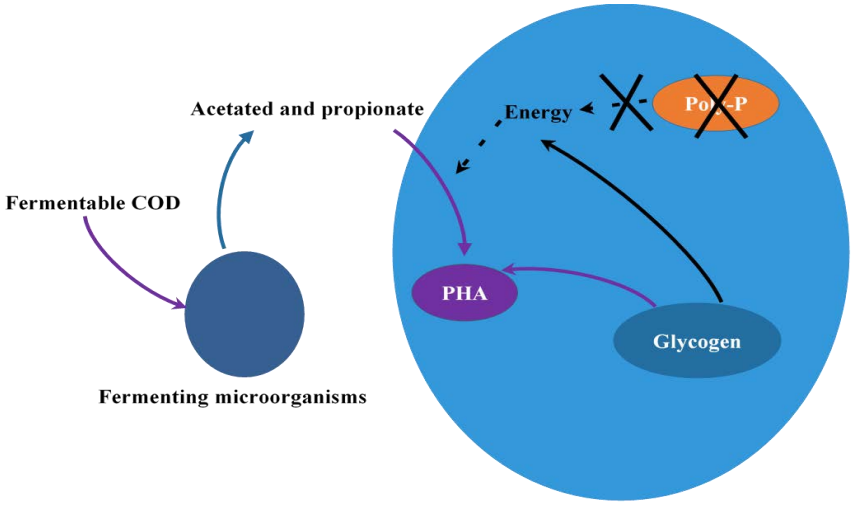


Figure 1.3. Simplified biochemical model for the anaerobic metabolism of GAO.

Different operating and environmental conditions have been identified as important factors to understand the PAO-GAO competition: type of carbon source (acetate and/or propionate), pH, temperature and influent P/COD ratio. For example, *competibacter* (GAO) have a competitive advantage against PAO at temperatures over 20 °C (Nielsen et al., 2012). Likewise, PAO use acetate and propionate at a similar rate and in contrast, GAO seem to be less efficient for propionate uptake (Oehmen et al., 2005). The foregoing shows that operating and environmental parameters can be redirected to create favourable conditions for PAOs and disadvantageous for GAO.

## 1.8. Configurations of wastewater treatment plants

As it was mentioned previously, EBPR process is achieved by recirculating the enriched-biomass PAO through anaerobic zone, followed by an aerobic zone. In many cases, an anoxic zone is incorporated for simultaneous removal biological of N and P (Metcalf & Eddy, 2003). From the basic EBPR process, different configurations have been developed aiming mainly at achieving simultaneous P removal alongside the nitrification-denitrification process. The process schemes can be divided in two main groups; i) the mainstream process and ii) the side-stream process.

### 1.8.1. Mainstream processes

In the Mainstream processes, P removal occurs along the main plant flow, where P is concentrated at high levels in the sludge. Sludge with a high phosphorus content is removed and is recycled from a final sedimentation tank to the head of an anaerobic zone. Some examples of mainstream processes are described below (Metcalf and Eddy, 2003).

#### 1.8.1.1. Phoredox process (A/O)

This is the most basic configuration for biological P removal and it consists of a sequence of anaerobic and aerobic zones with the aim of promoting carbon oxidation and P removal (Figure 1.4). The nitrification does not occur by employing this configuration. A/O process has the advantage that it can be operated at low sludge retention time (SRT) ranging between 2 to 3 days at 20 °C (Grady, 1999).

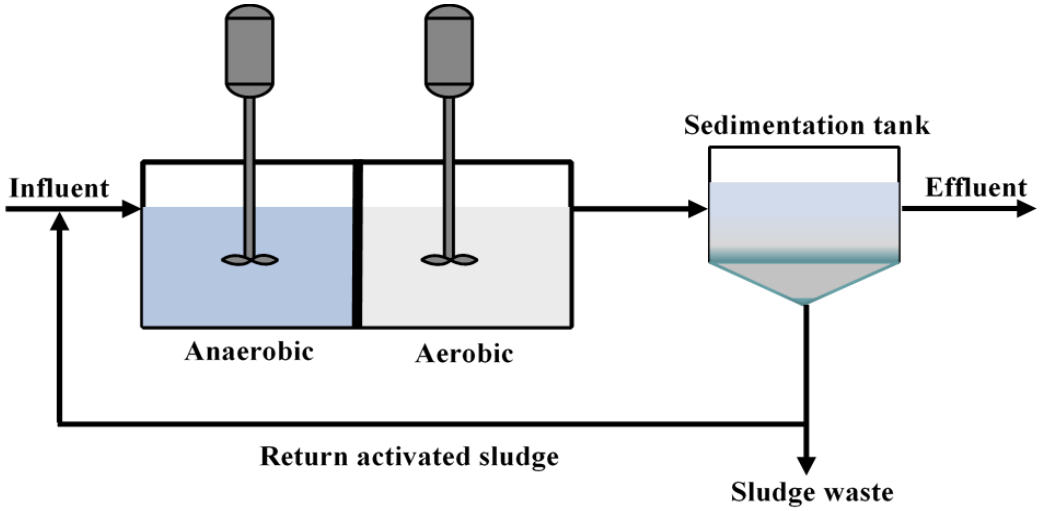


Figure 1.4. PHOREDOX (A/O) configuration.

### 1.8.1.2. Anaerobic/Anoxic/Aerobic process ($A^2/O$)

The return activated sludge is directly recirculated to the anaerobic reactor in both, the $A^2/O$ process and the A/O process. However, the difference between these two processes is that in the $A^2/O$ process the mixed liquor from the aerobic reactor is recycled into the anoxic reactor, resulting that the amount of nitrate fed to the anaerobic reactor is minimal (Figure 1.5) (Che, 2011). Precisely this configuration was created with the aim of minimizing the concentration of nitrate entering in the anaerobic reactor. Since the presence of nitrate in the anaerobic reactor (or phase) has a markedly deleterious influence on the P removal. If nitrate is recycled to the anaerobic reactor, the OHO are able to utilize VFA for energy and growth up using the oxygen of nitrate, in consequence the amount of VFA available to the PAO is reduced (Henze et al., 2008).

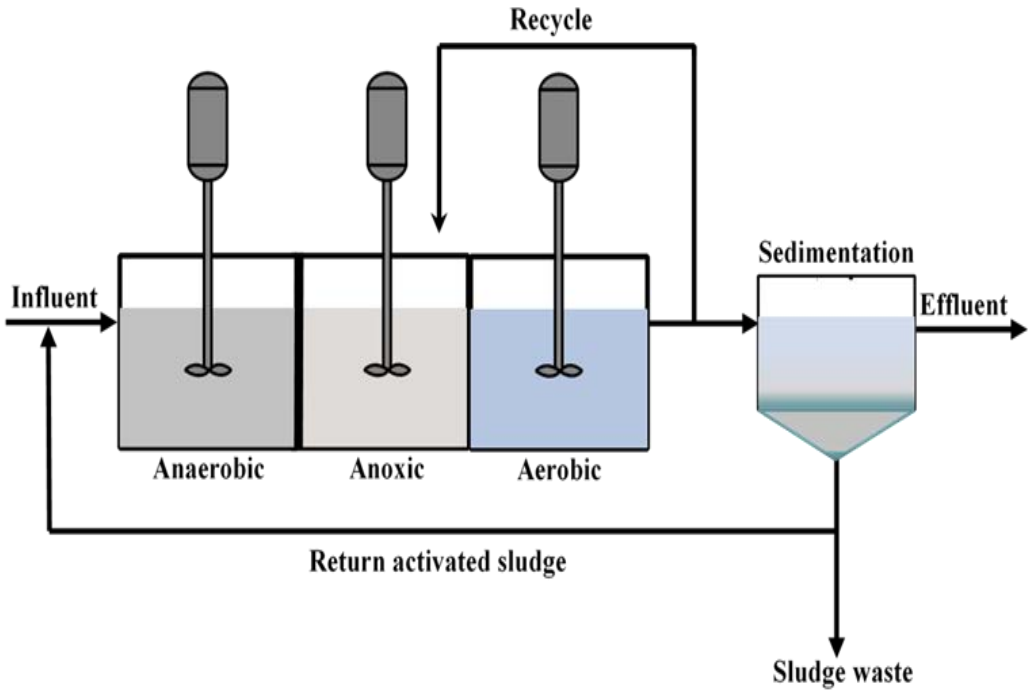


Figure 1.5. $A^2/O$ configuration.

### 1.8.1.3. University of Cape Town (UCT) process

An additional modification to the PHOREDOX process gave origin to the university of Cape Town process (UCT). In the UCT process, the activated sludge return to the anoxic zone instead of the anaerobic zone, which eliminates nitrate or nitrite from the anaerobic

zone by denitrifying the returned activated sludge in the anoxic zone before the biomass is recirculated to the anaerobic reactor (Che, 2011). In addition, the recirculation of the mixed liquor from the anoxic zone to the anaerobic zone provides optimum conditions for the formation of short chain fatty acid fermentation products (Figure 1.6).

A disadvantage of the UCT process is that it requires large additional internal recycling systems and large fractions of anaerobic reactor volume, which increases the requirements for pumping and maintenance (Metcalf and Eddy, 2003).

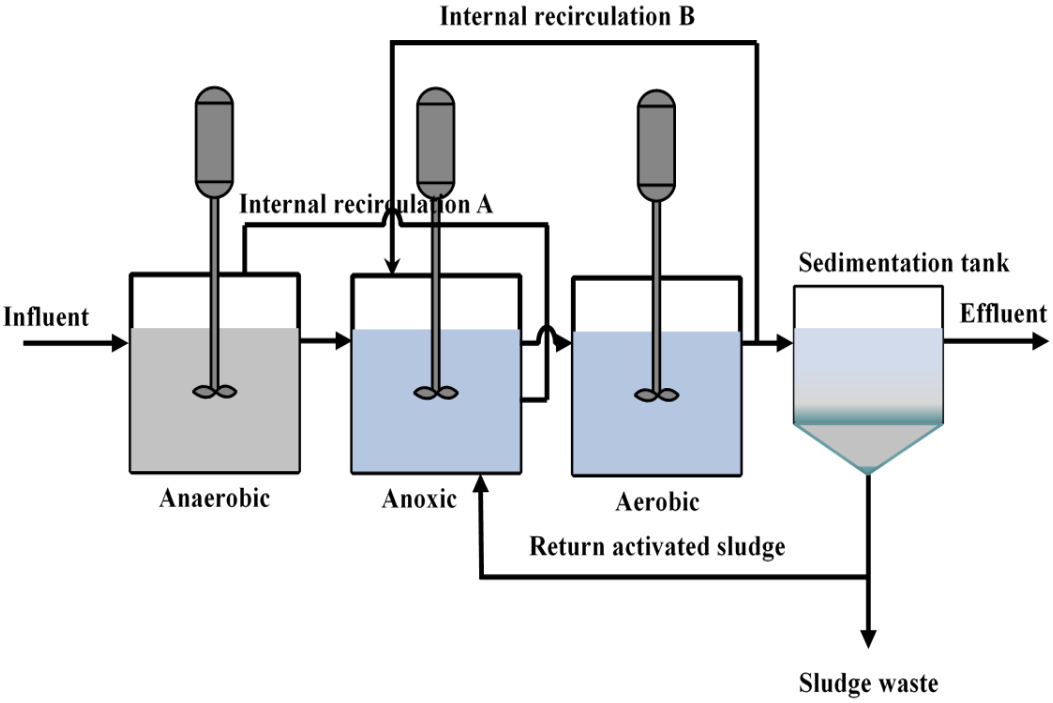


Figure 1.6. University of Cape Town (UCT) process.

### 1.8.1.4. Modified UCT process

In this configuration, the anoxic zone is divided into two compartments. By one side, the return activated sludge (with nitrate) is fed in the first anoxic reactor, where the nitrate is reduced, and the mixed liquor produced is recirculated back to the anaerobic zone. The second anoxic reactor allows denitrification of the recirculated mixed liquor from the aerobic stage (Figure 1.7). The modified UCT process provides better protection of the anaerobic tank from nitrate recycling. Because return activated sludge (RAS) is fist denitrified and recirculated back to the anaerobic stage before the consecutive anoxic stages for denitrification of the nitrate recirculation from the aerobic stage. One disadvantage of this

process is that it requires large additional internal recycle systems, which therefore increase pumping energy and maintenance requirements (Metcalf and Eddy, 2003).

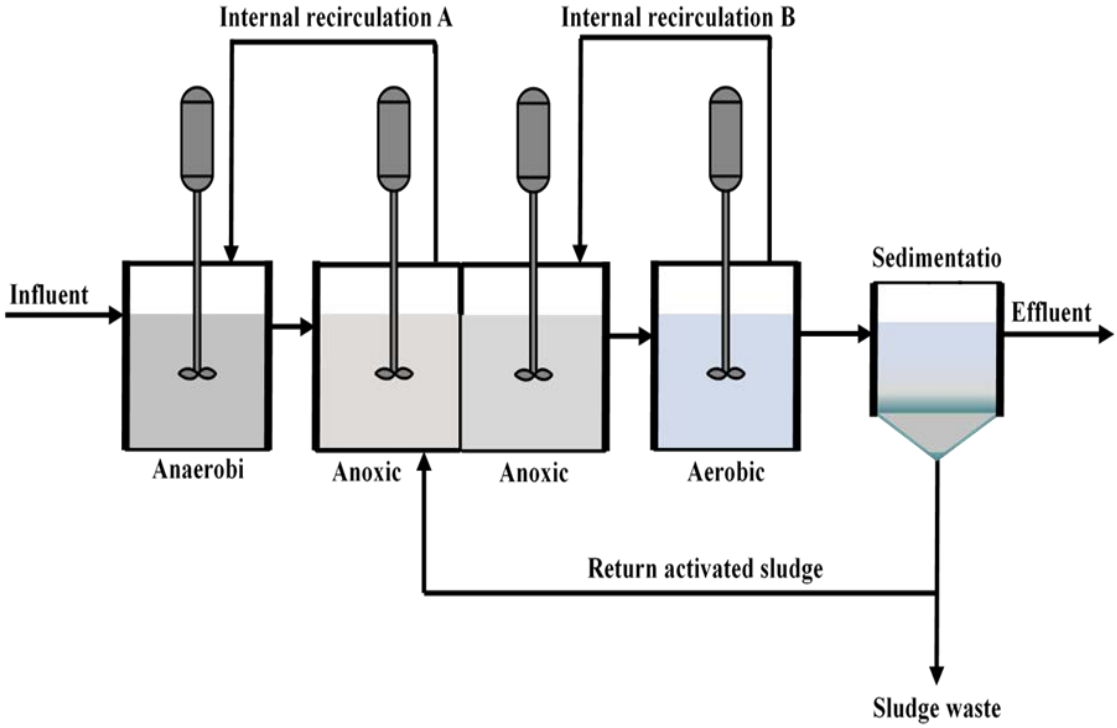


Figure 1.7. Modified UCT process.

### 1.8.2. Side-Stream processes

Conversely to mainstream process, in the side-stream processes P removal occurs in a sidestream as opposed to the main treatment plant flow. In side-stream configurations the release of P occurs in the sludge recycle stream. After the anaerobic treatment of the sludge recycle stream, the P is removed either chemically or biologically (Metcalf and Eddy, 2003).

#### 1.8.2.1. PhoStrip process

The PhoStrip process combines chemical and biological P removal. This process consists of an aerobic reactor with clarifier; a side-stream from the underflow of the clarifier passes to an anaerobic stripping tank where the sludge settles, and P is released from the sludge into the bulk solution. The stripped sludge is returned to the activated sludge system, while the supernatant is dosed chemically in a precipitator tank, to precipitate released P which is

settled and wasted. The supernatant from the precipitator tank is returned to either the influent or the effluent flow (Figure 1.8) (Henze et al., 2008).

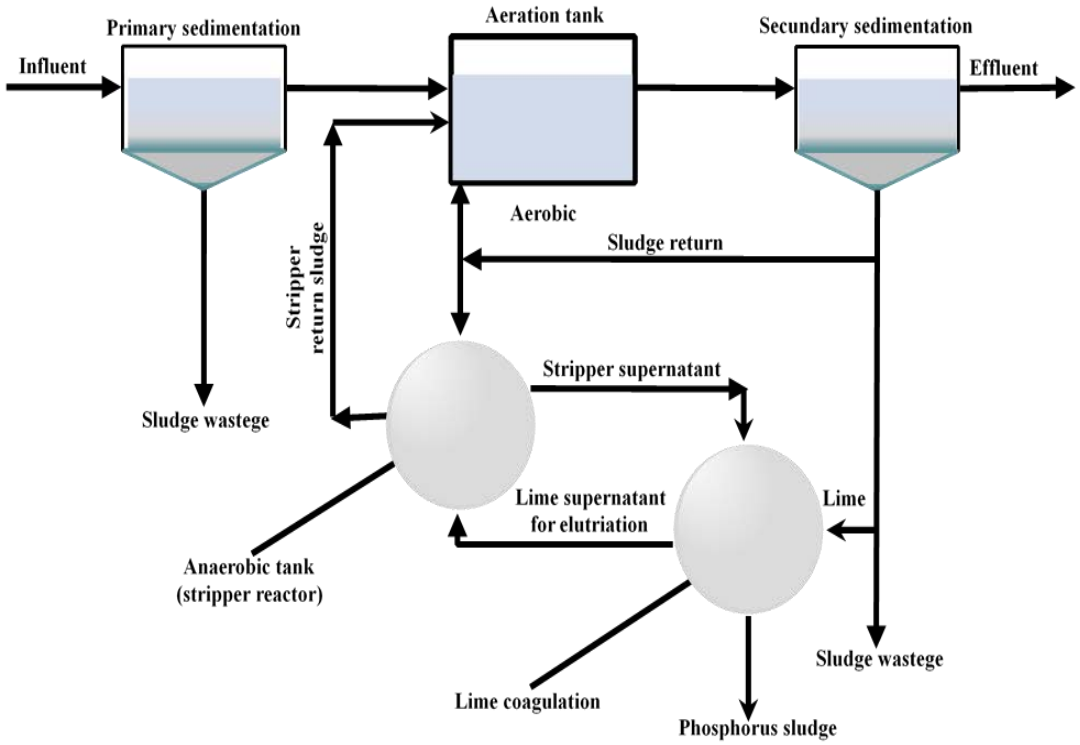


Figure 1.8. Graphic representation of Phostrip process.

### 1.8.3. Sequenced batch reactors (SBR) for EBPR

The use of sequenced batch reactors (SBRs) gained popularity through the 1970-80s because of hardware advancements (PLC controllers, improved instrumentation, etc.) (Brett et al., 1997). Today, this technology is a good option for employing anaerobic-aerobic contacting for biological phosphorus removal. This technology has been successfully applied in WWTPs (Lee et al., 2004).

Basically, the SBR system is a fill-and draw activated sludge system where reaction and settling take place in a single tank (Figure 1.9). The first operation step is a fill period where influent is diverted to the SBR tank. After the fill period, the reactor contents are mixed but not aerated to provide the anaerobic conditions. The next step is the aeration period followed by a settling time when both aeration and mixing are stopped. Finally, the

effluent is then withdrawn and, depending on the influent flow rate, a variable length idle time may occur.

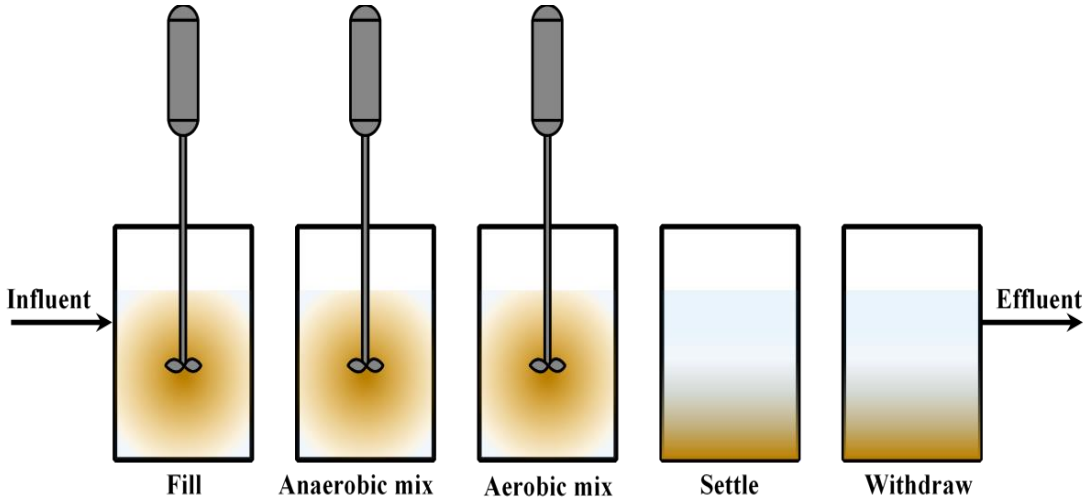


Figure 1.9. Sequenced batch reactor configuration for the EBPR process

## 1.9. Effect of operating factors on the EBPR process

The efficiency of the EBPR process is strongly related to the presence of PAO in the system and, therefore, it is important to promote the optimal conditions to give PAO a competitive advantage over other organisms (common heterotrophic organisms and GAO).

When operated successfully, the EBPR process can be an inexpensive option to reach relatively high P removal efficiency. However, the stability and reliability of EBPR can be a problem. It is widely documented that EBPR plants may experiences process upsets. There are several important factors that affect the performance of the PAO in the EBPR systems and normally, are related to the wastewater characteristics (carbon source and the presence of nitrates), the design parameters (SRT and the COD/P ratio) as well as with environmental factors (e.g. dissolved oxygen (DO), temperature and pH) (Bernard et al., 1975; Oehmen et al., 2007).

### 1.9.1. Substrate availability

One of the critical factors to consider when evaluating the EBPR potential of any wastewater, is its composition and characteristic. It has been demonstrated, in both, lab-scale

experiments as well as in modelling studies, that the VFAs are essential for a successful EBPR (Bernard, 1985; Wentzel et al., 1991; Smolders et al., 1994); it has been reported that 7-9 mg of VFAs are needed to remove 1.0 mg of P(Mulkerrins et al., 2004). Since soluble chemical oxygen demand (sCOD) is the precursor of VFAs and most of domestic and municipal wastewater are VFA-deficient, the sCOD concentration in the influent is one of the problems affecting EBPR efficiency.

The VFA concentration in the effluent could be increased by encouraging previous reactions in fermentation clarifiers (pre-fermentation) or by feeding external carbon source directly to the anaerobic reactor, such as acetate, propionate, ethanol, methanol or wastewater with a high soluble COD concentration (Reddy, 1998; Henze et al 2002).

### 1.9.2. Effect of the presence of nitrate in the anaerobic phase

In general, it has been accepted that the presence of nitrate, as well as other electron acceptors (e.g. nitrite), in the anaerobic zone are detrimental for EBPR process (Reddy, 1998). Since the presence of nitrate in the anaerobic reactor favour the use of VFAs by denitrifying bacteria reducing thus the amount of VFAs available for PAO. However, recently Guerrero et al. (2012) demonstrated that depending on the usual operational conditions of each system, the response of PAO to the presence of nitrate is different. They observed that the PAO population developed in an anaerobic-Aerobic SBR, showed a reduction of EBPR activity according to an increase in nitrate concentration. In contrast, the PAO population developed in an A2/O plant were able of coexisting with nitrate without an inhibitory effect.

### 1.9.3. Influence of carbon (C)/P ratio

The ratio between the organic carbon to P concentration in the influent determines the ability of biomass to remove P. This factor has a major impact on the performance of the EBPR systems and should be considered in the design of a treatment plant (Che, 2011). Generally, it is necessary a BOD: P ratio greater than 20: to achieve low P concentrations in the effluent. (e.g. 1.0 to 2.0 mg/L) (Metcalf &Eddy, 2003).

### 1.9.4. Effect of sludge retention time (SRT)

This parameter is normally considered for the design and operation of biological nutrient removal systems and is one of the keys for an efficient EBPR process. The SRT is directly related with the microorganisms growth rate (Smolders et al., 1995) and its effect on P removal is complex. Henze at al. (2008) reported that for SRT < 3 days, the P-removal increases with increase SRT. However, for SRT > 3 days, P-removal decrease with increase SRT. They explained that this is because an increase in the SRT causes an increase in the system OHO mass, which in turn causes an increase in fermentable COD conversion and, therefore, an increase in P release and P uptake. However, the increased SRT also causes a decrease in P uptake due to the lower PAO active biomass (and its associated P content) wasted per day. At SRT < 3d, the former effect dominates the P removal, while at SRT > 3d the latter dominates.

Bacteria populations involved during nutrient removal have different requirements of SRT. For example, the decay rate of PAO is relatively lower (0.15 to 0.2 $day^{-1}$) than the one of other microorganisms (0.3 to 0.7 $day^{-1}$) (Lee et al., 2007), which means that with a longer SRT, a greater abundance of PAO will be found in the active biomass. However, some adverse effects on phosphorus removal efficiency are associated with processes with long SRT; first, the higher the SRTs the higher the endogenous decay rate. Secondly, at long SRT values, PAO will deplete their intercellular storage products (e.g. glycogen) and less efficiencies of COD uptake and PHA storage will occur during the anaerobic phase (Metcalf and Eddy, 2003). In addition, a long SRT would lead to the decrease of biomass yield and the amount of excess sludge, which implies a lower amount of phosphorus removal, due to, the phosphorus removed is proportional to the amount of sludge wasted (Li et al., 2016).

Conventional wastewater treatment process for biological nutrient removal are operated under long SRT of 10-20 days. Currently, there has been a great interest in operating EBPR systems with low SRT to improve the energy efficiency of this process (Ge et al., 2013).

### 1.9.5. Effect of dissolved oxygen (DO)

EBPR process involves different oxygen demands and, depending on where it is present, can disturb or improve the performance of the process. It has been believed that in

the anaerobic zone the oxygen must be avoided (0.0-0.2 mg/L oxygen) as the presence of electron acceptors will negatively affect the EBPR process. However, Pijuan et al. (2004) observed that EBPR activity can be maintained under exclusively aerobic conditions. They observed that when substrate was fed under aerobic conditions, PAO could uptake it in a similar way as under anaerobic conditions, linking this uptake to P release and glycogen degradation.

For the aerobic zone, DO concentrations between 3.0 - 4.0 mg/L are required for successful EBPR (Shehab et al., 1996). Since DO concentrations of > 4 mg/L do not appear to further stimulate phosphorus removal, the maintenance of oxygen concentration above this level can represent a waste of energy. (Mulkerrins et al., 2004).

### 1.9.6. Effect of temperature

The biological reactions involved in EBPR process are closely related to the temperature because of its influences on the metabolic activities of the PAO. In addition, temperature strongly effect factors such as gas-transfer rates and settling characteristics (Metcalf &Eddy, 2003).

There are several works reporting the effect of temperature on the efficiency of EBPR but with contrasting results. For example, improved EBPR efficiency at higher temperatures (20 to 30 C°) was observed by Wentzel et al. (2008) and Converti et al. (1995). In contrast, good or even comparatively better P-removal efficiency at lower temperatures (5 to 15 °C) was reported by Mulkerrins et al. (2004). However, the results reported regarding to the EBPR kinetics have been more consistent. It has been reported that the growth and substrate consumption rates as well as the P-release and/or p-uptake rates increase as temperature does (10 to 33 °C) (Spatzierer et al., 1985; Mamais and Jenkins, 1992; Wentzel et al., 2008).

Henze et al. (2008) point out that this contrasting results is because temperature influences a variety of processes in activated sludge systems (e.g. lysis, fermentation, nitrification, etc.) which may influence EBPR process. These effects complicate the determination of the effect of temperature on EBPR. Also, they pointed that the different results on the temperature effect can be explained by the use of different substrates, activated sludge and measurement methods.

### 1.9.7. Effect of pH

The pH exerts a major influence on EBPR process. Smolders et al. (1994) reported that at pH values of 5.0 EBPR activity is inhibited. The optimum PH range for EBPR is between 6.5 and 8.0, which is typical for full-scale and lab-scale operations (Liu et al., 1996).

However, it is important to considerer the optimum pH in simultaneous phosphorus removal and nitrification systems, since the optimum pH range for PAOs and nitrifiers is different (pH 8.0 to 9.0 pH).

## 1.10. Redefining the role of WWTPs: EBPR process as a sink for resources

Although conventional activated sludge (CAS) systems have made significant contributions to the wastewater sanitation in the past 100 years, they still being systems with low cost-effectiveness and recovery potential, and with a high environmental foot print and electricity demand (Verstraete and Vlaeminck, 2011). This fact has led to the necessity to turn CAS into energy self-sustained processes, suitable to produce wastewater able for reuse, and allow the recovery of sub-products (Batstone et al., 2015), and all of this while effluents with the required quality are obtained (e.g. P< 1 mg/L) (Guest et al., 2009). This aim is currently addressed by scientific research, and is attracting increasing attention (Verstraete et al., 2009; McCarty et al., 2011; Batstone et al., 2015).

The A/B process is a viable option to achieve the objectives mentioned above, since it offers the possibility of developing processes that reduces energy consumption during wastewater treatment, and also the possibility to achieve nutrients recovery. In the A/B process, the first stage (A-stage) is used to maximize the capture of organic matter for direct anaerobic digestion prior to biological oxidation, whereas B-stage is mainly used for nitrogen removal (partial nitrification) (Wan et al., 2016). Although the A/B process is specifically put forward for maximizing the chemical energy recovery from domestic wastewater, and not for nutrients recovery, it could be modified to achieve it. In this context, the A-stage seems to be the most appropriated place to include the nutrient recovery, since less organic matter (COD) would enter to B-stage.

For example, P recovery can be achieved initially through an EBPR process. As, the effluent of this process is a P-enriched sludge, it can be treated by using anaerobic digestion,

which generates a P-enriched supernatant that is suitable for struvite precipitation. Obviously, the P recovery would require the incorporation of EBPR process in the A/B technology, implying a slight increase of the SRT needed to remove the organic matter. The advantages of such integration are as follows; i) it would make the process more sustainable in an economical and environmental point of view, ii) it would allow to reduce the precipitation costs and iii) it would open the door to the recovery of P as struvite.

### 1.10.1. The potential of the EBPR process to become an energy sustainable process

Nowadays, the energy consumption in the CAS process is estimated to be 3.2 kJ/g COD (Yan et al., 2013), whereas, theoretically, the potential energy in a typical domestic wastewater is supposed to be 16.2 kJ/g COD (Shizas and Bagley, 2004). This suggests that the potential chemical energy available in the raw municipal wastewater influent exceeds the electrical energy requirements for the treatment process by a factor of 5 (Rahman et al., 2016). So, If a 20% of the total energy in domestic wastewater could be recovered, the WWTPs will be energy self-sufficient (Wan et al. 2016)

The central point towards energy recovery is to recover as much as possible organic matter from the wastewater influent prior to its biological oxidation. There exist several options in order to redirect organic carbon to possible energy generation. However, they have certain limitations, such as the mineralization of a large fraction of the carbon in the influent. One successful process that has been used for carbon redirection is the high-rate activated sludge process (HRAS) (Jimenez et al. 2015). The HRAS technology employed the use of low SRT (between 1 and 4 days) with short hydraulic retention time (HRT) between 2 and 4 h (Grady et al., 211), which generates a sludge with a high degradability compared to the normal secondary sludge (Jimenez et al., 2015).

The HRAS technology is a relatively affordable method for removing particulated and soluble carbon and it can be designed and operated to reach the secondary effluent standards or as carbon adsorption processes when used as the first step (A-stage) in A/B process (Jimenez et al., 2015). The high-rate operation of the A-stage results in concentrating the influent particulate, colloidal and soluble COD to a waste sludge. The original purpose of the A/B process is to achieve a maximal organic matter recovery (A-stage) and the removal of the remaining biochemical oxygen demand (BOD) and ammonium (B-stage) (de Graaff et al. 2016), leaving aside the phosphorus removal.

In seeking to convert the conventional EBPR process into a suitable technology that not only remove resources from wastewater (organic carbon and phosphorus), but make them available for reuse, many researchers have studied the feasibility of adapting the EBPR process into the A-stage. The main obstacle for this integration is the SRT. The A-stage operates with short SRT which contrast with the high SRT used in the EBPR process (that is necessary to promote PAO growth). While it is difficult to operate an EBPR systems at such low SRT, it has been showed that it is possible to reduce the conventional SRT used in the EBPR process without losing the capacity of the system for P removal.

Ge et al. (2015) developed a high-rate biological phosphorus removal process operated at SRTs of less than 4 days. The results obtained demonstrated that the EBPR activity is stable at SRT between 2-2.5 days, achieving P removal up to 90 %. As it was expected, further reducing the SRT (1.7 days) resulted in a loss of the Bio-P activity. Besides, the waste activated sludge generated by this system was subject to anaerobic degradability; the lower the SRT, the higher the degradability (2 days SRT= 85%, 3 days SRT = 73% and 4 days SRT = 63%) (Ge et al., 2013).

### 1.10.2. The strategy of phosphorus recovery from the EBPR process

As phosphorus has an important role in food production (is a key ingredient in fertilizers to sustain high crop yields), guarantee its availability is probably one of the greatest challenges of the 21 st century (Cordell et al., 2011). Phosphorus scarcity has led scientists to find alternative sources of P, such as wastewater. A 20% of the consumed phosphorus is excreted by humans and hence recoverable (Cordell et al., 2011). However, P recovery from domestic wastewater is a challenge due to its low phosphorus content (10 mg/L), since its recovery as struvite requires wastewaters with a higher P content. This challenge has been faced by the use of P-capture technologies, such as chemical precipitation and EBPR process that enable concentration of this nutrient by 10-50 times. Although they were created for removing P from municipal wastewaters, they will not necessarily be effective in recovering it. Therefore, it is important to redefine the main objective of these technologies, focusing not only on the removal of P, but also on capturing it so that it can be recovered.

Rittmann et al. (2011) summarized the overall P-recovery in wastewater as follow: the first step is converting organic P into inorganic P and then remove the inorganic P from wastewater in a form that can be reused (Figure 1.10).

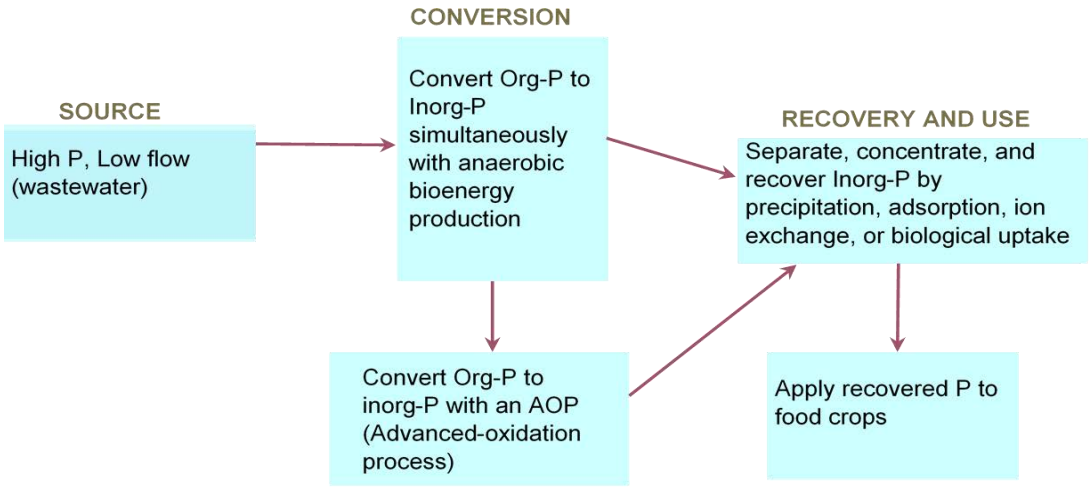


Figure 1.10. Diagram of the different ways to remove P from wastewater.

Nowadays, the most recommended technology for phosphorus recovery is the struvite ($MgNH_4PO_4.6H_2O$) crystallization. This technology might be suitable for treating sludge liquors from EBPR processes. However, the accumulation of P occurs in the sludge as all the P that is removed from wastewater is incorporated into the biomass (phosphorus can form 15 to 20 % of cell dry weight) (Yuan et al., 2012). Therefore, a previous treatment is necessary for both, the release from P-rich sludge and to separate undesired components (e.g. heavy metals, organic contaminants and pathogens) before P can be recuperated. P in EBPR sludge can be released by using biological methods, such as anaerobic digestion. One of the negative points of this method is the uncontrolled precipitation produced inside the digestor, which limits P availability in the liquid phase for its subsequent recovery (Yuan et al., 2012).

Recently, there has been increasing the interest in the P recovery as P stream in the water line using different technologies (Xia et al., 2014; Qiu et al., 2014). The advantage of obtaining a P-enriched supernatant is that it may be directly subjected to a phosphorus recovery process without the need for a pre-treatment.

The P-enriched liquid can be obtained directly from a sequencing batch reactor (SBR) performing EBPR by withdrawing it at the end of the anaerobic phase (after a

decanting phase). This extraction can result in the deterioration of EBPR process, due to the reduction of the main PAOs energy source. However, it has been showed that under adverse conditions, such as a low P concentration, PAOs are able to stay active during short periods (Acevedo et al., 2012).

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# CHAPTER 2

# Objectives

## 2.1. Thesis objective

EBPR process is the best technology to remove efficiently the P present in wastewater. Its current implementation is currently focused on simultaneous carbon, (usually) nitrogen and P removal. However, new trends in WWTP management involve a redesign of these systems in order to promote both energy and resource recovery. Hence, wastewater treatment facilities are adopting a role as resources recovery plants instead of nutrient removal systems.

The overall aim of this thesis is to evaluate the opportunities of EBPR integration in this new approach of future WWTP operation in order to optimise biological P removal and to study the possibilities of including strategies for P recovery. The specific objectives included are:

1) Assess the long-term impact of anaerobic extraction on the performance of an EBPR system with side stream generation of a P-enriched liquid (SBR-EBPR$_{RE}$) suitable for P recovery strategies such as chemical precipitation of struvite.
2) Assess the minimal SRT required to maintain the EBPR activity under a conventional anaerobic-aerobic configuration. This in view of a possible integration of EBPR process into more energy efficient systems such as the A/B process.
3) Investigate, with long and short-term experiments, the combined effects of temperature and SRT on EBPR activity. Special attention was paid to the low SRTs.
4) Assess the methane production in anaerobic digestion of biomasses containing different PHA levels obtained at different SRTs.

# CHAPTER 3

# Materials and methods

## 3.1. Lab-scale SBR

The system consisted of two fully monitored SBR (10L) with oxygen (Hamilton, Oxyferm 120 probe), pH (Hamilton, polilyte pro120 probe) and temperature. These reactors were controlled by a PLC (program logic control) (Siemens SIMATIC S7-226), which controlled the inlet and outlet pumps, the air and nitrogen electrovalves, the mechanical stirring, the pump for acid and base dosage. The PLC was connected via RS-232 to a PC, which monitored and stored the data received through a software programmed with Visual Basic (Figure 3.1).

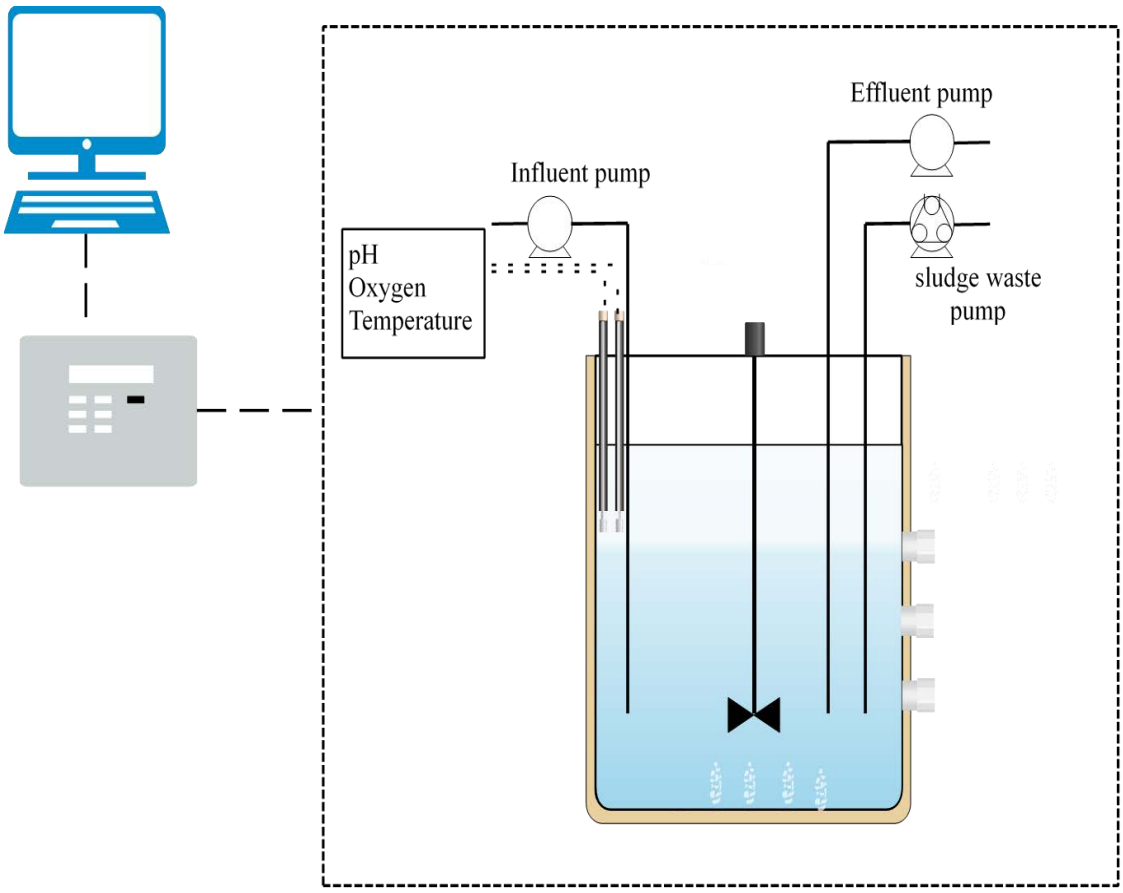


Figure 3.1. Schematic configuration of the SBR.

The reactors were inoculated with a seed sludge obtained from a pilot P removing treatment plant operated with an $A_2O$ process configuration. The reactors were operated with four cycles (6 h per cycle) per day. Each cycle consisted of a 120 min anaerobic period, a 210 min aerobic period, a 25 min settling and a 5 min for extraction (5L) (this configuration was modified in chapter 4). A volume of 5 L of synthetic wastewater was added during the

first five min of the anaerobic phase, producing a hydraulic retention time (HRT) of 12 h. The SRT was different for each chapter, varying from 3.5 to 15 days. The SRT values were maintained by daily wasting at the end of the anaerobic period but before the mixing was stopped.

For each SBR, homogenous conditions during reaction times were achieved with a stirring device. Aerobic conditions were achieved by injecting compressed air. The dissolved oxygen (DO) was controlled from 3.5 to 4.5 mg/L using an ON-OFF control by means of gas electro-valve. pH was controlled at 7.5 ± 0.1 (with an ON-OFF controller) by dosing HCL (1M) or NaOH (1M). The temperature was controlled at 25 ºC for the chapter 4, 5 and 7. In chapter 6, the temperature was adjusted to 20 ºC and varied in each batch experiment. During anaerobic phase, nitrogen was sparged 30 seconds of each 5 min to avoid oxygen transfer from the surface.

## 3.2. Wastewater (synthetic media)

As mentioned above, a volume of 5 L of synthetic wastewater was fed to the reactors along with 30 ml of a micronutrient solution and 68.5 ml of EDTA 0.5 M. Table 3.1 shows the composition of the synthetic wastewater. Propionic acid was used as the carbon source. The initial COD concentration was adjusted according to the main objective desired in each chapter.

Table 3.1. Synthetic wastewater fed to the reactors.

| **Concentrated feed** | | **Nutrient solution** | |
|---|---|---|---|
| **Component** | **Concentration (mg/L)** | **Component** | **Concentration (g/L)** |
| $KH_2PO$ | 54.4 | $FeCl_3.6H_2O$ | 1.5 |
| $K_2HPO_4$ | 41.2 | $H3BO_3$ | 0.15 |
| $NaHCO_3$ | 30 | $CuSO_4·5H_2O$ | 0.03 |
| $NH_4C$ | 100 | KI | 0.18 |
| $MgSO_4·2H_2O$ | 43.9 | $MnCl_2·4H_2O$ | 0.12 |
| $MgCl_2·2H_2O$ | 160 | $Na_2MoO_4·4H2O$ | 0.06 |
| CaCl2·2H2O | 42 | $ZnSO_4.7H_2O$ | 0.12 |
| Allylthiourea (ATU) | 5 | $CoCl_2·6H_2O$ | 0.15 |

## 3.3. Sampling and sample analyses

The SBRs performance were assessed daily by measuring $PO_4^{3-}$-P and propionic acid in the reactors influent and effluent. The volatile solid suspended (VSS) and total solid suspended (TSS) were measured twice a week. A complete monitoring (including the analysis of PHA and glycogen) was carried out when required. Each cyclic study involved withdrawing samples (10 mL) from the reactors every 15-30 min during the entire 6-h cycle. The samples were immediately filtered using a 0.22 µm pore size (Millipore).

## 3.4. Chemicals and biochemical analyses

Different methods were used in this thesis to determine and quantify the relevant parameters in the liquid and solid phase of the reactors.

### 3.4.1. Phosphorus

Phosphorus concentration was measured by a phosphate analyser (115 VAC PHOSPHAX sc, Hach-Lange). This equipment determines phosphate concentration using the Vanadomolybdate yellow method, where a two-beam photometer with LEDS measured the phosphate specific yellow colour. The detection limits were from 0.05 mg/L to 15 mg/L of $P\text{-}PO_4^{-3}$. It had an accuracy and a reproducibility of 2% ± 0.05 mg/L.

### 3.4.2. Volatile fatty acids (VFA)

Propionic acid concentration was determined by gas chromatography (GC) in an Agilent Technologies 7820A apparel equipped with flame ionization detector (FID) and a BP21 SGE column (30 m x 0.25 mm x 0.23mm; length x internal diameter x film thickness). A sample of 1µL of volume was injected at a temperature of 275 ºC under pulsed split conditions (29 psi). The carrier gas was helium with a split ratio of 10:1 at 2.9 mL/min, the column temperature was set at 85ºC for 1 min, followed by an increase of 3 ºC/min to reach 130 ºC. A second ramp of 35 ºC was maintained to reach 220 ºC. A cleaning step at 230 ºC during 5 min was used to remove any residue in the column. The rum time was 13 min.

### 3.4.3. Solids

Total Suspend Solids (TSS) and Volatile Suspended Solid (VSS) were determined following the methods described in Standard Methods for the Examination of Water and Wastewater (APHA, 1995). For the determination of TSS, 25 mL of well-mixed liquor were filtered through weighted glass fibre filter (Whatman, GF/C, 4.7 cm of diameter, 1.2 µm of pore size) previously dried up and weighed ($W_0$). The residue retained on the filter is dried to a constant weight (approximately during 2 h) at 100 ºC until achieving a constant weight. The increase in weight of the filter represents the TSS content of the sample ($W_1$). Regarding the VSS, the filters with TSS content were placed in the furnace using a ceramic bowl during half an hour at 550 ºC and then in the desiccator for 2h before being weighted again ($W_2$). So TSS was the difference between W0 and W1 and VSS were quantified by the difference between W1-W2. Triplicate analyses were done for each sample.

### 3.4.4. Glycogen

Glycogen content in biomass was determined by a modification of the method proposed by Smolders et al. (1994) and detailed in Pijuan et al. (2004). 50 mL of mixed liquor sample was centrifugated at 2500 *xg* for 20 min and then the extraction of the supernatant. The biomass obtained was frozen at -80 ºC during 24h before being lyophilised (24h). 20 mg of lyophilized biomass sample was placed in screw topped glass tube and a volume of 5 mL of HCl 0.6 M was added for each 20 mg of lyophilized sludge sample. Then, the tubes were heated at 100 ºC for 6h. The heated samples were left to cool at the room temperature, and then filtered through 0.22 µm Millex-HV filters. The concentration of glucose was measured using an YSI Model 2700 Select Biochemistry Analyser (Yellow Spring Instrument). Each sample was analysed three times.

### 3.4.5. Poly-hydroxyalkanoates (PHA) - biomass samples treatment and PHA extraction process

The different fractions of PHA (PHB, PHV AND PH2MV) content in the biomass were measured according to Oehmen et al. (2005).

0.6 mL of formaldehyde was added to each sample in order to stop biological activity and then centrifugated at 2500 *xg* for 20 min. The supernatant was removed, and the obtained biomass was frozen at -80ºC during 24h and then lyophilized during 24h.

For the extraction of PHA from the sludge, 40 mg of freeze-dried biomass were weighed and placed in Pyrex® tubes (for triplicate). 1.5 mL of butanol and 0.5 mL of hydrochloric acid were added. The tubes were capped and incubated at 100 ºC for 8h. The same procedure is required for the standards. The standards for calibration were performed using 3-hydroxybutiric acid and 3-hydroxyvaleric acid copolymer (Fluka, 98%) as standard for PHA and PHV and 2-hydroxycaproic acid (Sigma-Aldrich, 98%) as standard for PH2MV. After cooling, 2.5 mL of hexane and 4 mL of deionised water were added, the content was vortexed for 10s and then allowed to stand for 15 minutes to achieve phase separation. The upper solvent phase was taken and transferred to 15 mL Falcon tubes, then 4 mL of deionised water was added. The tubes were vortexed for 10s and then centrifugated at 2500 *xg* for 10 min (with the objective to remove solid debris from the sample). Finally, the upper solvent phase was taken and filtered through 0.22 µm Millex-HV filters into standard 2mL GC vials.

#### 3.4.5.1. GC analysis

A volume of 1 µL of sample was injected at GC system (7820 A) equipped with a FID detector and a HP-InnoWax column (30 m x 0.53 mm x 1 µm). Helium gas was used as carrier gas at 54 mL/min. The temperature of the injector was 220 ºC and the temperature of the detector was 275 ºC. The oven temperature was set to 70 ºC for 2 min, increase at 10 ºC/min to 160 ºC and held for 2 min. More information about the system is given in Table 3.2.

Table 3.2. Gas chromatographic method for the determination of PHAs.

| | |
|---|---|
| **Column:** | Agilent J&W HP-INNOWax (19095N-123)<br>Maximum temperature: 250 ºC<br>Length (m): 30 m<br>Diameter (mm): 0.53 mm<br>Film: 1 µm |
| **Liner:** | HP 5183 – 4711 |
| **Injector:** | |
| Mode | Split |
| Split ratio | 2:1 |
| Temperature (ºC) | 220 |
| Carrier gas | Helium |
| Pressure (psi) | 2 |
| Injection volume (µl) | 1 |
| Syringe (µl) | 10 |
| Sample washes | 3 |
| Sample Pumps | 4 |
| Pre-injection solvent washes (methanol) | 4 |
| Post-injection solvent washes (methanol) | 4 |
| **Oven:** | |
| Initial temperature (ºC) | 70 |
| Hold time at initial temperature (min) | 2 |
| Ramp 1 (ºC/min) | 10 |
| Final temperature (ºC) | 160 |
| Hold time at final temperature (min) | 2 |
| Run time (min) | 13 |
| **Detector (FID):** | |
| Temperature (ºC) | 275 |
| Hydrogen flow (ml/min) | 45 |
| Air flow (ml/min) | 450 |
| Makeup Flow (He) (mL/min) | 30 |

## 3.5. Microbial analysis

### 3.5.1. Fluorescence *in situ hybridisation* (FISH)

Fluorescence *in situ* hybridization (FISH) together with confocal laser scanning microscopy (CLSM), is one of the most widely used tools for direct detection and quantification of microbial population structures of activated sludge in wastewater research (Jubany et al., 2009). The principle of FISH is based on the tendency of RNA or DNA to bind specifically to its complementary sequences (hybridize) in target cells (Figure 3.2). If the complementary sequence for the oligonucleotides is not present in the 16S rRNA in the ribosome, stable hybridisation does not occur, and the oligonucleotide is washed out from the cell. Otherwise, if the complementary sequence is present, the oligonucleotide hybridises

the cell jointly with a fluorochrome. Cells, with the fluorescently-labelled oligonucleotide can be directly visualised in a CLSM.

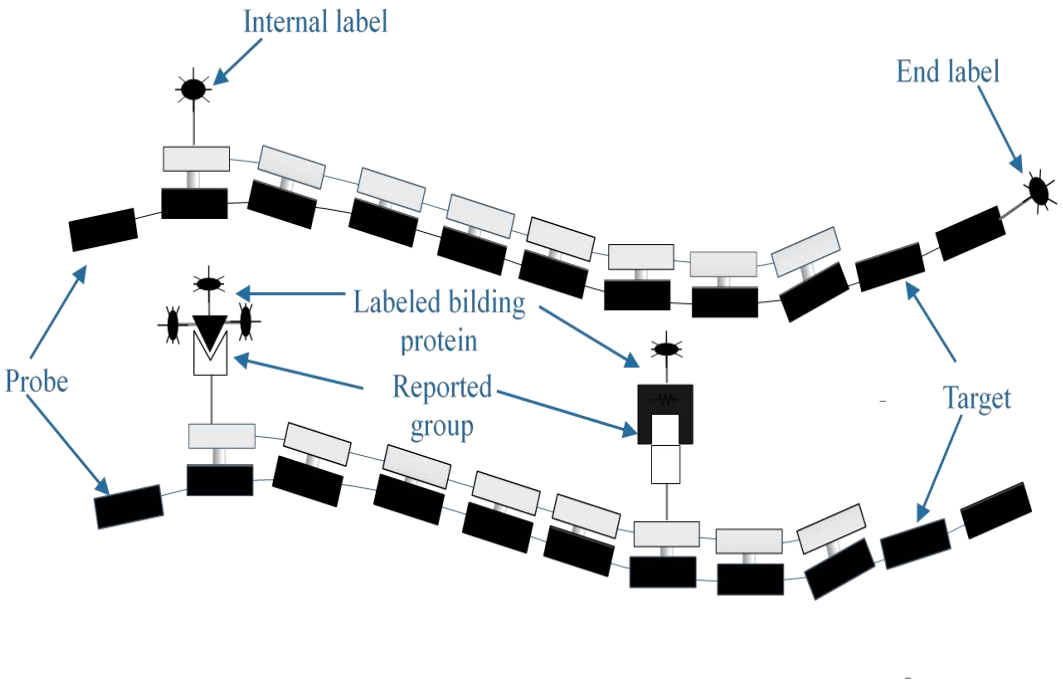


Figure 3.2. Diagrammatic oligonucleotide probe binding to rRNA molecule (source: Stahl and Amann, 1991).

Samples were withdrawn from the reactor at the end of the anaerobic phase in order to perform the FISH technique as detailed in Jubany et al. (2009). The probes (fluorescence dyes, also as fluorochromes) used in this thesis were: Cy3-labelled PAOMIX for "*Candidatus Accumulibacter phosphatis*" , comparing equal amounts of probes PAO462, PAO651 and PAO846; Cy5-labelled PAO I for cluster of "*Candidatus Accumulibacter phosphatis*", using Acc-II-444; Cy3-labelled GAOMIX for "*Candidatus competibacter phosphatis*", comprising equal amount of probes GAOQ431 and GAOQ989; Cy3-labelled DFIMIX for cluster I of "*Defluvicoccus vanus*" GAO, comprising TFO_df218 and TFO_DF618 probes; and Cy3-labelled DFIIMIX for cluster II of "Defluvicoccus vanus" GAO, comprising DF988 and DF1020 proves plus helper probes H966 and H1038.

Table 3.3. Oligonucleotide probes used in this thesis.

| **Probe** | **Specificity** | **Fluoro-chrome** | **Reference** |
|---|---|---|---|
| EUB338 | Many but not all bacteria | Cy5 | Amann et al. (1995) |
| EUB388-II | Planctomycetales | Cy5 | Daims et al. (1999) |
| EUB338-III | Verrucomicrobiales | Cy5 | Daims et al. (1999) |
| PAO462 | "*Candidatus Accumulibacter phosphatis*" | Cy3 | Crocetti et al. 2002) |
| PAO651 | "*Candidatus Accumulibacter phosphatis*" | Cy3 | Crocetti et al. 2002) |
| PAO846 | "Candidatus Accumulibacter phosphatis" | Cy3 | Crocetti et al. 2002) |
| **Acc-I-444** | "Candidatus Accumulibacter phosphatis" cluster I | Cy5 | Flowers et al. (2009) |
| Acc-II-44 | "*Candidatus Accumulibacter phosphatis*" cluster II | Cy5 | Flowers et al. (2009) |
| GAOQ431 | "*Candidatus Competibacter phosphatis*" | Cy3 | Crocetti et al. 2002) |
| GAOQ989 | "*Candidatus Competibacter phosphatis*" | Cy3 | Crocetti et al. 2002) |
| TFO_DF218 | "*Defluviicoccus-related TFO*" | Cy3 | Wong et al. (2004) |
| TFO-DF618 | "*Defluviicoccus-related TFO*" | Cy3 | Wong et al. (2004) |
| DF988 | "*Defluviicoccus-vanus*" cluster II | Cy3 | Meyer et al. (2006) |
| DF1020 | "*Defluviicoccus-vanus*" cluster II | Cy3 | Meyer et al. (2006) |
| H966 | Helper probe | - | Meyer et al. (2006) |
| **H1038** | Helper probe | - | Meyer et al. (2006) |

### 3.5.2. FISH protocol

FISH analysis was carried out in four main steps.

a) Sample fixation:

The morphology of the cells is fixed with aldehydes (formalin, paraformaldehyde, and glutaraldehyde) and/or alcohol (methanol, ethanol). This step is essential to make the cells more permeable to oligonucleotide probes. In this thesis the sample fixation solution used was solution of paraformaldehyde (PFA). It was prepared as follow: 4 g of paraformaldehyde was added to 65 mL of high purity water (Milli-Q water) heated to 60 ºC. Then, 2 M NaOH solution was added drop by drop and stirred immediately until it was nearly clarified (approximately 1-2 min). The solution was removed from the heat source and 33 mL of 0.03 M PBS solution was added. Afterwards, the pH was adjusted (7.2) by

addition of HCl. Any remaining crystal was removed by sterile filtration (0.22 µm). The solution was cooled to 4 ºC and stored at this temperature for no longer than 2 days; otherwise, it should be stored at -20 ºC in 1.5 mL aliquots. For sample fixation, 3 volumes of paraformaldehyde solution (1.5 mL) were added to 1 volume of sample (0.5 mL) and it was hold at 4 ºC for 1 to 3h. Afterwards, the cells were pelleted by centrifugation (5000g) and the fixative was removed. The cells were washed twice with 1 M PBS (centrifuging each time). Finally, the cells were resuspended and mixed in 0.5 mL of 1 M PBS and 0.5 mL of ice-cold ethanol.

b) Probe hybridization

Before the hybridization step, around 5-20 µm of fixed samples was added to each well in the glass slide and let air dry or in a heater (max. 60 ºC). Then, samples were dehydrated in ethanol series (3 min each): 50%, 80%, and 98% and let air dry. The cells are hybridized to the probe so that the probe binds to the complementary sequence in the target organisms. The hybridization buffer was prepared in 2 mL microcentrifuge tubes (Table 3.4). 8 µL of this hybridization buffer was added to each well on the slide. The remainder was poured in the 50 mL hybridization falcon tube that contained cellulose tissue. 1 µL of probe working solution was added and mixed carefully on each well. The slide was placed into the 50 mL tube containing the moistened tissue and was closed and put in the hybridization oven at 46 ºC for 2h. The working formamide concentration was 35%, but generally, it depends on the probe used.

Table 3.4. Composition of hybridization buffer.

| **Component** | **Volume to prepare 2 mL** |
|---|---|
| 5M NaCl (autoclaved) | 350 µL |
| 1M Tris/HCl (autoclaved) | 40 µL |
| 10 % SDS (not autoclaved) | 2 µL |
| Formamide | 700 µL |
| Milli-Q water | 898 µL |

c) Washing step

This step is essential to decrease or partly remove the fluorescence signal from non-specifically bound probes. The composition of washing buffer is given in Table 3.5. This solution was heated at 48 ºC in a water bath during the hybridization process. After 2h of

hybridization, the slides were carefully removed from the tube and immersed into the washing buffer tube and, then, into the water bath at 48 ºC for 10-15 min.

Once washed, the slide was gently rinsed in cold Milli-Q water. Water is directly above wells and allowed to flood over them. Both sides of the slide were washed to remove any salt, which is highly autofluorescent. After the washing step, all water droplets had to be removed from the wells by applying compressed air direct at the side of the slide.

Table 3.5. Composition of washing buffer.

| **Component** | **Volume to prepare 50 mL** |
|---|---|
| **5M NaCl (autoclaved)** | 80 µL |
| **0.5M EDTA (autoclaved)** | 500 µL |
| **1M Tris/HCl (autoclaved)** | 2 µL |
| **10% SDS (not autoclaved)** | 50 µL |
| **Milli-Q water** | 43.8 µL |

Finally, a few drops of anti-bleaching reagent (Citifluor AF1) were applied to the wells on slides. Slides were covered with a large coverslip that had to be pressed down gently to remove excess regent.

d) Identification and quantification

The fluoresce signal from the probe-targeted cells was detected by epifluorescence, with a CLSM (Olympus Fluoview 1000). By CLSM, the hybridized cells can be visualized at different wavelengths which bases upon the fluorescence dyes used to label the probes (Amann et al., 1998).

The quantification of the different cells hybridized as a proportion of all bacteria was done using image analysis techniques as described in Jubany et al. (2009). 40 randomly chosen CLSM fields from different x, y, and z coordinates were treated using the MATLAB Image Processing Toolbox.

## 3.6. Biochemical methane potential test

A biochemical methane potential (BMP) test was performed to quantify the biogas production of the biomass obtained in the anaerobic and aerobic phases of the EBPR process (chapter 7). The activated sludge used in the tests was generated from the SBR-EBPR during periods corresponding to a sludge age of 15, 10 and 5 days. Inoculum used in this experiment was collected from a full-scale anaerobic digester located in Rubi, Spain. Prior to the anaerobic sludge digestion test, the substrate (EBPR-biomass) and inoculum were subject to analysis of PHA, glycogen, VSS and TSS, $NH^{4+}$, pH, and COD. In addition, the inoculum was degassed by incubation at 37 ºC before use for 3 days to ensure that methane production from inoculum is as low as possible.

### 3.6.1. Substrate/Inoculum ratio

The determination of the amount of inoculum added is an important factor for the success of the experiment. It is usually desired (or recommended) a low amount of inoculum as inoculum also contributes to biogas formation. On the other hand, a smaller amount of inoculum than the necessary can lead to overload the process as a result. A substrate/inoculum ratio of approximately 0.5 volatile solid suspends (VSS) basis was maintained in all the test.

## 3.6.2. Determination of methane potential

In order to determine the biomethane potential for biomass with different SRT, anaerobic biodegradability tests were performed in batch essays according to the methodology proposed by (Angelidaki and Sanders 2004).

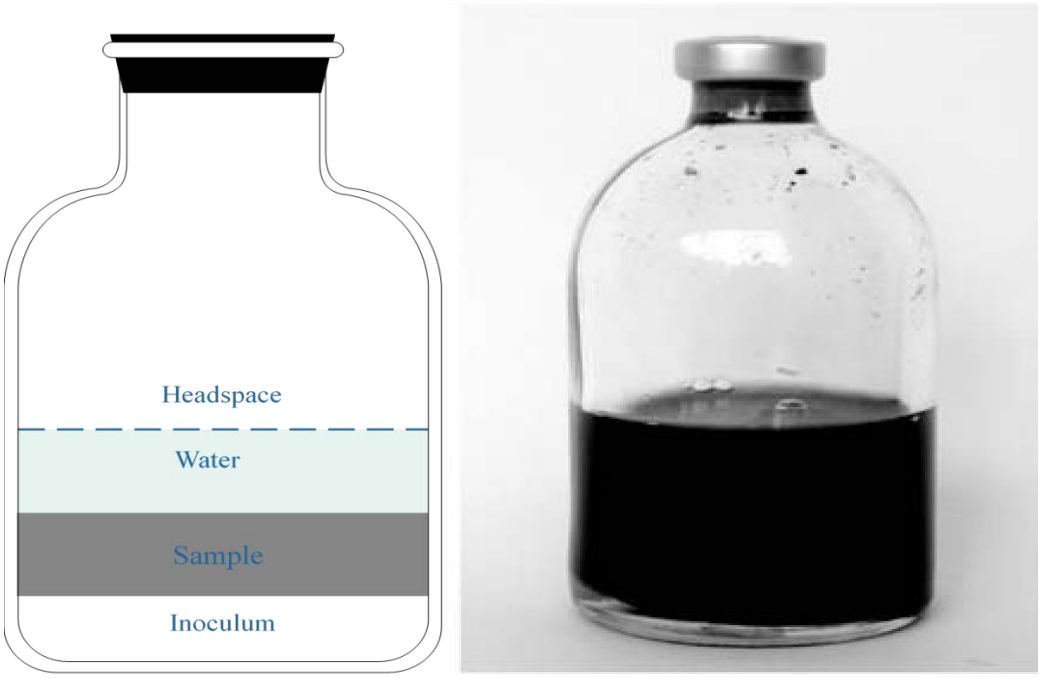


Figure 3.3. Experimental set-up for BMP tests.

The BMP test was performed in 160 mL sealed glass serum bottles. The total working volume was 125 mL, which resulted in a headspace of 35 mL (21.87%). The bottles consisted of a mixture of substrate, inoculum (the amount of substrate and inoculum added in each bottle was directly related to the substrate/inoculum ratio desired), a grow medium containing macro and micro-nutrients (Table 3.6), and a volume of Milli-Q water to complete the working volume (Figure 3.3). The pH was measured, and the bottles were flushed with high purity nitrogen gas for 3 min sealed with a rubber stopper retained with an aluminium crimp-crap and stored in temperature-controlled incubators. For each type of substrate, blank controls were performed. Blanks contained only inoculum and Milli-Q water to determine the background methane produced from the inoculum. All the tests were carried out in triplicates. The volume of biogas produced was determined by the variation of pressure inside the glass flask by means of a pressure transducer and, its composition, by gas chromatography.

Table 3.6. Macro and micro-nutrients solution.

| **Reagents (g/L)** | **Amount** |
|---|---|
| $NH_4Cl$ | 10.0 |
| NaCl | 10.0 |
| $MgCl_2.6H_2O$ | 10.0 |
| $CaCl_2.2H_20$ | 5.0 |
| $K_2HPO_4.3H_2O$ | 200.0 |
| Resazurin | 0.5 |
| **Trace-metal and selenite solution (g/L)** | |
| $FeCl_2.4H_2O$ | 2.0 |
| $H_3BO_3$ | 0.05 |
| $ZnCl_2$ | 0.05 |
| $CuCl_2.2H_2O$ | 0.038 |
| $MnCl_2.4H_2O$ | 0.05 |
| $(NH_4)_6Mo7O_{24}.4H_2O$ | 0.05 |
| $AlCl_3$ | 0.05 |
| $CoCl_2.6H_2O$ | 0.05 |
| NiCl2.6H2O | 0.092 |
| ethylenediaminetetraacetate | 0.5 |
| HCl | 1 ml |
| $Na_2SeO_3.5H_2O$ | 0.1 |
| **Vitamine mixture (mg/L)** | |
| Biotin | 2.0 |
| Folic acid | 2.0 |
| Pyridoxine acid | 10.0 |
| Riboflavin | 5.0 |
| Thiamine hydrochloride | 5.0 |
| Cyanocobalamine | 0.1 |
| Nicotinic acid | 5.0 |
| P-aminobenzoic acid | 5.0 |
| Lipoic acid | 5.0 |
| Pantothenic acid | _ |

## 3.7. References

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# CHAPTER 4

## Performance of an EBPR system oriented to obtain a liquid highly enriched with phosphorus

## 4.1. Introduction

Global reserves of P, essential for agriculture, could run out in 50 to 100 years (Rittmann et al., 2011), which would endanger food security. This has led to the need to find alternative sources of P, such as municipal wastewaters. It has been estimated that 15-20 % of world demand for phosphate rock could theoretically be satisfied by recovering P from this source (Yuan et al., 2012). Although phosphorus recovery from municipal wastewater is an important option, it can be a challenge due to its low phosphorus concentration (7-10 mg P/L) (Parsons and Smith, 2008). Existing phosphorus recovery processes are only suitable for wastewater streams with phosphorus content higher than 50 mg/l, such as liquor of dewatered EBPR sludge and side stream of an anaerobic digester (Wong et al., 2013). Up to now, the most widely proposed technology for phosphorus recovery is through struvite crystallization ($MgNH_4PO_4.6H_2O$). Struvite precipitation occurs readily once phosphorus reaches 50-200 mg/L and as long as ammonium is present (Matassa et al., 2015). Most full-scale struvite plants use anaerobic liquor originating from primary and secondary sludge digestion (Moerman en al., 2009), with the sludge obtained from EBPR process being the best option (this sludge contains normally 0.06-0.15 mg P/ mg VSS) (Wang et al., 2016).

The EBPR process removes up to 90% of the soluble phosphorus present in wastewater in the form of sludge. In this process, phosphorus is largely captured in solid form, which can be separated from wastewater. There are several methods to recover the phosphorus present in the liquors generated in the EBPR process, which can be classified into three treatment categories: biological, wet-chemical and thermal. While wet-chemical and thermal methods can increase the extent of P-release from the sludge to a liquid phase, it is also clear that they have challenges related to capital cost, energy consumption, chemical use and corrosion (Rittmann et al., 2011; Sartorius, 2012). In biological treatments, the sludge produced from EBPR is treated in an anaerobic digester to solubilise the phosphorus in the biomass. During anaerobic digestion, phosphorus is released from the biomass to the liquid phase (as well as ammonium and magnesium), resulting in supernatants with high phosphorus, ammonium and magnesium content (Martí et al. 2010). A complication of the anaerobic digestion process is the re-precipitation of phosphorus with calcium or magnesium that takes place in the digester, limiting the availability of phosphorus in the liquid phase (Chaparro et al., 2011).

Phosphorus recovery is possible from both the liquid phase and the solid phase (sludge) (Cornel and Schaum, 2009). However, due to the disadvantages of existing methods for releasing phosphorus from sludge, researchers in the field believe that recovery of phosphorus directly from the water line is the best option (Sartorius 2012). The recovery of phosphorus directly form wastewater has been shown to be feasible using methods such as adsorption (Westholm, 2006), ion exchange (Kuzawa et al. 2006) and osmosis (Qiu and Ting 2014). Nevertheless, these methods involve high operating costs and large reactor volumes (Rittmann et al. 2011). In view of this, a viable alternative for the recovery of phosphorus from wastewater is to use biological methods such as the EBPR process. However, as mentioned above, the P content of wastewaters is relatively low, which makes it unsuitable for P recovery. Unless the P content is increased (concentrations greater than 50 mg/L), direct recovery of this element from domestic wastewater is an economic and technical challenge (Wong, 20016). Some researchers have addressed this challenge. For example, Xia et al. (2014) used a novel approach based on the EBPR process to carry out phosphorus recovery. They operated two reactors, the first for the production of P-enriched sludge (EBPR system) and the second for P-release. When steady state was reached in the first reactor, some of the sludge was removed and added together with external carbon to the second reactor for P-release. The mixed liquor was then filtered, and the filtered sludge was returned to the first reactor and the supernatant was used for P-recovery. They were able to recover 79 % of phosphorus through chemical precipitation. One of the disadvantages of this proposed method is that it was operated at an SRT of approximately 60 days, which increases energy requirements while reducing the faction of recoverable carbon.

Taking into account the previous background, this chapter studies a new strategy based on EBPR to obtain a highly P-enriched stream directly from the process line. In this strategy, the conventional SBR-EBPR configuration (anaerobic-aerobic-settling) was modified by adding a settling period after the anaerobic phase, resulting in the following configuration: anaerobic-settling-aerobic-settling. A stream highly enriched in P is obtained by withdrawing part of the supernatant at the end of the anaerobic stage (after the settling period). However, phosphorus is the main source of energy for PAO to capture carbon in the anaerobic phase, so phosphorus extraction would reduce the availability of energy for PAO to perform this task. This would lead to a deterioration of the EBPR process. Hence, this study explores the long-term impact of anaerobic phosphorus extraction on the new EBPR configuration.

## 4.2. Materials and methods

### 4.2.1. Experimental design

This study was divided into two main periods. In the first period, a conventional EBPR configuration was used to obtain a robust EBPR process. In the second period the configuration was modified to include the extraction of P-enriched anaerobic supernatant.

For the first period, a laboratory-scale SBR-EBPR with a working volume of 10 L was operated with a conventional A/O configuration with 6 h cycles. Each cycle consisted of an anaerobic phase (120 min), an aerobic phase (210 min), a settling phase (25 min) and a withdrawal phase (5 min). The pH was regulated at 7.5 ± 0.05 by dossing 0.1 M HCl or 0.1 M NaOH. Stirring was provided during the anaerobic and aerobic phases. Aeration was carried out during the aerobic phase to maintain an oxygen concentration (DO) between 2.5 and 3.5 mg DO/L. SRT was set at 8 days and HRT at 12 hours. SRT was controlled by biomass wasting at the end of the aerobic phase. During the feeding phase, a synthetic wastewater with propionic acid (200 mg COD/L) as the sole carbon source was fed to the reactor within the first 5 minutes. The synthetic wastewater solution (5L) is described in chapter 3.

For the second period, the conventional EBPR configuration was modified by adding a settling phase after the anaerobic phase, resulting in the following configuration: anaerobic/settling/aerobic/settling (SBR-EBPR$_{REC}$) (figure 1). The amount and frequency of the supernatant extracted varied according to the final objective sought. To evaluate the effect of the influent COD/P ratio on the performance of SBR-EBPR$_{REC}$ and to promote the maximum enrichment of the anaerobic supernatant with phosphorus (maximum P-release) the SBR-EBPR$_{REC}$ was operated with four different strategies (Table 1). For this purpose, the COD concentration in the influent was kept constant at around 300 mg COD/L while the P concentration in the influent gradually increased from 20 mg P/L in the first stage to 40 mg P/L in the last stage.

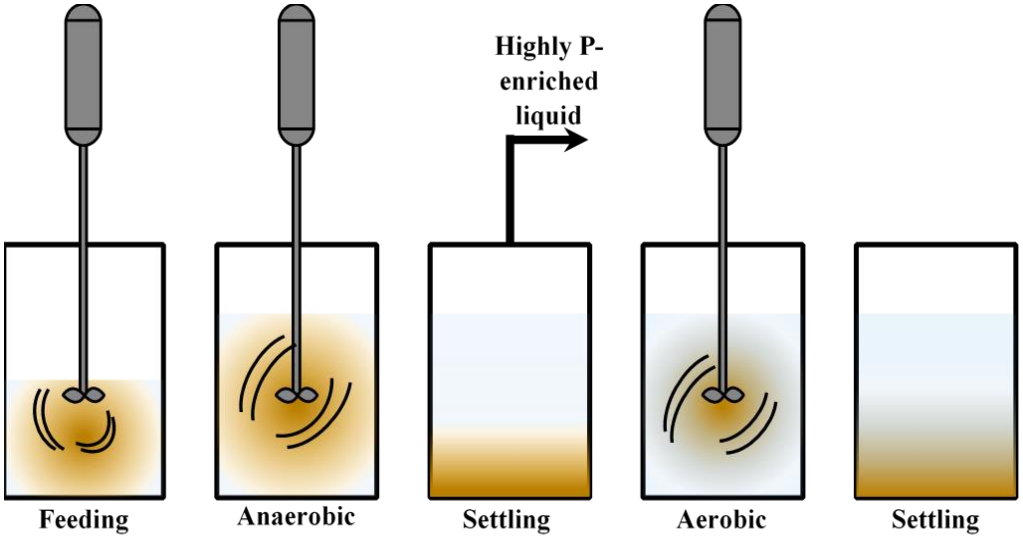


Figure 4.1. SBR configuration to obtain a P-enriched liquid.

Table 4.1. Different stages studied during the SBR-EBPR$_{REC}$ operation.

| Stage | Volume of supernatant extracted (L) | COD (mg/L) | P (mg P/L) | COD/P |
|---|---|---|---|---|
| **I** | 1 per cycle | 300 | 20 | 15 |
| **II** | 1 per cycle | 300 | 30 | 10 |
| **III** | 1.5 per cycle | 300 | 30 | 10 |
| **IV** | 1 per cycle | 300 | 40 | 7.5 |

### 4.2.3. Analytical methodology

The SBR-EBPR performance was assessed daily by measuring P-$PO_4^{-3}$ and propionic acid in the reactor. TSS and VSS were measured twice a week. PHA and glycogen were evaluated during each operational change in the reactor.

For chemical analyses, mixed liquor samples were taken at the end of each anaerobic and aerobic phase. The samples were immediately filtered with 0.22 µm pore size Millipore filters and analysed for soluble P-$PO_4^{-3}$ and propionic acid. The concentration of P was measured by a phosphate analyser (115 VAC PHOSPHAX sc, Hach-Lange) based on the Vanadomolybdate yellow method, where a two-beam photometer with LEDS measured the phosphate specific yellow colour. The propionic acid was measured using a gas chromatograph (GC Agilent Technologies 7820 A) equipped with a BP21 SGE column (30 m 0.25 mm 0.22 mm; length internal diameter film thickness) and a flame ionisation detector (FID). Sludge samples collected for PHA and glycogen measurements were treated as

follows: immediately after collection, 0.6 ml of formaldehyde was added to the samples to stop biological reactions; the samples were centrifuged and then lyophilized. PHA extraction was done using hexane and butanol. The different types of PHA were measured with a GC (Agilent Technologies 7820 A). 3-hydroxybutiric acid and 3-hydroxyvaleric acid copolymer was used as standard for polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) and 2 hydroxycaproic acid as standard for polyhydroxy-2-methylvalerate ($PH_2MV$). Glycogen analysis was performed by a modification of the method proposed by Smolders et al. (1994). A volume of 5 mL of 0.6 M HCl was added to each 20 mg of lyophilised sludge sample, and then heated to 100 ºC for 6 hours. Once the samples were cooled they were filtered with a 0.22 µm filter (Millipore). An YSI model 2700 select Biochemistry Analyser (Yellow Springs Instrument, Yellow Spring, Ohio, USA) was used to determine glucose.

### 4.2.4. Calculations

The calculation of some parameters used as performance indicators are explained in this section.

The calculation of specific removal efficiency (SRE) was as follows:

$$SRE = \frac{P_{in} - P_{eff}}{VSS \cdot V_{SBR}}$$

Where $P_{in}$ and $P_{eff}$ are the amount of P in the influent and efluent (mg P), Vss is the concentration of VSS (g VSS/L) and $V_{SBR}$ is the SBR volume (L). This parameter proportions information on the sludge PAO activity. It relates the net P removal capability of the System to the amount of biomass inside the reactor (VSS).

The net P removal per cycle (NPR) is the mass of phosphorus removed per cycle in mg P/L and the removal efficiency (RemE) is the porcentage of imput P removed. The recovery efficiency (RecE) is the percentatge of P present in the anaerobic supernatant extracted relative to the influent P and therefore is the màximum ratio of P that could be recovered as struvite. Finally, the biological removal efficiency (BioE) is the ratio of P biologically removed and was calculate as the percentatge of P removed that was not extracted.

## 4.3. Results and discussion

### 4.3.1. Obtaining a stable EBPR system

In the first part of the study, the conventional EBPR configuration (anaerobic-aerobic-settling) was used to obtain a robust EBPR system. Figure 2 shows the performance of a typical EBPR cycle under stable operation.

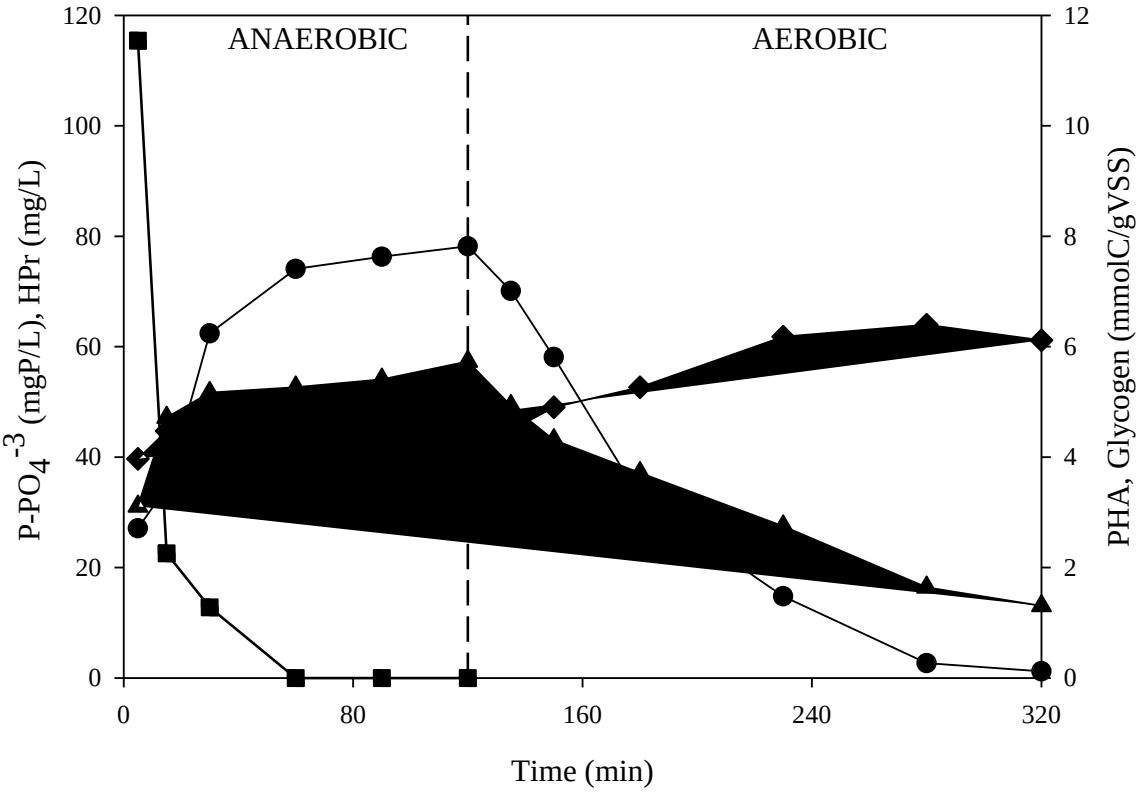


Figure 4.2. Typical EBPR performance under stable operation. (●) P, (■) HPr, (▲) PHA, (♦) glycogen.

It can be seen from Figure 4.2 that during the anaerobic period, propionic acid was taken up and stored as PHA, using internally stored P and glycogen degradation. This results in P-release into the bulk solution and a decrease in glycogen content in the biomass. Under aerobic conditions, most of the internally stored PHA was consumed, P-uptake almost led to complete P depletion, and glycogen was recovered. PAO activity can be reflected through the evolution of these measurements. Considering the typical ratios observed in the literature (Oehmen et al. 2007), the ratios obtained demonstrate a typical PAO phenotype: $P_{rel}/VFA_{upt}$ = 0.4 molP/molC, $PHA_{prod}/VFA_{upt}$ = 1.27 molC/molC, and $Gly_{deg}/VFA_{upt}$ =0.63 molC/molC. The results obtained from the weekly phosphorus monitoring showed a gradual increase in

P-release and P-uptake rates from 0.95 to 1.24 mol $P\text{-}PO_4^{-3}$/g VSS. h and 0.32 to 0.42 mol $P\text{-}PO_4^{-3}$/g VSS/h, respectively, indicating an increase in PAO activity during this period. Subsequently, the P-release and P-uptake rates remained relatively stable (1.17 ± 0.10 mol $P\text{-}PO_4^{-3}$/g VSS/h and 0.37 ± 0.07 mol $P\text{-}PO_4^{-3}$/g VSS/h), indicating that the SBR achieved stable operation. Once stable operation was observed (day 20-25), the conventional EBPR configuration was changed to the $SBR\text{-}EBPR_{REC}$ configuration (Figure 4.1).

### 4.3.2. Influence of COD/P ratio on the $SBR\text{-}EBPR_{REC}$ performance

The concentration of phosphorus in the anaerobic supernatant extracted from the $SBR\text{-}EBPR_{REC}$ system depends on the P-release in the anaerobic phase, which in turn depends on the PAO metabolism. Therefore, factors affecting the PAO population, such as the influent COD/P ratio, must be considered to obtain a highly P-enriched supernatant without compromising EBPR efficiency. The COD/P ratio has been shown to influence the PAO population and, consequently, the EBPR performance (Oehmen et al., 2005; Broughton et al., 2008). In this chapter the effect of different COD/P ratios on $SBR\text{-}EBPR_{REC}$ performance was evaluated. Table 4.2 and Figure 4.3 show the typical operating parameters obtained for $SBR\text{-}EBPP_{REC}$ operation.

Table 4.2. Characteristics of anaerobic and aerobic periods of $SBR\text{-}EBPR_{REC}$.

| Stage | COD/P[a] | $P_{rel}$[b] | $P_{upt}$[b] | $P_{upt}/P_{rel}$[c] | RemE (%) | Volume of supernatant extracted (L) |
|---|---|---|---|---|---|---|
| **I** | 15.0 ± 0.1 | 38.9 ± 7.5 | 53.3 ± 6.6 | 1.41 ± 0.01 | 98% ± 1 | 1 |
| **II** | 10.0 ± 2.3 | 67.5 ± 17.9 | 86.3 ± 17.2 | 1.27 ± 0.01 | 67% ± 10 | 1 |
| **III** | 10.0 ± 2.5 | 40.2 ± 14.3 | 60.8± 14.4 | 1.51 ± 0.06 | 82% ± 14 | 1.5 |
| **IV** | 7.5 ± 0.9 | 36.2 ± 8.5 | 53.9 ± 7.5 | 1.48 ± 0.02 | 77% ± 6 | 1 |

a= mg COD/mg P; b = mg/L; c= mg P/mg P

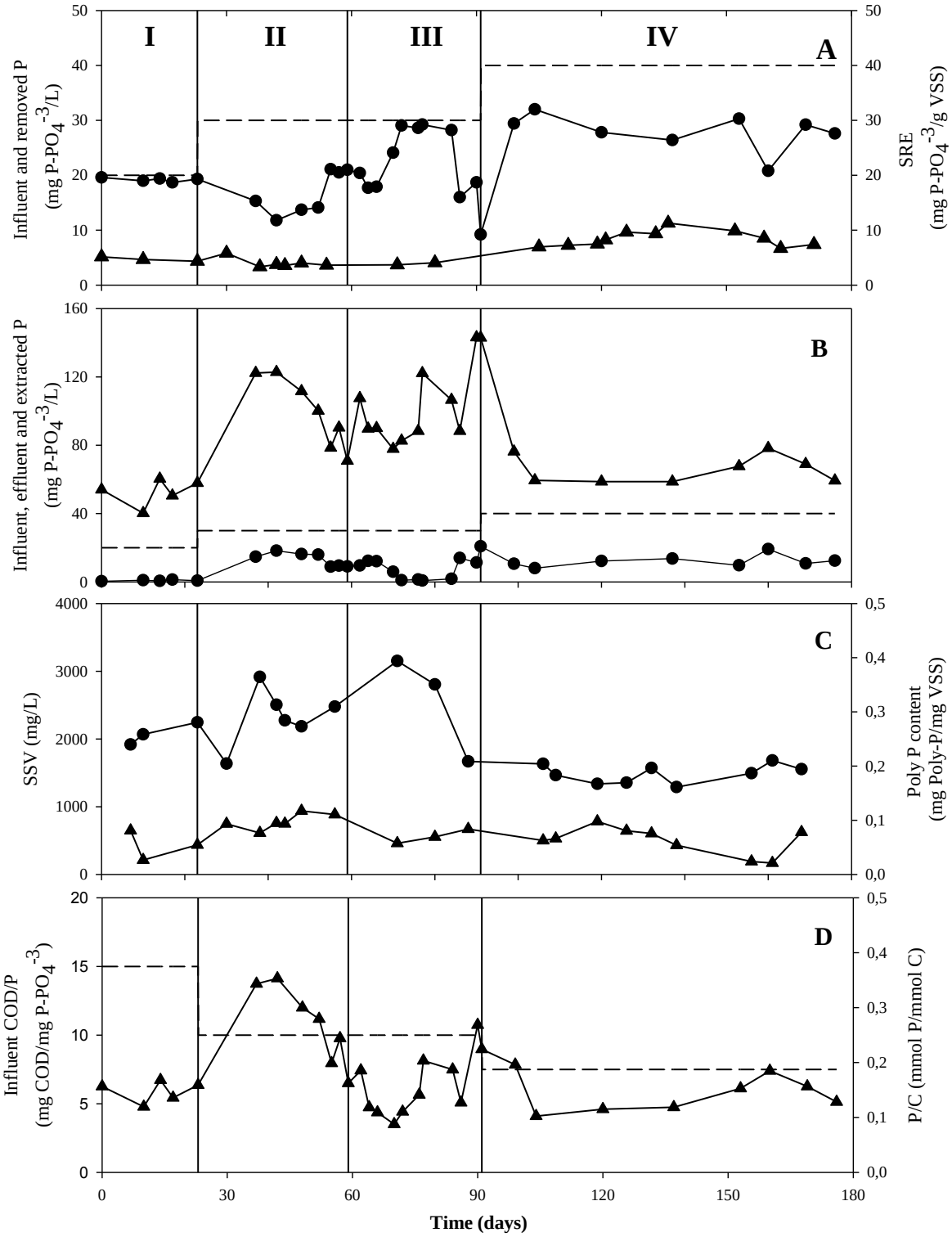


Figure 4.3. Evolution of different parameters during the whole experimental period at different P load. A: P influent (solid line), SRE (▲), P removed (●); B: P influent (solid line), P content in the anaerobic liquid (▲), P effluent (●); C: VSS (●), P content (▲) and D: influent COD/P (dashed line), P/C ratio (▲).

In order to assess the capacity of SBR-EBPR$_{REC}$ to treat high P loads without increasing the organic load, that is, influent with low COD/P ratios, the P concentration in

the influent was increased progressively (Table 4.1). Despite the low COD/P ratio applied in stages II, III and IV, an average P-removal of 17, 21 and 28 mg P/L was observed, respectively (Figure 4.3A). These values represent between 57 and 70 % of net-P-removal in these stages, which was reflected in periods with elevated P levels in the effluent (Figure 4.3B). This means that in these stages the biological P removal efficiency (BioE) was low (Table 4.3).This means that the influent COD/P ratio in these stages was limited. For example, when the COD/P ratio of the influent was decreased to 7.5 (stage IV), the COD was not sufficient for complete P-removal. In spite of this, approximately 30 mg P was removed, which means that the minimal COD/P ratio of our system is around 10.

BioE is a parameter that represents the amount of P biologically removed. This parameter was calculated for all periods (Table 4.3) and may be used in order to assess the PAO activity: a high BioE value indicates high PAO activity, whereas a low value may be indicative either of low PAO enrichment of the sludge or low PAO activity. The stage I was the only in which successful net-P removal was achieved (98% ± 1) despite showing a low PAO activity (BioE = 45%). This is because BioE only takes into account the P captured by PAO and not the P removed during the anaerobic extraction. The good EBPR performance observed in this stage could be attributed to the adequate COD/P ratio used (COD/P = 15). Broughton et al. (2008) and Kapagiannidis et al. (2012) point out that a COD/P ratio in the range of 13-20 is necessary to obtain good P removal efficiency. Therefore, it can be assumed that the low P removal efficiency observed during stages II and IV is caused by the low COD/P ratio (10 and 7.5) (In this case, stage III is not considered since the high volume of anaerobic supernatant extracted may also have contributed to the loss of EBPR activity). According to Yagci et al (2003), this can be explained by the fact that the required influent COD/P ratio is related to the stoichiometry of P storage. In other words, if the COD/P ratio in the influent is higher than the minimum required, the existing organic carbon is in excess so that the net result is total P removal, whereas when COD/P ratio is lower than the minimum required, the P storage capacity is limited and there is always a certain P concentration in the effluent.

Table 4.3. Fate of P in the influent in the different operational periods.

| Stage | Influent P (mg P/cycle) | NPR (mg P/cycle | RemE (%) | RecE (%) | BioE (%) | Effluent P (%) |
|---|---|---|---|---|---|---|
| I | 100 | 97.7 ± 1.4 | 98 ± 1 | 53 ± 8 | 45 ± 10 | 2 ± 1 |
| II | 150 | 99.9 ± 15.0 | 67 ± 10 | 66 ± 14 | 0 ± 23 | 33 ± 10 |
| III | 150 | 122,8 ± 21.7 | 82 ± 14 | 69 ± 16 | 13 ± 25 | 18 ± 14 |
| IV | 200 | 154.3 ± 12.8 | 77 ± 6 | 33 ± 4 | 44 ± 9 | 23 ± 6 |

NPR: net phosphorus removal per cycle
RemE: removal efficiency
RecE: recovery efficiency,
BioE: biological removal efficiency

Two main elements are key to quantifying the degree of P recovery: the volume of anaerobic supernatant (addressed in the following section) and its P concentration. The concentration of P in the anaerobic supernatant is related to the amount of P released in the anaerobic phase. Therefore, the high amount of P released during the anaerobic phase is expected to lead to high percentages of P-recovery (RecE). A comparison between stages I and II, in which the same amount of anaerobic liquid was extracted (1L) and different P influent concentrations were used (20 and 30 mg P/L, respectively), shows that the highest RecE and P- release were obtained in stage II (Table 4.2 and 4.3). The results obtained in this chapter, together with the bibliography, show that there is a clear relationship between the influent COD/P ratio and P recovery efficiency (Table 4.4). That is, the higher the influent COD/P ratio, the higher the percentage of P recovery. However, municipal wastewater lacks readily biodegradable COD, and hence the results obtained with systems operating with low influent COD/P are more realistic. While it is true that with a COD/P ratio of 10, approximately 66-69% of the influent P was recovered, however, complete P removal was not achieved. Therefore, a COD/P ratio greater than 10 must be applied to ensure a P rich anaerobic stream and good EBPR performance.

Table 4.4. Reports of the amount of phosphorus diverted to recovery observed in previous studies and comparison to this work.

| **Works** | **Influent COD/P** | **Influent P** | **P-recovery[a]** | |
|---|---|---|---|---|
| | (g COD/g P) | (g P/m$^3$) | (g P/m$^3$) | (%) |
| **Period I** | 15 | 20 | 40-60 | 54 |
| **Period II** | 10 | 30 | 71-123 | 66 |
| **Period III** | 10 | 30 | 52-95 | 69 |
| **Period IV** | 7.5 | 40 | 59-78 | 33 |
| (Acevedo et al. 2015) | 14 | 7.5 | 70-50 | 59 |
| | 50[b] | 7.5 | 200-150 | 81 |
| (Zou, Lu, and Li 2014) | 25 | 10 | 28,3 | 70 |
| (Barat and van Loosdrecht 2006) | 54[c] | 3.7 | 60 - 70 | ~60 |
| (Zou and Wang 2016) | 40 | 5 | -[d] | 59 |

[a] Calculated as the fraction of the influent extracted for its recovery.
[b] The COD/P was 50 for the cycles when extraction was performed. During the normal operation the COD/P ratio was 14.
[c] Only COD as VFA and fermentable organic matter is taken into account for COD/P ratio calculation.
[d] P recovered as HAP.

### 4.3.3. The influence of volume of anaerobic extraction on the SBR-EBPR$_{REC}$ system performance

Sporadic anaerobic extraction of supernatant as a strategy for obtaining a highly P-enriched stream has been shown not to have a negative effect on EBPR activity (Acevedo et al., 2015). Furthermore, Baeza et al. (2017) predicted, through simulation, that it is possible to extract up to 4.3% of the working volume of an SBR-EBPR in each cycle without compromising the P-removal efficiency. However, the effect of long-term anaerobic extraction on the EBPR process has not been evaluated experimentally prior to the present work. For this purpose, automatic anaerobic supernatant extraction was implemented in each cycle over a long-term operation. Before proceeding with the analysis of the results, it is important to understand the fate of the P entering the system. A mass balance in the system shows that input-P has three different pathways: i) the anaerobic supernatant, ii) as part of the biomass in the purge and iii) the reactor effluent (Figure 4.4).

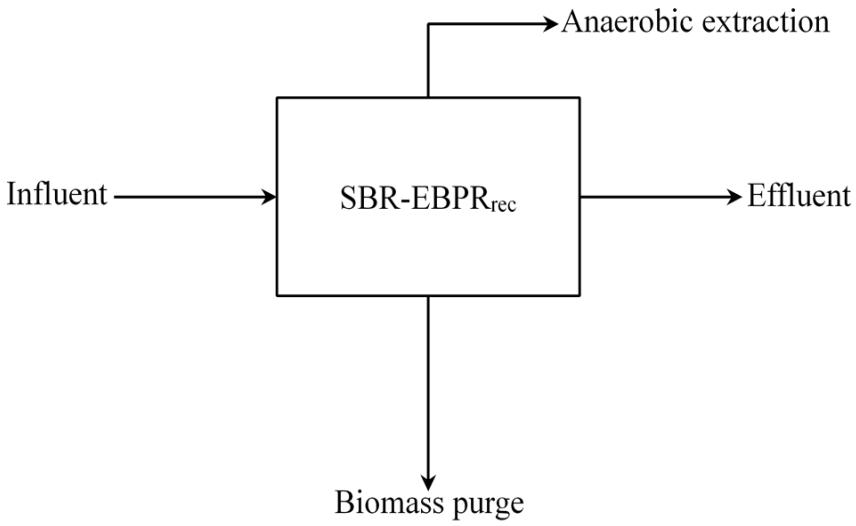


Figure 4.4. Fate of influent phosphorus.

In stage I, a volume of 1 L of anaerobic supernatant per cycle was extracted from the system. EBPR activity was not negatively affected under this condition, since successful P removal was observed (98%). At this stage, the P concentration in the influent was 20 mg P/L (COD/P ratio of 15), i.e. 100 mg of P/cycle entered the system. Based on the mass balance, approximately 53 % of the input-P was present in the anaerobic supernatant and could therefore be recovered (RecE). The rest of the influent P was mostly removed through the EBPR activity (BioE = 45%) and therefore purged with the biomass and approximately 2% was present in the effluent (values mentioned in Table 4.3). These results are interesting since they show that good EBPR performance can be maintained despite the continued decrease in the availability of P for PAO (due to supernatant extraction), in contrast to earlier reports where continuous anaerobic extraction of P could not be sustained in the long term as it led to deterioration of the EBPR process (Acevedo et al. 2015).

In stage II, the volume of anaerobic supernatant extraction remained at 1L per cycle, but the P load was increased to 30 mg /cycle (COD/P ratio of 10), meaning that 150 mg of P entered the system at each cycle. The increase of P load led to an increase of P released in the anaerobic phase, however, the absolute P-removal decreased to a value of 67% (Table 1). High anaerobic P-release led to high percentages of RecE (66%). In other words, during this stage an enriched stream of approximately 100 mg $P\text{-}PO_4^{-3}$/L was obtained (i.e. more than three times the influent concentration), which is an adequate concentration for possible recovery by chemical precipitation. To improve the amount of P recovered, in stage III the

extraction volume was increased to 1.5 L per cycle (COD/P = 10). During the first days, both the P-release and P-uptake increased, indicating increased PAO activity (Figure 4.3.A). However, this high extraction could not be sustained in the long term and the system was about to fail at this stage (Figure 4.3.A). Despite this increase in the volume of supernatant extraction, the RecE obtained (69%) was only slightly higher than that obtained in stage II; as a result, the concentration of P in the anaerobic supernatant extracted (103.5 mg $P\text{-}PO_4^{-3}$/L) was similar to that of stage II. In order to restore system stability, in stage IV the extracted volume was returned to the previous value of 1 L and the concentration of P in the influent was increased to 40 mg P/L (COD/P = 7.5). However, the unfavourable influent COD/P ratio (7.5) led to a decrease in RemE (77%). On the other hand, the P-release decreased, leading to a low amount of RecE (about 33%). This resulted in an anaerobic stream with a P concentration of 65.9 ± 8.1 mg/L. In this sense, Stage IV had the lowest RecE in spite of the high effluent P.

Overall, the anaerobic supernatant obtained in the different operational stages of the $SBR\text{-}EBPR_{REC}$ system contained up to 100 mg $P\text{-}PO_4^{-3}$/L (i.e. up to three times higher than that of the influent), which makes it suitable for use as raw material in the struvite precipitation process, since the minimum value for struvite precipitation is 50 mg $P\text{-}PO_4^{-3}$/L (Cordell et al., 2009; Kodera et al., 2013). However, in most of these stages full P removal was not achieved (II, III and IV), which was reflected in the high P concentration in the effluent (Figure 2A). Therefore, we can assume that the high extraction of P during the anaerobic phase (stage III) and the low COD/P ratio in the influent (stage II and IV) (discussed above) were the main factors that affected the performance of $SBR\text{-}EBPR_{REC}$. As mentioned above, Baeza et al. (2017) used modelling techniques to evaluate the impact of anaerobic extraction on the EBPR activity of a new SBR-EBPR configuration aimed at P-recovery from the main treatment line. Different percentages of volume extraction were studied in the range of 0.5-20% of the total working volume. Although they observed that the proposed system could remove P in the range of the above values, the highest recovery efficiency was obtained by extracting 4.3% of the total volume of the reactor. In this study, the volume percentages of the anaerobic supernatant extracted from the $SBR\text{-}EBPR_{REC}$ system (10-15%) are within the range of values modelled in the work of Baeza et al. (2017), however, they are higher than the anaerobic optimum extraction volume with which the highest percentage of P recovery was obtained without affecting EBPR activity (4.3%).

As can been seen in Figure 4.3.B, stage I was the only one in which phosphorus was completely removed, which means that the amount of phosphorus extracted during the anaerobic phase did not affect the Poly-P reserve of PAO, so that the PAO were able to store all the VFA as PHA, thus avoiding being outcompeted by ordinary heterotrophic organisms (OHO). Furthermore, as mentioned above, the influent COD/P ratio (15) for this stage favoured the growth of PAO. However, the efficiency of P-recovery achieved in this stage was 17% lower than that obtained with the optimal percentage of anaerobic extraction (4.3%) proposed by Baeza. This observation is in agreement with Baeza et al. (2017), who pointed out that the concentration of soluble compounds at the end of the anaerobic phase is greatly affected by the volume of supernatant extracted. When the volume of supernatant extracted is less than 5%, a maximum concentration of around 61 mg P/L is obtained. Extraction volumes above 5% decreased P concentration. Although the same amount of anaerobic supernatant was extracted during the stages II and IV, the P removal efficiency obtained in these stages was up to 31% less than that obtained in stage I, suggesting that the main factor affecting EBPR activity during these stages was the low COD/P ratio (discussed above). With regard to stage III, we can assume that the high anaerobic supernatant extracted in this stage (15%) limited the poly-P reserves of PAO, so that PAO could not take up all the VFA under anaerobic conditions, favouring that part of the COD was consumed by the OHO under aerobic conditions and, moreover, the COD/P ratio used in this stage was not the optimal to favour the EBPR activity.

## 4.4. Conclusions

Recently, there is increasing interest in the recovery of P from wastewater. In this chapter, a new strategy for P recovery by a modified EBPR process was evaluated, in which a highly P-enriched supernatant was obtained. Taking into account the results obtained in this chapter, the following can be concluded:

- Excellent EBPR performance was achieved by extracting 10% (1L) of supernatant at the end of the anaerobic phase. However, extraction volumes above 10 %, in this case 15%, led to a considerable decrease in EBPR activity.
- The COD/P ratio influenced EBPR performance. The studied system achieved a successful performance with a COD/P ratio of 15. When the COD/P ratio was reduced to 10 (influent P =30 mg/L) the EBPR activity decreased.

- The P content of the anaerobic supernatants obtained in each of the operational stages of the system was three times higher than the P content in the influent, making them suitable for use as raw material in P-recovery processes, such as struvite precipitation.

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# CHAPTER 5

# Factors affecting the integration of EBPR process in A-stage systems: low sludge retention time

## 5.1. Introduction

Municipal wastewater treatment has evolved extraordinarily during the last years. However, it is an energy-consuming process that should be improved to make it more sustainable. A widely proposed system for this task is the two-stage A/B WWTP configuration (Böhnke 1977; Böhnke et al., 1998; Versprille et al., 1985). In this system, the first phase (A-stage), is designed to remove particulate and organic matter at very short Sludge Retention Time (SRT), while the second phase (B-stage) is designed to remove N. According to previous reports, WWTP operated with this configuration have reported up to 65% of organic load elimination in the A-stage with a maximum ammonia effluent concentration of 5 mg/L after the B-stage (Wett et al., 2007).

The A-stage is designed for carbon harvesting which can be directed to an anaerobic digestion system for the production of biogas. To achieve this, the reactor (A-stage) has to be designed at low SRT to prevent nitrification, to produce a large amount of sludge biomass (high biomass yield) and to avoid excessive carbon mineralization in view of maximizing energy recovery (i.e. to divert most of the entering carbon to the anaerobic digestion) (Ge et al., 2013). This secondary biomass should have higher anaerobic biodegradability (Batstone et al., 2003). Gossett and Belser (1982) estimated a decrease of 10% of sludge biodegradability for each extra day of SRT at low SRT values. However, at SRT values higher than 8 days, this decrease in degradability was only 3-8% when extending the sludge age by an additional 5 days (Batstone et al., 2015). This opens a new research focus on short SRT systems where carbon sequestration (i.e. either as biomass growth, internal storage or even as adsorption) and settleability are the main parameters to consider (Batstone et al. 2015; Jimenez et al. 2015).

In the initial design of A/B system, the EBPR process was not included, so the removal of P is carried out by chemical precipitation. If EBPR is to be included in an A/B configuration, the A-stage seems to be the most adequate place. The integration of EBPR in an A-stage system requires the inclusion of an anaerobic phase (this phase is necessary to obtain a sludge enriched with PAO) which hinders the possibility of working at very low SRTs. EBPR in full scale WWTPs is usually operated at high SRT (around 10 days) which is adequate to promote PAO growth. In this context, few studies have been reported about the performance of EBPR systems under low SRT conditions. Brdjanovic et al., (1998) demonstrated through metabolic modelling that the kinetics for the utilization of the stored

PHA were more limiting when estimating the minimal SRT for EBPR than the kinetic for the kinetic of PAO growth. Rodrigo et al. (1999) studied the effect of SRT (from 11 to 65 days) on the performance of an A2O pilot plant and concluded that GAO could be favoured at long SRT. More recently , Ge et al. (2015) a SBR-EBPR system at very low SRT (between 0.5 and 2 days). They obtained a stable system with SRT of 2 days with 80% of COD and P removal. Valverde-Pérez et al. (2016) investigated the operation of a single SBR at low SRT. They studied the interaction between PAO, nitrifiers and sulphate reducing bacteria (SRB) when operated at different SRT.

Considering these previous studies, the main objective of the present study was to evaluate, with long-term experiments, the minimal SRT required for *Accumulibacter* PAO to maintain its EBPR activity under a conventional SBR operation with A/O configuration. For this aim, different experiments were conducted with *Accumulibacter* enriched sludge at different SRT and both the SBR performance and the microbial evolution was monitored to gain insight on the EBPR integration in A-stage systems.

## 5.2. Materials and methods

### 5.2.1. Equipment

Three anaerobic/aerobic SBRs with EBPR configuration, named as SBRI, SBRII and SBRIII, were operated at different SRT during 163, 203 and 115 days, respectively. Four 6-h cycles were performed per day which consisted of 120 min for anaerobic phase (the first 5 minutes were utilized for feeding), 210 min for aerobic phase, 25 min for settling and 5 min foe effluent withdrawn. The SBRs were stirred at 120 rpm during the anaerobic and aerobic phases. A volume of 5 L of synthetic water per cycle was fed to the SBRs in order to maintain a hydraulic residence time (HRT) of 12h. The synthetic water used in this study is given in chapter 3. Propionic acid was used as the carbon source with an initial concentration in the reactor of 226 mg $COD.L^{-1}$. In some cases, COD concentration was adjusted as explained below.

pH was controlled (7.5 ± 0.05) by dosing HCl or NaOH through peristaltic pumps. Dissolved Oxygen (DO) was also controlled between 2.5 and 3.5 mg DO/L by using an on/off controller activating an electrovalve that allowed an aeration flowrate of 3 l/min in on position. The temperature was kept at 25ºC by recirculating thermostated water by the reactor jacket. A constant nitrogen gas flow (1 L/min) was applied to the SBR during the

anaerobic phase to ensure anaerobic conditions. SRT was strictly monitored considering both the biomass in the effluent and in the reactor and controlled by manipulating the volume of sludge extracted. Different SRTs were studied (Table 1.9). The exact amount of sludge was removed by means of a controlled peristaltic pump at the end of the anaerobic phase of each cycle before turning off the stirrer.

### 5.2.2. Chemical and biochemical analysis

Samples for propionic acid and phosphate were filtered with a 0.22 µm filter (Millipore). Phosphorus concentration was measured by a phosphate analyser (115 VAC PHOSPHAX sc, Hach-Lange). Propionic acid was analysed with a gas chromatograph (GC Agilent Technologies 7820 A) equipped with a BP21 SGE column (30 m 0.25 mm 0.22mm; length internal diameter film thickness) and a flame ionisation detector (FID). Measurements of mixed liquor volatile suspended solids (VSS) and mixed liquor total suspended solids (TSS) were analysed according to Standard Methods (APHA 1995).

Glycogen and PHA content in the biomass were quantified. Glycogen analysis was performed by a modification of the method proposed by Smolders et al. (1994). The concentration of glucose was measured using an YSI model 2700 Select Biochemistry Analyser (Yellow Springs Instrument, Yellow Spring, Ohio, USA). PHA was extracted from lyophilised samples using hexane and butanol. Afterwards, PHA was determined with a GC (Agilent Technologies 7820A). As standards were used 3-hydroxybutyric acid and 3-hydroxyvaleric acid copolymer for polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) and 2-hydroxycaproic acid as standard for polyhydroxy-2-methylvalerate (PH2MV). Fluorescence in situ hybridization (FISH) analyses were performed in order to study the microbial community evolution in the SBRs. The distribution of PAO and GAO-labelled biomass was quantified using confocal microscopy and image analysis as detailed in Jubany et al. (2009). Hybridizations were performed using Cy3-labelled specific probes and Cy5-laballed EUBMIX for most bacteria; Cy3-labelled GAO MIX for "*Candidatus Competibacter Phosphatis*"(GAOQ 431 and GAOQ 989); Cy3-labelled DF1MIX for cluster I of "*Defluvicoccus vanus*" GAO; Cy3 labelled DF2 for cluster II of "*Deflivicoccus vanus*" GAO; Cy3-labelled PAOMIX for "*Candidatus Accumulibacter phosphatis*" (PAO 65, PAO 846 and PAO 462); Cy5-labelled PAO I for cluster I of "*Candidatus Accumulibacter phosphatis*" and Cy5-labelled PAO II for cluster I of "*Candidatus Accumulibacter phosphatis*". All probes were used at a 35 % formamide concentration.

### 5.2.3. Calculation of the observed yield

The real observed yield ($Y_{obs}$) under different SRT conditions was carefully calculated using experimental biomass production data obtained under stable operation periods. Biomass production (PX, gVSS·d−1) was calculated using Equation (1) with data from stable operation at a given SRT for each reactor

$$P_X = Q_W X_E + Q_E X_E \qquad (1)$$

where $Q_W$ and $Q_E$ are the flowrates of purge and effluent (L/d), while $X_W$ and $X_E$ are the VSS concentration in the purge and in the effluent (g VSS/L). $X_W$ and $X_E$ were measured from integrated samples collected during one day from the purge and effluent flows.
The experimental observed yield (Yobs, g $COD_X$/g $COD_S$) was calculated with Equation 2, where 1.416 is a stoichiometric factor (g $COD_X$/g VSS), $Q_{IN}$ is the daily influent flowrate (L·d-1) and $COD_{IN}$ is the COD concentration in the influent wastewater (g $COD_S$/L), which is assumed to be fully consumed during a cycle.

$$Y_{obs} = \frac{P_X .1.416}{Q_{IN}.COD_{IN}} \qquad (2)$$

The experimental data was fit to the typical model of Yobs as a function of SRT, as detailed in Tchobanoglous et al. (2003), following Equation 3.

$$Y_{obs} = \frac{Y}{1 + k_d.SRT} (1 + f_d . k_d.SRT) \qquad (3)$$

where Y is the biomass growth yield coefficient, $f_d$ = 0.1 is the fraction of biomass that remains as cell debris and $k_d$ is the endogenous decay coefficient (d-1).

## 5.3. Results

### 5.3.1. SBR-EBPR systems operation

The possible integration of EBPR into the A-stage process could be very favourable in view of sustainability and economics. However, the main bottleneck of this integration

lays on the SRT, since the designs for A-stage are aiming at the lowest SRT possible to decrease carbon mineralization and enhance biomass growth: SRT values around 12 h have been tested (Jimenez et al., 2015; de Graaff et al., 2016). A priori, it seems impossible that EBPR could be sustained at this low SRT. In order to find the minimum SRT whit which the EBPR activity can be maintained and to understand the effect of low SRT on PAO-enriched systems, three different SBRs (namely $SBR_I$, $SBR_{II}$ and $SBR_{III}$) were operated at different SRTs.

The starting point of the experiments was a steady-operation at a SRT higher than 10 days, which is a SRT adequate to achieve simultaneous carbon and P removal (Lee et al., 2007; Zheng et al., 2014). And given that, in full-scale WWTP, EBPR process is generally implemented along with nitrification/denitrification, this high SRT is adequate to avoid nitrifiers washout. After steady state was reached, SRT was gradually decreased and the effect of each SRT reduction was quantified in terms of SBR performance and potential microbial population shifts.

Figure 5.1 compares the desired SRT with the experimental SRT (the purge flow was the manipulated variable for the SRT control). The VSS variations either in the reactor or in the effluent caused the differences between the SRT targeted and the real SRT. As can be seen, the three experimental periods were very long (5, 6 and 4 months) and the last SRT tested was 3 days which, in all cases, led to the reactor washout.

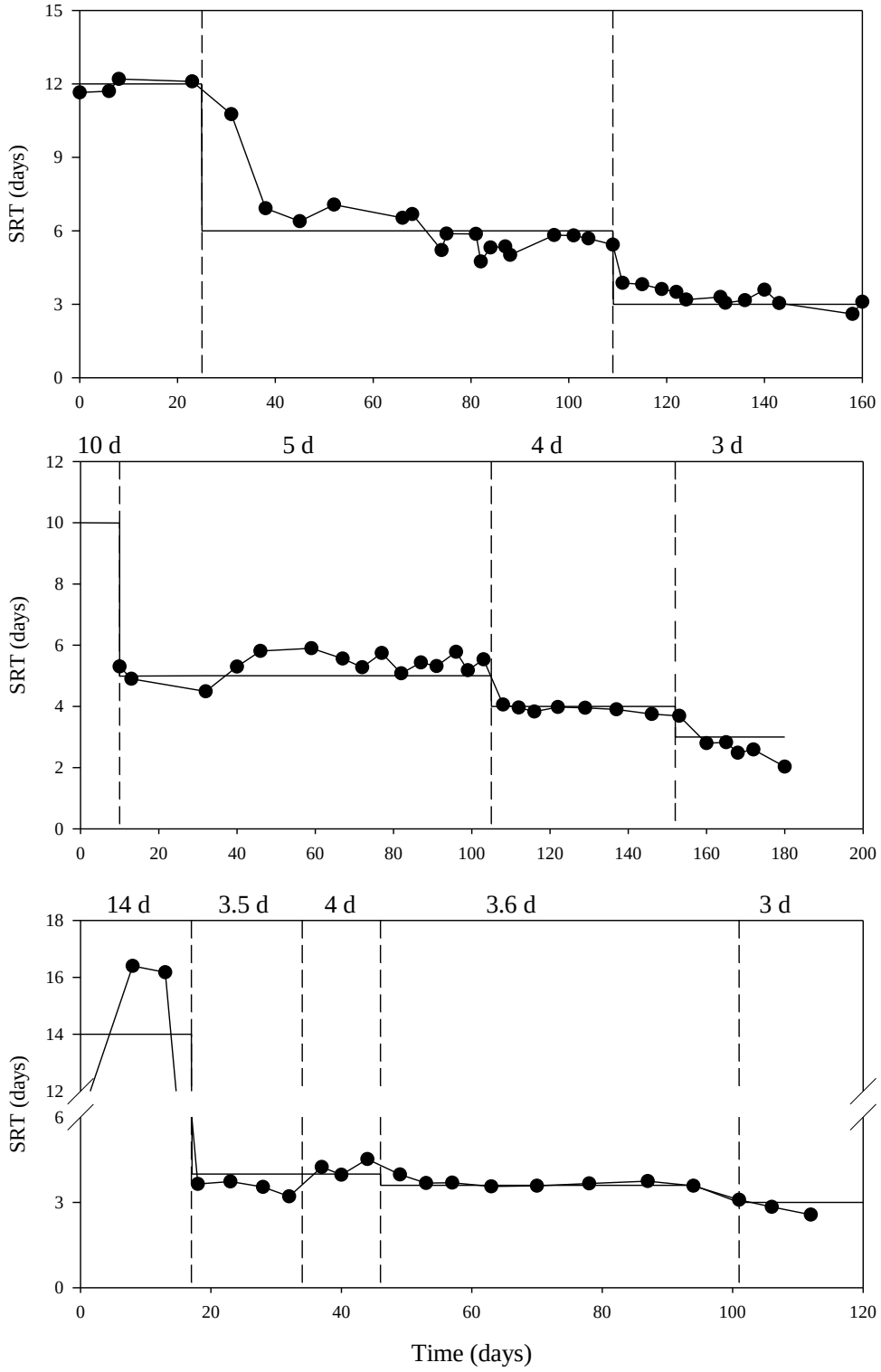


Figure 5.1. Target SRT (line) vs real SRT (●) for the three experimental periods. SBRI (up), SBRII (middle) and SBRIII (down).

For the case of the $SBR_I$, it can be observed that the system had a successful performance at SRT of 12 days, with a P/C around 0.3, anaerobic P-release values of 70 mg P/L and P removal higher than 90% (Figure 5.2). When the SRT was reduced to 6 days, the EBPR activity was not affected and the P/C did not decrease (except for a single accident with the PHA control). Once the system was stabilized at SRT of 6 days, the SRT was again decreased to 3 days which led to the system failure after around 30 days of operation. The SBRI failed when the SRT was removed from 6 to 3 days.

From the results of $SBR_I$, it was decided to conduce a new SBR ($SBR_{II}$) in order to assess more precisely the minimal SRT feasible for our system and to understand the behaviour of the microbial community under such low SRT values. $SBR_{II}$ (Figure 5.3) was successfully operated at 10 days and, then moved to 5 days. The system suffered a couple of periods with low P removal and low VSS but around day 60 the system was fully recovered with a high P-release, a high P/C ratio (Figure 5.3C), and a successful net-P removal (Figure 5.3A). The conventional cycles performed also showed adequate P profiles for a cycle from an EBPR-SBR system. Then, the SRT was reduced to 4 days in order to push the limits of the system and the activity was not affected during the first 10 days with high P/C and high net-P removal. However, the slight decrease of the biomass concentration was crucial and caused that not all the COD could be degraded under anaerobic conditions and, thus, part was being transferred to the aerobic phase. To avoid this problem, the initial COD concentration in the system was reduced from 226 to 190 mg/L and the system became more stable, although some short periods with lower P-removal were also observed (Figure 5.3). The SRT of the system was decreased again from 4 to 3 days and the system failed again under the same parameters as $SBR_I$, without recovering EBPR activity even after 40 days of operation.

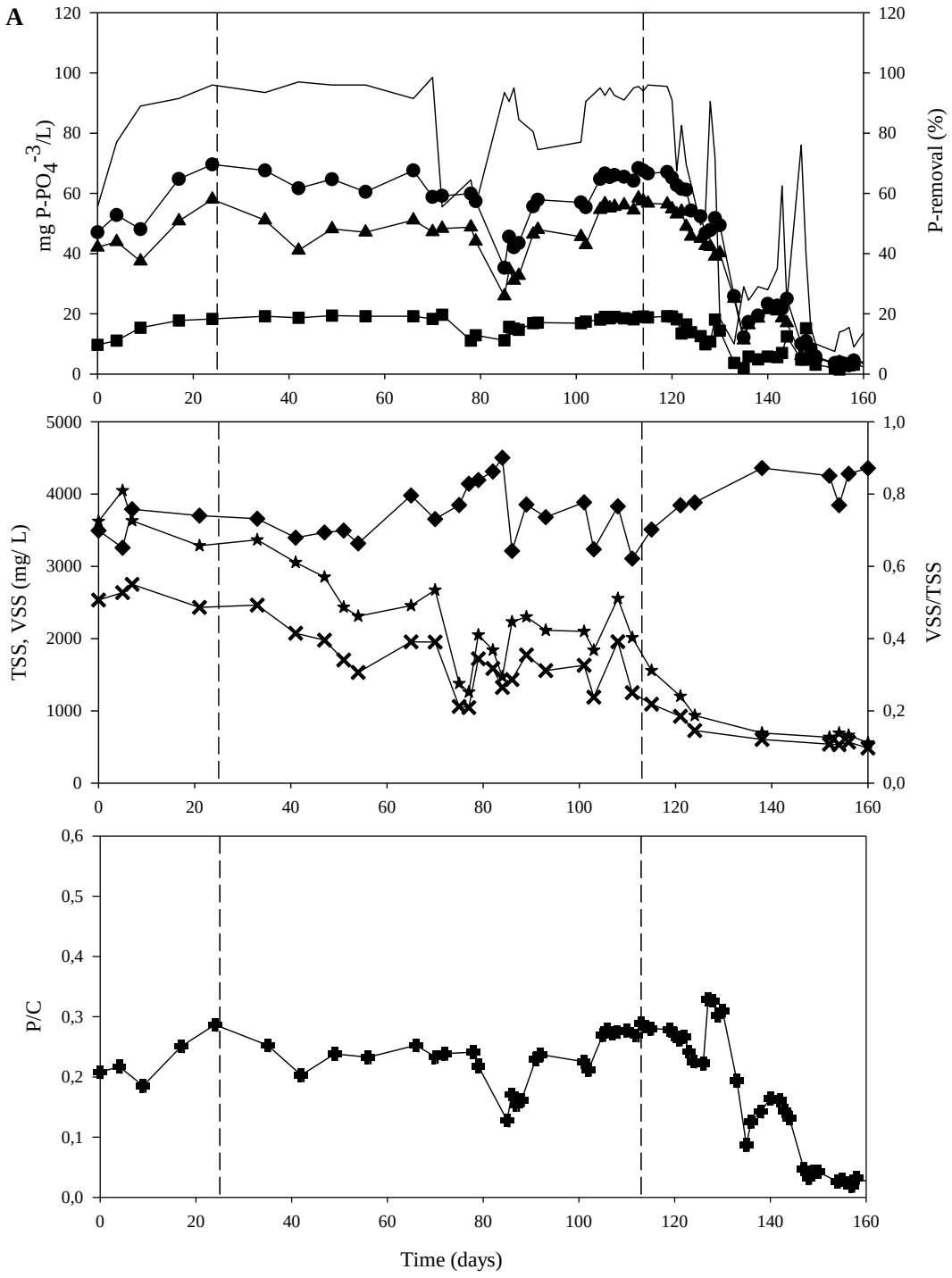


Figure 5.2. $SBR_I$ operation. A): Evolution of P-release (▲), P-uptake (●), net P-removal (■) and percentage of P removal (solid line); B): Profile of TSS (★), VSS (×) and VSS/TSS (♦) ratio; C): P/C ratio.

A third SBR ($SBR_{III}$) was conducted aiming to find more precisely the minimal SRT possible. A system with a successful performance operating at high SRT values was again achieved in two weeks. Then, the SRT was decreased drastically to 3.5 days. The system was operated at its boundary conditions, robustness was very fragile, and any slight parameter variation could drive the SBR to its failure. The SRT was increased again to 3.6

days and the SBR maintained a good SBR activity during almost two weeks. Again, some COD was left at the end of the anaerobic phase. Initial COD was decreased to reduce this problem and the system recovered successfully. This was the limit SRT, but the system was not showing a reliable performance at such low SRT. The SRT was again decreased to 3 days and, likewise $SBR_I$ and $SBR_{II}$, the PAO biomass of $SBR_{III}$ was wash-out.

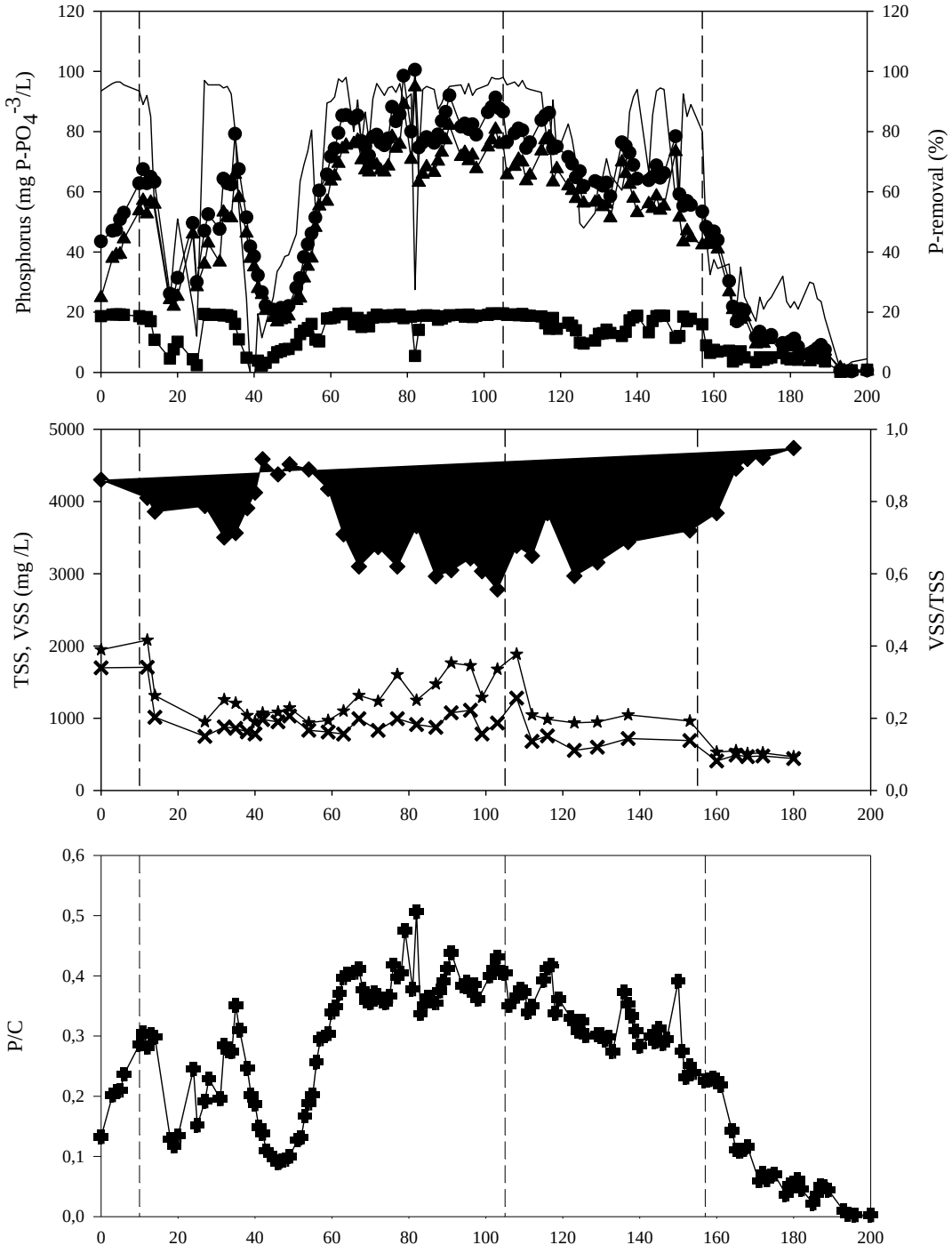


Figure 5.3. SBRII operation. A): Evolution of P-release (▲), P-uptake (●), net P-removal (■) and percentage of P removal (solid line); B): Profile of TSS (★), VSS (×) and VSS/TSS (♦) ratio; C): P/C ratio.

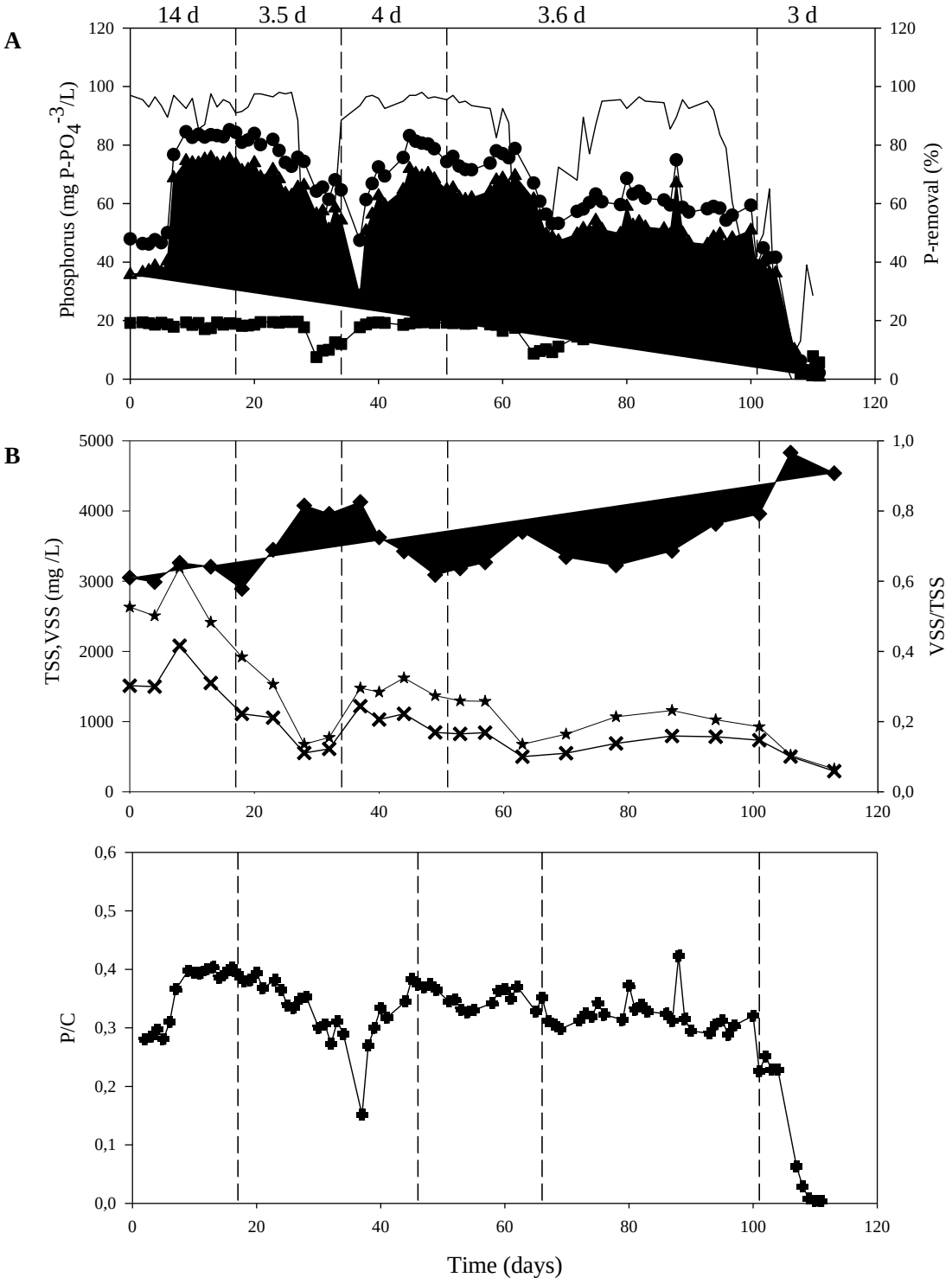


Figure 5.4. A): $SBR_{III}$ operation. Evolution of P-release (▲), P-uptake (●), net P-removal (■) and percentage of P removal (solid line); B): Profile of TSS (★), VSS (×) and VSS/TSS (♦) ratio; C): P/C ratio.

For each SBR-EBPR system, fully monitored cycles were performed. These cycles allow a better understanding of the influence of SRT on metabolic behaviour of PAO. Figure 5.5 shows these cycles. As can be observed, the profiles obtained at both high and low SRT

are very similar with anaerobic propionic uptake and glycogen degradation linked to P-release and PHA storage. The opposite trends were observed under aerobic conditions, i.e. PHA degradation led to glycogen recovery and P-uptake.

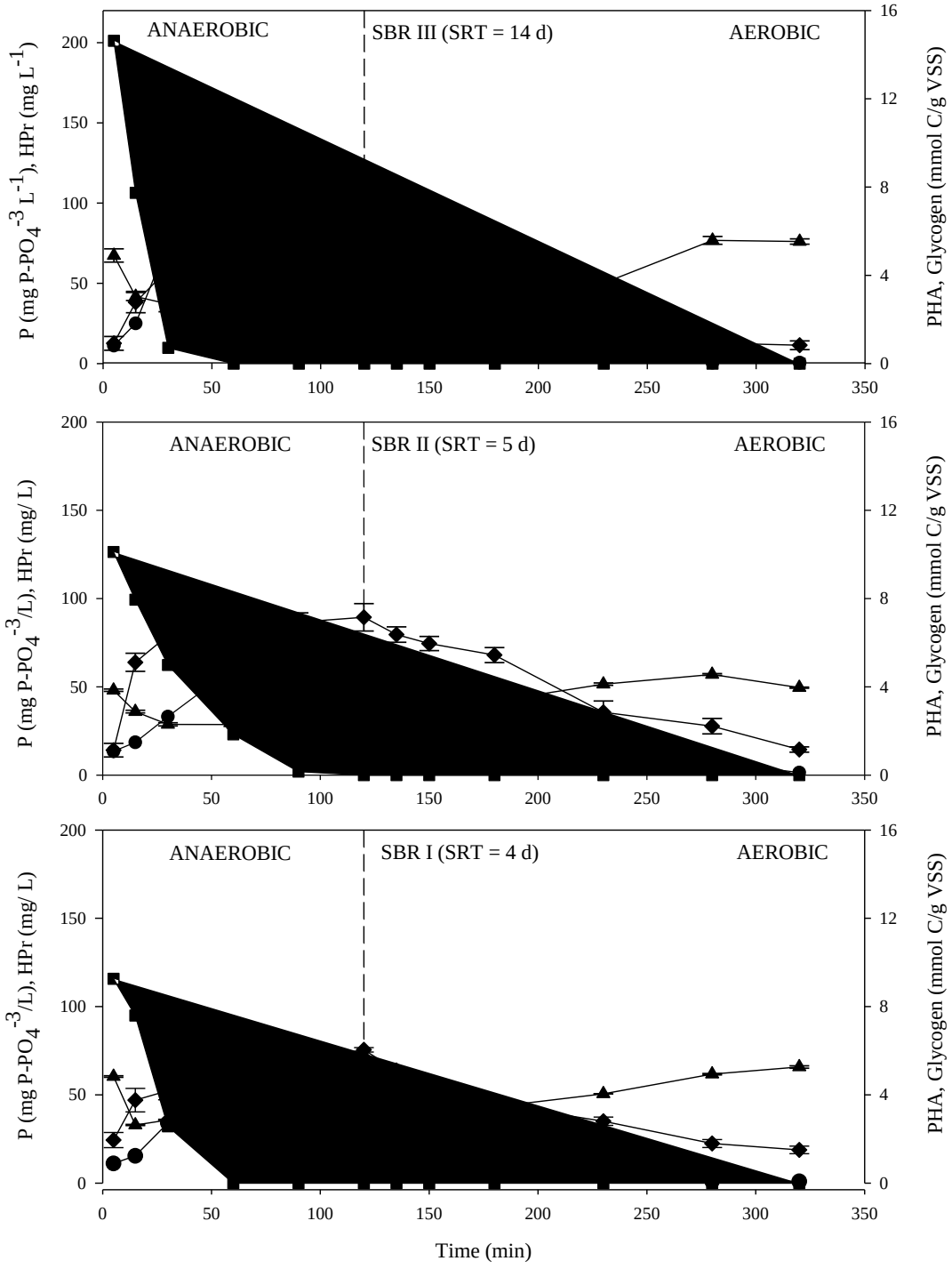


Figure 5.5. Experimental profiles obtained from fully-monitored cycles at different SRTs (14, 5 and 4 days). Phosphorus (●), Propionic acid (■), glycogen (▲) and PHA (♦).

Table 5.1 and 5.2 summarise the main ratios and rates obtained in full-monitored experiments. Both P/C ratio and the Gly/C ratio dot not seem to be very affected by the STR. However, higher PHA/C and, therefore, higher CRR ratios are obtained at lower SRTs. Regarding the rates, a general trend is that higher specific rates (i.e. per gram of VSS) are obtained at lower SRTs in all the characteristic EBPR process: P release and uptake, PHA production and glycogen degradation.

In all the SBR-EBPR systems studied, the periods operated under SRT of 3 days led to the reactor washout with common indicators: i) increase of the ratio of VSS/TSS which tended to 1, ii) decrease of the P/C ratio, iii) decrease of the VSS, iv) decrease of the P-release and P-uptake and v) COD not being completely consumed under anaerobic conditions and part of it being transferred to the aerobic phase. We have observed that the P-uptake capacity was lost before than the P-release capacity, so an increase of effluent P was an indicator that the system was headed to the failure. In addition, by operating the reactors at low SRTs, the concentration of biomass in the reactors is reduced. However, this biomass shows higher ability to store PHA, which means that higher PHA/C ratio and, thus, higher carbon recovery ratio are obtained.

Table 5.1. Typical EBPR ratios measured in each experimental period.

| **SBR** | SRT | P/C[a] | PHA/C[b] | Gly/C [b] | CRR |
|---|---|---|---|---|---|
| **I** | 12 | 0.35 | 1.10 | 0.62 | 0.68 |
| | 6 | 0.36 | 1.14 | 0.56 | 0.81 |
| **II** | 5 | 0.35 | 0.89 | 0.43 | 0.62 |
| | 4 | 0.36 | 1.38 | 0.64 | 0.84 |
| | 14 | 0.37 | 1.21 | 0.68 | 0.72 |
| **III** | 4 | 0.36 | 1.49 | 0.52 | 1.03 |
| | 3.6 | 0.36 | 1.33 | 0.59 | 0.84 |

a= mol P/mol C, b= mol C/mol C

Table 5.2. Typical EBPR rates obtained in each experimental period.

| **SBR** | SRT (days) | $P_{rel}$[a] | $P_{upt}$[a] | $PHA_{prod}$[b] | $GLY_{prod}$[b] | $GLY_{deg}$[b] |
|---|---|---|---|---|---|---|
| I | 12 | 0.011 | 0.005 | 0.012 | 0.0097 | 0.011 |
| | 5 | 0.026 | 0.008 | 0.029 | 0.0016 | 0.015 |
| II | 5 | 0.035 | 0.010 | 0.036 | 0.0143 | 0.014 |
| | 4 | 0.018 | 0.004 | 0.037 | 0.0106 | 0.014 |
| III | 14 | 0.029 | 0.009 | 0.022 | 0.0169 | 0.017 |
| | 4 | 0.036 | 0.014 | 0.042 | 0.0143 | 0.016 |
| | 3.6 | 0.037 | 0.012 | 0.033 | | 0.014 |

a = mmol P/g VSS/ min, b = mmol C/g VSS /min

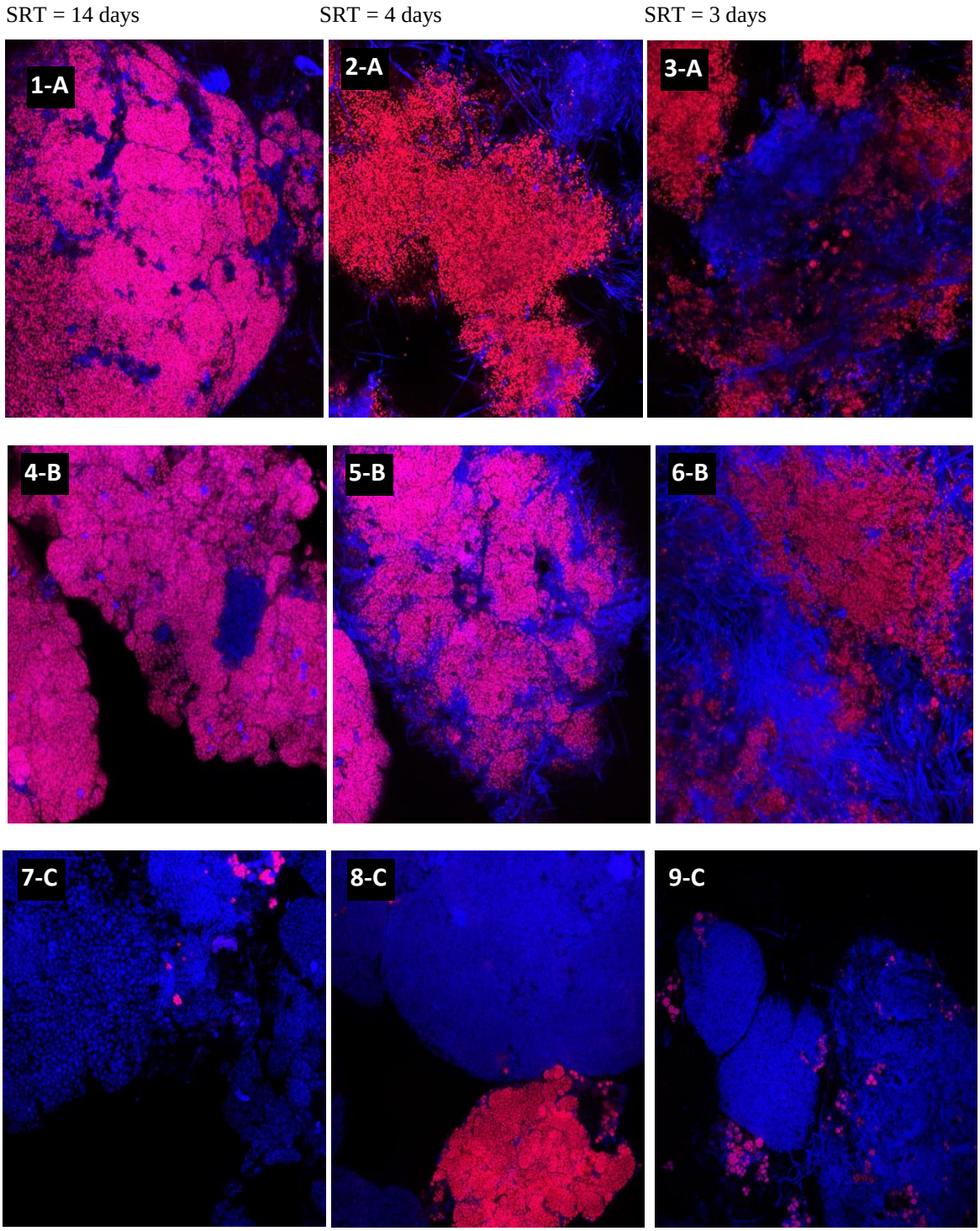


Figure 5.6. FISH representative images of the biomass from $SBR_{III}$ during the experimental period. A. PAOMIX, B. PAO II and 3. DFI. Specific probe is shown in pink and EUBMIX in blue.

Finally, the evolution of the different PAO and GAO populations in the systems was measured through FISH measurements. The results obtained indicate that our system, at high SRT, was mainly enriched in PAO (87 %), which were mostly PAO I. This high PAO percentage decreased as the SRT was reduced since part of the PAO was washed out from

the system. No GAOMIX was detected due to the synthetic wastewater without acetate and only containing propionate as carbon source. The only additional microorganisms detected in the SBR sludge with our FISH probes was DF I, although the higher concentrations observed was 3.2 ± 0.3 %. These results demonstrate that our study was specifically focused on the effect of reduced SRT on *Accumultibacter* PAO. Figure 5.6 shows the evolution of the FISH quantification during the experimental period of $SBR_{III}$.

As mentioned above for all the systems studied, the 3-days SRT led to the reactor washout, while SRT = 3.6 days allowed to maintain EBPR activity (Figure 5.4). Obviously, this SRT value is a higher value than the minimal SRT of an A-stage aiming at only organic matter removal (around 0.5 days). However, it is low enough to be considered as feasible from a practical point of view: the potential disadvantages of being higher than the optimum SRT for only organic matter removal (i.e. higher volume needs, higher biomass growth and higher carbon mineralization) can be overcome by the possibility of P removal (i.e. less cost than the chemical precipitation). Moreover, it should be considered that the low SRTs reported in the literature as minimal values for organic matter removal could lead to operational issues since we have seen that extreme values would lead to fragile systems where any minimal low variation (solid concentration, sudden temperature or pH changes) may results in biomass washout. Hence, real systems could be always be operated at a safety SRT, which could be closer to 4 days. This safety SBR would also lead to a more stable and robust operation with lower effluent variations and more adequate for a real scenario. The minimum SRT limit observed in this thesis is higher than the reported in the literature (Ge et al. 2015), where EBPR activity could be maintained at SRT = 2-2.5 d. Differences in wastewater characteristics (abattoir wastewater), temperature (studied in the next chapter) and microbial communities could explain this discrepancy. Another important aspect to consider is the oxygen demand of these systems. Including EBPR in an A-stage also be beneficial from this point. Part of the influent COD would be stored in the biomass under anaerobic conditions and, thus, without oxygen requirements. Moreover, the reaction time would be increased because of the addition of an anaerobic phase but the aerobic retention time would not be significantly increased.

### 5.3.2. Influence of SRT on biomass production

One of the goals of the A-stage is related to the promotion of sludge production (i.e. yield), which could be diverted to the anaerobic digester for methane production. Literature sources indicates that different SRTs should lead to different observed biomass growth yield values ($Y_{obs}$) according to equation 3 (Tchobanoglous et al., 2003). Experimental $Y_{obs}$ were calculated by means of the procedure detailed in methodology section, using data from stable operational periods at fixed SRT for each reactor. Figure 5.5 shows the $Y_{obs}$ values obtained versus the experimental SRT used. Equation 3 was fit to the experimental data, obtaining the parameters Y = 0.39 ± 0.05 g $COD_X$/g $COD_S$ and $k_D$ = 0.06 ± 0.04 $d^{-1}$. A good agreement was observed, despite the experimental data were noisy because they depend on the VSS measurements, which have important variability. The parameters obtained differ from the default parameters for PAO (Y = 0.625 g $COD_X$/g $COD_S$, $k_D$ = 0.2 $d^{-1}$) reported in the ASM2d model (Henze et al., 2000). The model prediction using these default parameters is also presented in Figure 5, demonstrating that the new parameters allow a better prediction of Yobs, especially at low SRT.

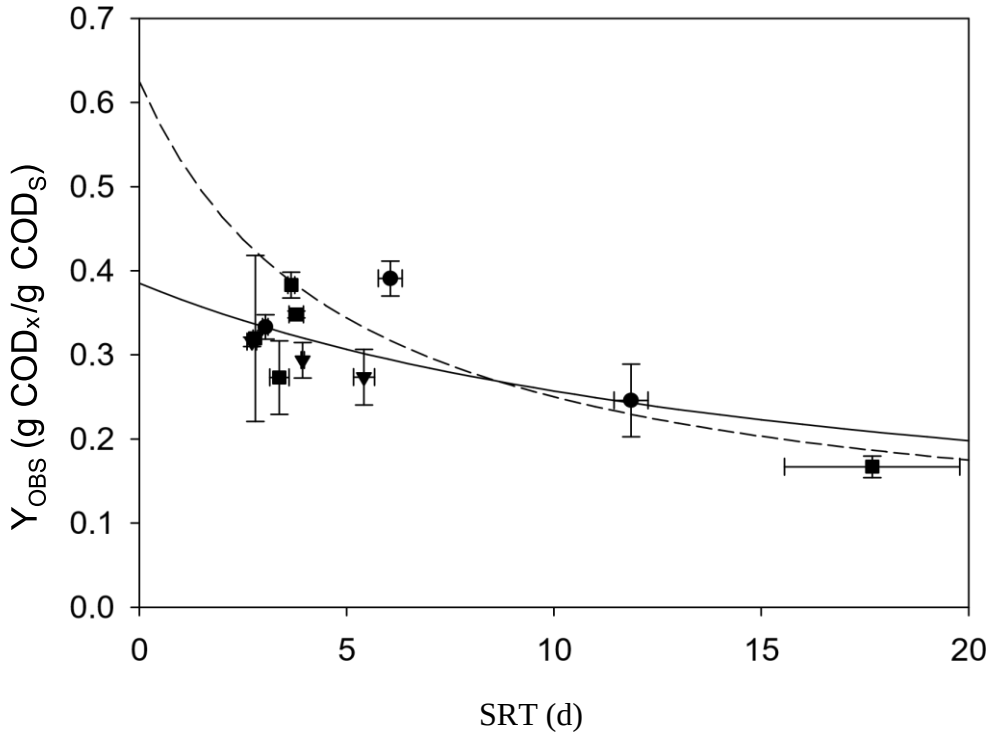


Figure 5.7. Observed biomass yield ($Y_{obs}$) as a function of operational SRT. Experimental data: SBRI (●), SBRII (▼) and SBRIII (■). Dashed line: model prediction with default parameters (Y = 0.625 g $COD_X$/g CODS, $k_D$ =0.2$d^{-1}$) and Solid line: model prediction with fitted parameters (Y = 0.39 ± 0.05 g $COD_x$/g $COD_s$, $K_D$ = 0.06 ± 0.04 $d^{-1}$).

The observed yield would increase from 0.257 to 0.319 g $COD_x$/g $COD_s$ when decreasing SRT from 10 to 4 days, i.e. a 24% increase in the amount of biomass generated. This is a good improvement with respect to typical EBPR systems, but it should be compared to the observed yield obtained in A-stage systems operating at much lower SRT. Jimenez et al., (2015) report $Y_{obs}$ = 0.50 $gVSS \cdot g^{-1}COD$ = 0.71 $gCOD_X \cdot g^{-1}COD_S$ when operating at the lowest SRT of 0.1 d. Adsorption plays a more important role at this SRT and the conventional model in equation 3 does not provide realistic values. On the other hand, lower yields when operating at SRT = 4 d would also have positive counterparts as higher stability of the effluent, because A-stage at very low SRTs has much higher effluent variability.

The biomass coming from an EBPR-A-stage reactor would have a higher PAO concentration and higher poly-P values. The distribution of internal polymers would also be different since PAO can store organic matter as PHA or glycogen. Conventional PAO, from the genus *Accumulibacter*, store most of carbon as PHA (which mainly be PHB or PHV depending on the nature of the carbon source. The higher PHA fraction in PAO biomass is expected to produce a higher methane yield because PHA has lower reduction state than biomass (studied in chapter 7), i.e. theoretical COD content in PHA and PHV is 1.674 and 1.920 g COD/g VSS, higher than the value for biomass 1.4416 g COD/g VSS.

This work shows that EBPR could be integrated in the A stage of an A/B system, i.e. carbon removal will be accomplished together with phosphorus removal at relatively los SRT values. The value we found (around 3.6 days) depends on the operational parameters of the plant (mainly temperature but also pH or aerobic DO set-point) and also on the nature of the carbon source used. The more complex the carbon is, longer anaerobic phases are required to degrade the influent carbon by the bio-P consortium. In this sense, the minimal SRT should be estimated according to the characteristics of the entering wastewater.

## 5.4. Conclusions

In this chapter, three SBR-EBPR systems were operated under conventional anaerobic/aerobic EBPR configuration and at different SRT. This chapter leads to the following conclusions:

- EBPR activity, which is related to PAO activity, and net P-removal can be maintained with a minimal SRT of 3.6 days at 25 ºC.

- SRT of 3 days drastically reduced P-release and P-uptake and it was linked to an increase of the VSS/TSS ratio, decrease of the P/C ration and an important change of biomass characteristics, related to PAO washout.
- A 24 % increase of biomass yield is predicted for the decrease of SRT from 10 to 4 days.

## 5.5. References

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# CHAPTER 6

# Evaluation of EBPR limits in different temperature scenarios

## 6.1. Introduction

There is a growing interest in converting wastewater treatment plants (WWTPs) into energy self-sufficient systems and in considering wastewater as a source of energy and materials. As for energy, the chemical energy in the wastewater is stored mainly as organic matter. Different authors have recently taken on the challenge of estimating the energy content of wastewaters and relating it to COD, and a correlation of 13-15 kJ/g COD seems to be a conservative approach (Shizas et al., 2004; Heidrich et al., 2011; Korth et al., 2017). In this sense, the objective is to recover the energy contained in the wastewater by capturing as much COD as possible before biological oxidation (Wan et al.,2016). The innovative configurations of the WWTPs focus on improving the quality of their sludge liquors (Ge et al., 2015), so that they can be used as raw material in the production of methane (Wett et al., 2007; Rahman et al., 2016). Examples of these novel approaches are High-rate activated sludge systems (HRAS) (Meerburg et al., 2015; Jimenez et al., 2015) or the A-stage of the A/B process (Smitshuijzen et al., 2016; Wan et al.,2016) to maximise the capture of organic matters from wastewater and divert it to anaerobic digestion. In both technologies, most of the influent organic carbon is stored by adsorption in the sludge, which in turn leads to the production of highly biodegradable biomass (up to 80%) with high methane yield (Ge et al., 2013).

In this regard, there has recently been interest in integrating the Enhanced Biological Phosphorus Removal (EBPR) process into the A-stage of the A/B process to avoid costly tertiary treatment of phosphorus (P). However, the A-stage is characterized by a high loading rate and a very short sludge retention time (SRT) (0.1-0.5 d) (Boehnke, 1998). Therefore, EBPR needs to be operated at the lowest possible value of SRT to maintain as much as possible the benefits of the A-stage (Boehnke, 1977; de Graaff et al., 2016). SRT is in fact the main obstacle to this integration, since EBPR is usually operated with high SRT, as it is combined with biological nitrogen removal. However, lower SRT values could be used if the system only focused on biological COD and P-removal. Previous studies have found that the minimum SRT with which EBPR activity can be preserved is in the range of 2.5-3 d at 25 ºC (Mamais and Jenkins 1992, Smolders et al.,1995, Ge et al., 2013, Valverde-Pérez et al., 2016, Chan et al., 2017).

However, SRT is extremely linked to temperature, as it affects reaction kinetics, gas-transfer rates, biomass growth and decay rates. There are several publications on the

influence of temperature on the EBPR process, which have focused on: i) the impact on the microbial population (Panswad et al., 2003; Lopez-Vazquez et al., 2008; Ong et., 2016) and ii) the impact on stoichiometry and kinetics (D Brdjanovic., 1997a; D Brdjanovic., 1997b; Krishna and Van Loosdrecht, 1999). In the latter case, the higher the temperature, the higher the biological rates. As for the microbial population, the PAO compete with other microorganisms, being the main competitor the GAO. These microorganisms have become a significant bottleneck in the EBPR process. The presence of GAO in EBPR systems increases carbon and chemical requirements, sludge production and total plant costs. The reported results regarding the efficiency of P-removal at different temperatures indicated that the higher the temperature the move favourable it will be for GAO. Therefore, although the kinetics of PAO are higher at hinger temperatures, the proliferation of GAO needs to be avoided. On the other hand, the success of EBPR in low temperatures ranges (i.e. 5-15ºC) has also been studied (Ydstebø et al., 2000; Erdal et al. 2006).

This chapter is part of the challenge of designing A-stage systems that include EBPR. In these systems, the relationship between SRT and temperature is fundamental. This relationship has already been studied both theoretically by modelling and experimentally. Brdjanovic et al. (1998) used a model-based approach to identify the temperature and minimum SRT required for the stable EBPR process, which was predicted as 8 d at 20 °C, increasing to 16 d at 10 °C and further to 31 d at 5 °C. Experimentally, Erdal et al. (2006) established that the EBPR performance was optimum in the SRT ranges of 16 to 24 d and 12 to 17 d for 5 °C and 10 °C, respectively. The washing of PAOs of the system occurred at 3.5 d SRT and 5 °C and 1.8 days at 10 °C. This work includes a wide range of short and long-term experiments to understand the effects of SRT and temperature on the EBPR process with the aim of assessing the feasibility of integrating the EBPR in A-stage systems, or in the best possible scenario, integrating it into the A/B process.

## 6.2. Materials and methods

### 6.2.1. Sequenced Batch Reactor (SBR) operation

Three laboratory scale SBRs ($SBR_A$, $SBR_B$ and $SBR_C$), each with an effective volume of 10 L, were operated with a typical EBPR cycle configuration with the following phases (minutes): 120 anaerobic, 210 aerobic, 25 settling and 5 effluent discharge. The

synthetic medium (5 L, per cycle, composition given in chapter 3) and the amount of propionic acid to obtain an initial concentration in the reactor of 150 mg L were pumped to the SBRs during the first 5 min of the anaerobic phase. Four 6-hour cycles per day were operated. SRT was controlled by automatic biomass wastage at the end of the aerobic phase of each cycle and was fixed at different values depending on the specific experiment (Table 6.1). The volume exchange ratio was maintained at 50 %, hence the SBRs operated with a constant HRT of 12 h. The SBRs were equipped with pH, temperature, conductivity and dissolved oxygen electrodes. The temperature in each SBR was controlled by recirculating water from a thermostatic bath through the reactor jacket. The pH was controlled at 7.5 ± 0.05 by automatic dosing of 0.1 M HCl or 0.1 M NaOH. DO was automatically controlled between 2.5 and 3.5 mg DO/L by manipulating an on/off solenoid valve that allowed an aeration flow rate of 4 L/min. The SBRs were stirred during both the anaerobic and aerobic phases at 120 rpm.

### 6.2.2. Experimental design

$SBR_A$ and $SBR_B$ were used for long-term test, while $SBR_C$ was used for short-term test. The specific conditions for each reactor and period are show in Table 6.1. In $SBR_A$, SRT was kept constant at 10 days and the temperature was set at 20, 15 and 10 ºC for different periods. $SBR_B$, was operated under three different conditions by setting the SRT at 10 or 5 days and temperature to 10 or 20 ºC. Finally, $SBR_C$ was operated in four periods at different SRTs (3.5, 5, 10, and 5 days). $SBR_C$ operated usually at 20 ºC, but during each SRT period the sludge was subject to sporadic changes in temperatures. These thermal shocks were performed by changing the water bath temperature the day before the experiment to acclimate the microorganisms to the new temperature and, at the end of the experiment, the temperature was returned at 20 °C. An extensive characterization (both liquid phase and biomass) was performed during each batch tests.

Table 6.1. Combinations of SRT and temperature studied in each reactor.

| Period | $SBR_A$ | | $SBR_B$ | | $SBR_C$ | |
|---|---|---|---|---|---|---|
| | SRT (days) | Temp (°C) | SRT (days) | Temp (°C) | SRT (days) | Temp (°C) |
| I | 10 | 20 | 10 | 20 | 15 | 20, 15, 10 |
| II | 10 | 15 | 5 | 20 | 10 | 20, 15, 10 |
| III | 10 | 10 | 5 | 10 | 5 | 20, 15, 10 |
| IV | - | - | - | - | 3.5 | 20, 15, 10 |

### 6.2.3. Analytical methodology

Total suspend solids (TSS) and volatile suspended solids (VSS) were determined following the methodology described in Standard Methods (APHA, 1995). Phosphorus concentration was measured by a phosphate analyser (115 VAC PHOSPHAX sc, Hach-Lange) based on the Vanadomolybdate yellow method, where a two-beam photometer with LEDS measured the phosphate specific yellow colour. Propionic acid was measured by gas chromatograph (GC agilent Technologies 7820 A) equipped with a BP21 SGE column (30 m 0.25 mm 0.22 mm; length internal diameter film thickness) and a flame ionisation detector (FID). Sludge samples for PHA and glycogen measurements were collected. The different types of PHA were measured with GC (Agilent Technologies 7820 A). As standards were used 3-hydroxybutiric acid and 3-hydroxyvaleric acid copolymer for polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) and 2 hydroxycaproic acid as standard for polyhydroxy-2-methylvalerate (PH2MV). Glycogen analysis was performed by a modification of the method proposed by Smolders et al. (1994). For the determination of glucose, was used an YSI model 2700 select Biochemistry Analyser (Yellow Springs Instrument, Yellow Spring, Ohio, USA).

### 6.2.3. Calculation of the temperature coefficient

The different P concentrations registered for a whole cycle, that is, P-release, P-uptake and net P-removal, were calculated as following: P-release = $P_{anEnd}$ - $P_{anBeg}$, P-uptake = $P_{anEnd}$ - $P_{aerEnd}$ and net P-removal = $P_{in}$ - $P_{aerEnd,}$ where $P_{anEnd}$ and $P_{anBeg}$ are the amount of P at the end and beginning of the anaerobic phase, respectively, $P_{aerEnd}$ = amount of P present at the end on the aerobic phase and finally $P_{in}$ = the influent P concentration.

The temperature coefficient (θ) was calculated by fitting the simplified Arrhenius equation. This coefficient describes the effect of temperature on the rate of reactions. The equation can be expressed as:

$$K(T) = K(20\ ^{o}C) * \theta^{(T-20\ ^{o}C)}$$

Where K(T) = active biomass specific reaction rate at temperature at 20 ºC; T = temperature in ºC; and θ = temperature coefficient

## 6.3. Results

### 6.3.1. Long-term experiments

The short- and long-term effects of SRT and temperature on the EBPR activity were investigated in order to integrate EBPR in a conventional A-stage. To this end, three SBRs ($SBR_A$, $SBR_B$ and $SBR_C$) with high EBPR activity and subject to alternate anaerobic and aerobic conditions were operated at different temperatures and SRTs at different experimental periods ($SBR_A$= 3.6 months, $SBR_B$= 2.7 months and $SBR_C$ = 5.9 months).

In the first part of the study, the long-term effect of a decrease in temperature on EBPR was evaluated. $SBR_A$ was kept at a constant SRT of 10 days and operated in three different periods at 20, 15 and 10 °C for a total of 100 days. Figure 6.1A shows the profile of P-release, P-uptake and net P-removal obtained. $SBR_A$ initially operated at 20 ºC (period I) to promote PAO growth, achieving a high P removal efficiency (86%). After 28 days of operation, the temperature was reduced to 15 °C (period II) and efficiency of P-removal decreased from 86% to 71%, and then decreased to 40% when the temperature was set at 10 ºC (period III). A similar pattern was observed for P-release and P-uptake: both were higher for period I (20 °C) than period II (15 °C) and III (10 °C). The mean P-release values were 61, 59 and 37 mg $P\text{-}PO_4^{3-}$/L for periods I, II and III, respectively, while the average P-uptake values were 71, 69 and 48 $P\text{-}PO_4^{3-}$/L, respectively.

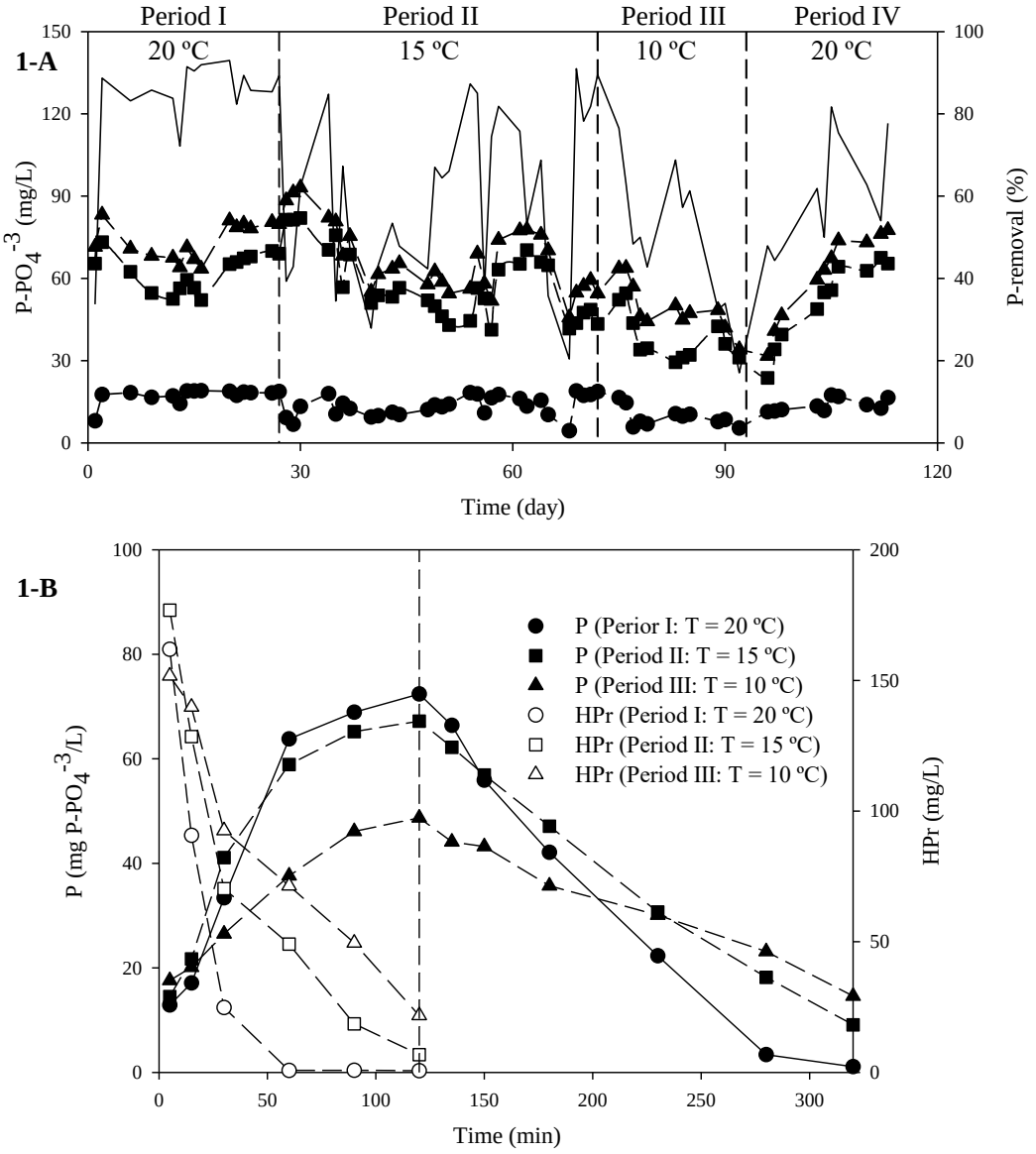


Figure 6.1. (A) Evolution of SBRA performance over the four-month period at 10-days SRT and different temperatures: net P-removal (●), P-uptake (▲), P-release (■), and P-removal efficiency (solid line). (B) Propionic (empty) and phosphorus (filled) concentrations.

A batch test was carried out in each period to better understand which processes limited the performance of the EBPR at lower temperatures. Figure 6.1-B shows the experimental profile of phosphorus and carbon in a typical anaerobic/aerobic cycle of periods I, II and III. The typical phenotype of a good-performing EBPR system (i.e. complete anaerobic uptake of C and high P-release, as well as complete P-uptake under aerobic conditions) was observed at 20 ºC, but this was not the case for cycles performed at 10 and 15 ºC where phosphorus was not completely taken up in the aerobic phase. Table 6.2 shows the rates obtained in the batch cycles and those of 20 ºC were higher than those of 15 and 10

ºC. For example, the specific P-release rate was about 1.4 times higher than that of 15 ºC and 2 times higher than that of 10 ºC. A similar correlation is observed when comparing carbon uptake rate due to direct stoichiometric coupling between those two metabolic processes (Brdjanovic et al., 1997). As for the aerobic phase, the specific P-uptake rate at 20 ºC was 45% higher than that obtained at 15 ºC and at 10 ºC.

Table 6.2. Maximum rates for carbon uptake, P-release and P-uptake obtained during specific cycles in SBRA and SBRB at different SRT and temperature.

| Period | $SBR_A$ | | | Period | $SBR_B$ | | |
|---|---|---|---|---|---|---|---|
| | $\mathbf{C_{upt}}^a$ | $\mathbf{P_{rel}}^b$ | $\mathbf{P_{upt}}^b$ | | $\mathbf{C_{upt}}^a$ | $\mathbf{P_{rel}}^b$ | $\mathbf{P_{upt}}^b$ |
| I (10 d; 20 ºC) | 0.124 | 0.022 | 0.011 | I (10 d; 20 ºC) | 0.119 | 0.024 | 0.013 |
| II (10 d; 15 ºC) | 0.096 | 0.015 | 0.006 | II (5 d; 20 ºC) | 0.138 | 0.026 | 0.020 |
| III (10 d; 10 ºC) | 0.045 | 0.012 | 0.005 | III (5 d; 10 ºC) | 0.060 | 0.017 | 0.006 |

a = mmol C/g VSS/min; b = mmol P/g VSS/min

EBPR activity was influenced by temperature. Temperature coefficients (θ) were obtained from these experiments, showing a medium temperature dependency for P-release rate (θ =1.075), P-uptake rate (θ = 1.05) and HPr uptake rate (θ = 1.07). However, as shown in the next section, through short-term experiments, the values of θ increase when working with lower SRTs. Similarly, Brdjanovic et al. (1997) indicated that θ values of at least 1.11 could be expected for the minimum SRT, which would imply different temperature influence as function of the SRT. This could further complicate the design of integrated EBPR systems into the A-stage process.

In the second part of the work ($SBR_B$), the impact of SRT on EBPR activity was firstly studied at a constant temperature (20 ºC). Figure 6.2A shows the changing trend in the P removal efficiency, P-uptake, P-release and net P removal throughout the $SBR_B$ operation. As in $SBR_A$, the operation started under favourable conditions for PAO growth (i.e. SRT 10 days and temperature 20 ºC). During this period, the average anaerobic P-release was 70 mg $P\text{-}PO_4^{-3}$/L, with a subsequent P-uptake of 82 mg $P\text{-}PO_4^{-3}$/L, resulting in an average P removal efficiency of 85 %. In the period II, the SRT was reduced to 5 days, while the temperature was kept at 20 ºC. This change had not a negative impact on EBPR activity

since the values of P-release, P-uptake and P removal efficiency (72 $P\text{-}PO_4^{-3}$/L, 85 $P\text{-}PO_4^{-3}$/L and 86%, respectively) obtained were very similar to those obtained in period I.

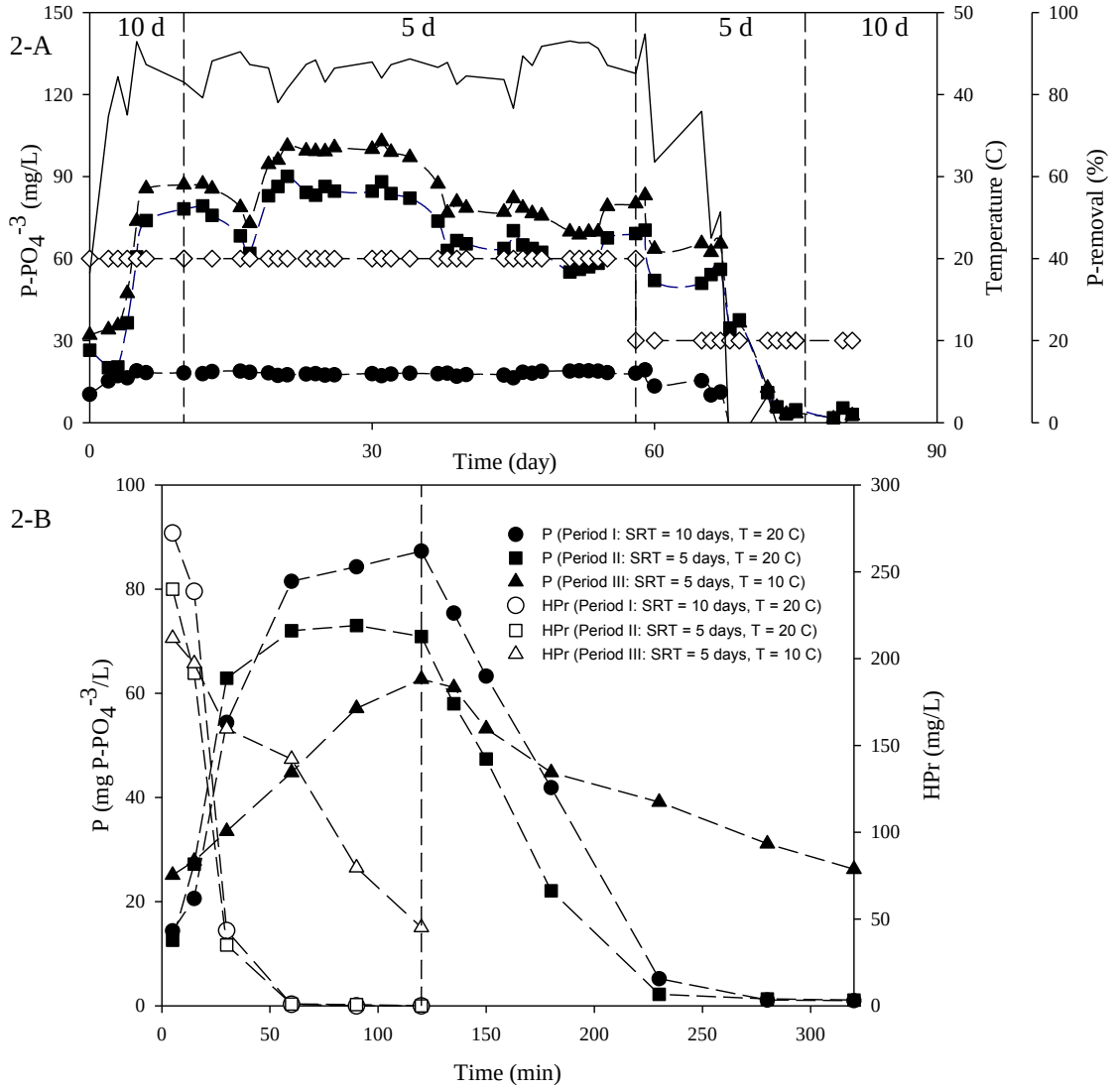


Figure 6.2. (a) Evolution of SBRB performance throughout the experimental period at different SRTs and temperatures: P-removal (●), P-uptake (▲) and P-release (■), % of the influent P removed (solid line) and temperature (♦). (b) Propionic (empty) and phosphorus (filled) concentrations during batch testing of SBRB at different temperatures.

Since the system was operating with good performance at T=20ºC and SRT of 5 days, the long-term effect of a temperature decrease was studied. SRT was maintained at 5 days and the temperature was decreased at 10 ºC (period III), as a typical low temperature found in many WWTP. This change produced a decrease in PAO activity that after approximately one week resulted in the cessation of EBPR activity, obtaining only 10% of P removal efficiency. This is consistent with the results of McClintock et al. (1993), who also observed that the EBPR activity was lost at a SRT of 5 days and a temperature of 10 ºC. Erdal et al.

(2003) pointed out that temperature affects EBPR activity and washout can occur at low temperatures and low SRTs. They operated two laboratory-scale UCT systems whose EBPR activity was completely lost at 5 ºC and 3.5 days of SRT. The loss of EBPR observed during the period III is in accordance with the low P/C ratio (0.22) and the high VSS/TSS relation (0.95) obtained, which indicate that PAOs were washout from the system at 10 ºC and 5 days of STT.

As already discussed, the minimum SRT necessary reported in literature for a successful operation of EBPR systems depends on the temperature. In our study, PAO could not sustain its activity at an SRT of 5 days at 10 ºC. Much lower SRTs are reported to be feasible at temperatures higher than 20ºC. For example, in chapter 4 was reported that at 25 ºC the minimum SRT is 3.6 days; similarly Valverde-Pérez et al. (2016) reported that at 20 ºC the minimum SRT is 3.0 days; even lower values of the minimum SRT have been reported by Ge et al. (2015) (SRT= 2.5 days at temperature of 20 ºC).

Figure 6.2-B shows the experimental carbon and P profiles obtained in three batch cycles of $SBR_B$ at periods I, II and III. The profiles obtained at 20 ºC and SRT of 5 and 10 days were in accordance with robust EBPR systems: high anaerobic P-release and complete aerobic P-uptake. However, in period III (SRT = 5 days) the activity of EBPR was strongly affected by the low temperature (10 ºC), which was reflected in the incomplete carbon uptake, the low anaerobic P-release and the incomplete poor level of P released during the anaerobic phase, and the incomplete aerobic P uptake. All rates decreased considerably at SRT = 5 days and temperature = 10 ºC (Table 6.2). Temperature coefficients (θ) were calculated from these batch cycles, showing a high temperature dependency for P-uptake ($\theta = 1.11$), P-release ($\theta = 1.08$) and carbon uptake ($\theta = 1.09$). When comparing the temperature coefficients for SRT of 5 and 10 days, it was observed that the lower the SRT, the greater the temperature dependence.

Finally, the operation of the $SBR_C$ (Figure 6.3) was also useful in evaluating the long-term effect of different SRT on EBPR activity, even though it was mainly devoted to investigating the short-term impact of temperature on the EBPR process in different SRTs. As expected, the P-removal efficiencies in the $SBR_C$ were significantly influenced by changes in SRT. Initially, the $SBR_C$ was operated a 15-day SRT, obtaining 87% average P-removal efficiency. When the SRT was reduced to 10 days, the efficiency of P-removal decreased slightly to 85%. This value was maintained at SRT of 5 days (85%). However, the

P-removal efficiency was severely affected when the SRT was reduced to 3.5 days (44 ± 25.9). In summary, the $SBR_C$ showed an unstable EBPR performance in the SRTs of less than 5 days. Although it was possible to maintain EBPR activity in the SRT of 3.5 days, the P-removal efficiency obtained was 44%, which led to high concentrations of P in the effluent.

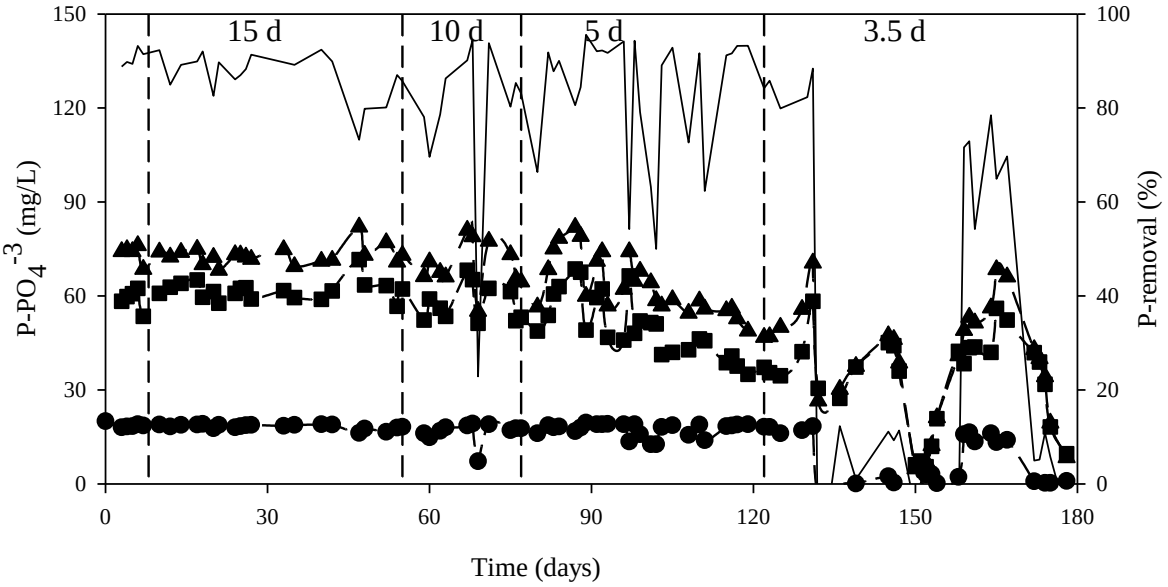


Figure 6.3. Evolution of SBRC performance throughout the experimental period at different SRTs. P-removal (●), P-uptake (▲), P-release (■) and % of the influent P removed (solid line).

In summary, the results obtained from long-term SBR operations clearly indicate that there is a relationship between temperature and SRT, i.e. for each temperature there is a minimum SRT or vice versa. Higher temperatures allow an efficient EBPR to be maintained at short SRT values. The optimum working temperature range found was 15-20 ºC, as long as SRT values were greater than 3.5 days. By individually exploring the impact of SRT on the EBPR process, we can observe that at a constant operating temperature (in this case 20 ºC) the EBPR activity exhibited an unstable performance at SRTs lower than 5 d (Figure 6.3). With regard to temperature, it was observed that EBPR activity could be unstable at temperatures below 20 ºC even in the "safe" SRT tested in this work (e.g. 10 days), resulting in periods of low P-removal efficiency (Figure 6.1-A). However, this does not mean that good EBPR activity cannot be achieved at temperatures below 20 ºC, as demonstrated by Brdjanovic et al. (1997) who reported good EBPR performance at 10 ºC and 16 days of SRT (to avoid PAO washout). However, the operation of an EBPR system at large SRTs makes

it incompatible with the drive towards energy self-sufficient WWTPs. For example, long SRTs would make it difficult to integrate the EBPR process into the treatment technologies that maximize the recovery of the full energy potential present in wastewater such as the A-stage.

### 6.3.2. Short-term experiments

As mentioned above, the $SBR_C$ was operated with the objective of evaluating the short-term effect of temperature on EBPR activity at different SRTs. To this end, the effect of sporadic temperature changes on stoichiometry and EBPR kinetics was studied by operating the $SBR_C$ at 20 ºC and then applying different thermal conditions (10 and 20 ºC) for less than 24 h in each of the periods at different SRTs. Figure 6.4 shows the results of 12 fully monitored and representative batch cycles of each condition. During the anaerobic phase, all organic carbon was completely taken up in almost all batch experiments, with the exception of those conducted at 10 ºC and 3.5-day SRT, where the carbon was not fully taken up. Following the typical PAO metabolism, COD uptake was related to glycogen degradation, production of PHA and P-release. Overall P-removal efficiencies were greater than 90%, resulting in P effluent concentrations of less than 1.2 mg $P\text{-}PO_4^{-3}$/L for most experiments. However, this was not the case for the 3.5 days + 10 ºC experiments, where net P-removal did not occur resulting in high concentrations of P in the effluent (up to 20 mg $P\text{-}PO_4^{-3}$/L). The P/C ratio obtained for the two above-mentioned batch tests (0.20) corresponds to the typical ratio found in a sludge enriched in GAO (Lopez-Vazquez et al., 2006). However, this low P/C ratio can be attributed to a temperature change rather than the population variations, as these variations do not occur over such short periods of time. In this sense, Erdal et al. (2006) showed that the inhibition of glycogen metabolism at low temperature (5 to 10 ºC) and SRTs (> 1.8 days) was the main cause of the decrease in EBPR activity, and not by population shift. They observed (through a series of batch tests) that at this temperature and SRT range, anaerobic carbon uptake was not complete, which was related to the poor glycogen consumption. This led to the substrate breakthrough into the aerobic phase, causing EBPR failure. A similar behavior was observed for the batch test performed at SRT of 3.5 days and 10 ºC (Figure 6.4-D).

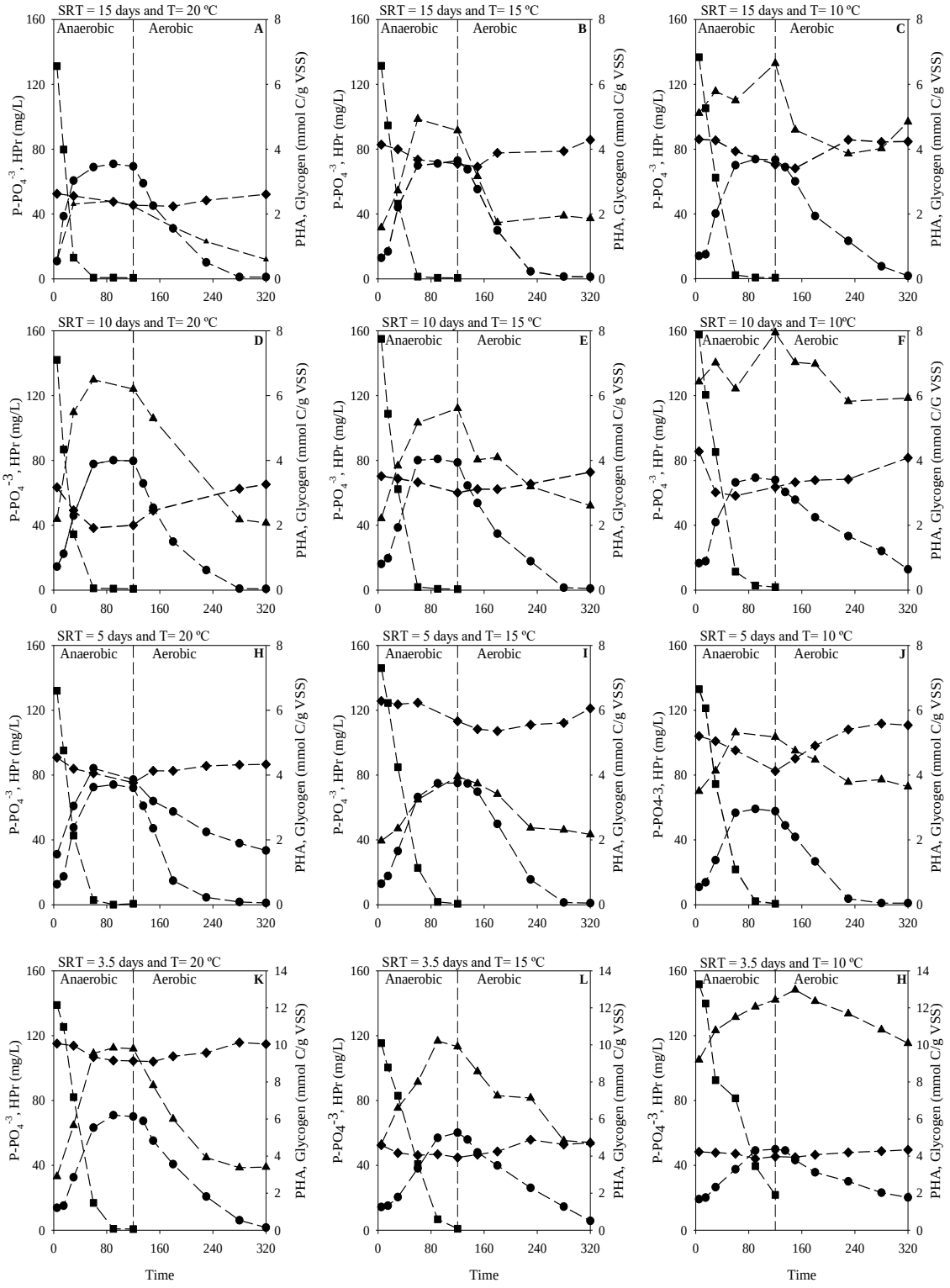


Figure 6.4. Phosphorus (●), PHA (▲), Glycogen (♦) and HPr (■) variations during the 12 short-term batch experiments at different SRT and temperature.

80 % of the incoming propionic was consumed under anaerobic conditions but the glycogen degradation decreased, in comparison with the same temperature but at different

SRT (e.g. 10 ºC and 5 days), from 1.4 to 0.27 mmol C/L (Table 6.3). Also, from the Figure 6.4-D it can be seen that higher accumulation of PHA occurred. Another reported cause of EBPR failure at lower temperatures is the low PHA utilization rate under aerobic conditions (Brdjanovic et al., 1998; Baetens et al., 1999). It has been pointed out that the PHA produced anaerobically must be consumed during the aerobic period. Otherwise, the PHA content in the cell will increase until a maximum level which will prevent acetate uptake under anaerobic conditions and, therefore, the deterioration of EBPR.

Brdjanovic et al. (1997) suggested that EBPR process stoichiometry was insensitive to temperature changes. However, in this thesis, the observed stoichiometric was significantly sensitive to temperature changes, especially when the system was operated at low SRTs (<5 days). For example, by considering only the SRTs of 3.5 (which led to the loss of EBPR activity) and 10 days (SRT used mainly in the conventional EBPR process) (Table 6.3), we can see that the ratios P-release vs HPr-uptake (P/C) and PHA-produced vs HPr-uptake obtained at SRT of 3.5 days decreased as the temperature decreased. However, this trend was only observed with PHA/C and Gly/C with the10-days SRT.A similar observation was done by Erdal et al. (2002) who postulated that cold temperatures suppress some of the metabolic pathways of EBPR organisms. In addition, Lopez-Vazquez et al. (2006) observed that anaerobic stoichiometric is insensitive to temperature changes from 20 to 35 ºC. But, it was significantly affected at low temperatures (e.g.10 ºC). The discrepancy with respect to the results of Brdjanovic et al. (1997) may be due to the fact that in that work the SRT values were increased as the temperature was decreased to avoid PAO washout.

Table 6.3. EBPR ratios measured in the 12 short-term experiments in each pair of SRT and temperature conditions.

| SRT (days) | T (°C) | P/C[a] | PHA/C[b] | $Gly_{deg}$/C[b] |
|---|---|---|---|---|
| **3.5** | 20 | 0.32 | 0.88 | 0.05 |
| | 15 | 0.26 | 0.82 | 0.09 |
| | 10 | 0.20 | 0.49 | 0.05 |
| **10** | 20 | 0.37 | 1.19 | 0,34 |
| | 15 | 0.36 | 1.01 | 0.12 |
| | 10 | 0.37 | 0.46 | 0.19 |

a= mol P/mol C, b= mol C/mol C

The process rates obtained in this short-term study are displayed in table 4. In overall, the batch experiments indicate that temperature have an influence on kinetics of metabolic processes of EBPR. Within the temperature range 10 to 20 ºC, a 10 ºC drop in temperature

results in a decrease in these rates. A similar trend was observed for the rates of P-release, C-uptake and P-uptake in the long-term experiments presented previously (i.e. $SBR_A$ and $SBR_B$) in agreement with previous reports (Brdjanovic et al., 1997, Choi et al., 1998, Baetens et al., 1999). Regarding the SRT, we can observe from Table 6.4 that there is a trend toward increasing the specific process rates as the SRT decrease from 15 to 3.5 days. Short SRTs might enhance the bio-P removal efficiency as long as the temperature is high enough to maintain EBPR activity. A similar pattern was found in the chapter 5 where the values of the kinetic parameters obtained at 25 ºC were markedly high at an SRT of 12 days compared with that at an SRT of 3.6 days.

Table 6.4. Specific EBPR rates measured in the 12 short-term experiments in each pair of SRT and temperature conditions.

| **Temperature (ºC)** | **SRT (days)** | **$P_{rel}$[a]** | **$P_{upt}$[a]** | **$C_{upt}$[b]** | **$PHA_{prod}$[b]** | **$PHA_{deg}$[b]** |
|---|---|---|---|---|---|---|
| **20** | 3.5 | 0.047 | 0.025 | 0.13 | 0.121 | 0.053 |
| | 5 | 0.037 | 0.021 | 0.12 | 0.036 | 0.012 |
| | 10 | 0.030 | 0.018 | 0.10 | 0.077 | 0.025 |
| | 15 | 0.028 | 0.012 | 0.09 | 0.070 | 0.010 |
| **15** | 3.5 | 0.021 | 0.013 | 0.07 | 0.063 | 0.026 |
| | 5 | 0.027 | 0.016 | 0.08 | 0.023 | 0.009 |
| | 10 | 0.026 | 0.014 | 0.06 | 0.053 | 0.019 |
| | 15 | 0.016 | 0.010 | 0.07 | 0.046 | 0.011 |
| **10** | 3.5 | 0.014 | 0.011 | 0.05 | 0.021 | 0.026 |
| | 5 | 0.024 | 0.012 | 0.07 | 0.025 | 0.009 |
| | 10 | 0,020 | 0.010 | 0.06 | 0.024 | 0.016 |
| | 15 | 0.017 | 0.009 | 0.05 | 0.020 | 0.011 |

a= mmol P/g VSS/min, b= mmol C/g VSS/min

### 6.3.3. Temperature coefficients (θ)

According to the IWAS ASM2 model (Henze et al. 2000), biological processes can be classified as a function of their temperature dependency: (i) none (θ = 1.00); (ii) low (θ = 1.04); (iii) medium (θ = 1.07); and (iv) high (θ = 1.12). Using the simplified Arrhenius equation for the temperature dependence, the temperature coefficients (θ) for anaerobic and aerobic kinetics were calculated based on values from Table 4.

Table 6.5 shows the temperature coefficients obtained. In a SRT of 3.5 days, the temperature dependency for C uptake (θ = 1.13), P release (θ 1.15), P uptake (θ = 1.11) and PHA consumption (θ = 1.11) were significantly higher than those observed for SRT of 15-days (C-uptake (θ = 1.07), P-release (θ = 1.08), P-uptake (θ = 1.03) and PHA consumption

(θ = 1.07)). Based on the classification of the coefficients in ASM2, the mean of the anaerobic and aerobic phases obtained at 3.5 and 15 days of SRT indicates that the EBPR process has a medium degree of temperature dependency (1.06 ± 0.022) at 15 days of SRT and a high degree (1.12 ± 0.019) at 3.5 days of SRT. Hence, the EBPR process is more sensitive to temperatures change at low SRT. As mentioned above, Brdjanovic, et at. (1997) predicted a value of temperature coefficient (θ) of at least 1.11 for the minimum SRT, which is similar to that obtained in this study. On the other hand, it can be seen that the temperature dependence of anaerobic rates, such as P-release and C-uptake (Table 6.5), are very similar for all SRTs tested in this study. This similarity is expected, as these two processes are stoichiometrically related. This behavior also was observed for aerobic kinetic rates, for example the effect temperature of P-uptake (θ = 1.06 ± 0.04) was similar to the PHA consumption (θ = 1.07 ± 0.03) since both processes are much related: polyphosphate storage requires energy from PHA degradation.

Table 6.5. Temperature coefficients (θ) calculated for EBPR rates related to P and C.

| SRT (d) | $P_{REL}$ | $P_{UPT}$ | $C_{UPT}$ | $PHA_{PROD}$ | $PHA_{DEG}$ |
|---|---|---|---|---|---|
| **3.5** | 1.15 | 1.11 | 1.13 | 1.12 | 1.11 |
| **5** | 1.05 | 1.05 | 1.07 | 1.07 | 1.05 |
| **10** | 1.07 | 1.05 | 1.07 | 1.11 | 1.05 |
| **15** | 1.07 | 1.03 | 1.06 | 1.11 | 1.07 |
| **Average** | 1.08 ± 0.05 | 1.06 ± 0.04 | 1.09 ± 0.03 | 1.10 ± 0.03 | 1.07 ± 0.03 |
| **Long-term*** | 1.07 | 1.05 | 1.07 | - | - |

*These data were obtained at an SRT of 10 d

## 6.4. Conclusions

The long-term and short-term effects of SRT and temperature on EBPR processes were evaluated using three SBR EBPR systems with A/O configuration. An EBPR system working at T = 20ºC was operated at 5 days with good performance. However, when the temperature decreased at 10 ºC, EBPR activity was lost. Another EBPR system operated at 10 days SRT maintained good EBPR activity (86% of P-removal) at 20 ºC, decreased its performance at 15 ºC (71 % of P-removal) and at 10 ºC progressively lost its EBPR activity.

The temperature coefficients obtained both in the short- and long-term experiments show that EBPR reaction rates have a high degree of temperature dependence in SRT of 3.5.

However, at SRTs of 5, 10 and 15-days, EBPR reaction rates have a medium degree of temperature dependence. From batch test we can elucidate that the deterioration of EBPR was probably caused by the suppression of certain metabolic process caused by the effect of low temperature and SRT.

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# CHAPTER 7

# Evaluation of methane production from bio-P sludge obtained at different SRT and different PHA content

## 7.1. INTRODUCTION

Wastewater treatment requires a high amount of energy. For example, a conventional WWTP requires an annual net energy input of about 40 kWh per population equivalent to meet the legal requirements for effluent discharge regarding organic matter, nitrogen and phosphorus (Meerburg et al. 2015). Fortunately, WWTPs can improve their energy efficiency through the use of energy recovery processes, such as anaerobic digestion (Stillwell et al., 2010). The primary and secondary sludge generated from WWTP operation is a good substrate to produce biogas which, in turn, is a possible fuel source for electricity generation. Currently, studies on anaerobic digestion of sludge generated from wastewater treatment systems have focused mainly on sludge obtained from short SRT systems for the following reasons:

i) High degree of degradability. Ge et al. (2013) observed that the degradability of sludge decreases with increasing SRT, with a measured degree of degradability of 85%, 73% and 63% at 2, 3 and 4 days SRT respectively. When sludge with 85% degradability was subjected to anaerobic digestion, the methane produced (350 mL of $CH_4$ per g VSS) was 17% higher than that obtained with the sludge with 63% degradability (300 mL of $CH_4$ per g VSS).

ii) High biogas production. According to Bolzonella et al. (2005), the anaerobic methane yield of activated sludge is enhanced with the reduction of SRT. They estimated, for example, that passing from 20 to 10 days of SRT in the activated sludge process represents an increase in gas production of around 25%.

iii) Systems operated at short SRTs can produce sludge with high PHA content. Experimental studies have shown that PHA exhibits a higher biochemical methane potential on a VSS basis compared with protein and carbohydrates (the main components of a cell) (Huda et al. , 2013).

Currently, studies on PHA are mainly oriented to their recovery as a source of biodegradable plastic (Perez-Feito and Noguera, 2006; Pijuan., 2009; Rodgers and Wu, 2010; Yuan et al., 2015). However, another approach has recently emerged, which focuses particularly on energy recovery through production of methane (Huda et al., 2013). In this context, some studies reported that the presence of PHA in the sludge improves the methane yield. Wang et al. (2016) predicted, through model-based analyses, an increase of 40% of

methane (from 192 to 274 mL $CH_4$/g VSS) when the PHA levels increased from 21 to 143 mg/g VSS. Since the PHA degradation is not taken into account in the current anaerobic digestion model (ADM1), increased PHA content could lead to an underestimation of methane generation. Similarly, Huda et al. (2013) reported that 25% more methane was produced from a sludge with PHA (PHB) accumulation in comparison to that of sludge without PHA accumulation.

Smolders et al. (1995) pointed out that the PHA content of the biomass is determined by the conversions taking place during anaerobic and aerobic periods of the cycle and the ratio between substrate addition and biomass present in the reactor. With respect to the second point, in a constant substrate fed to the reactor, the PHA content will be higher if the substrate is anaerobically taken up by a small amount of biomass, and lower if there is a high biomass concentration in the reactor. The biomass concentration in the reactor can be controlled by manipulation of SRT. Therefore, it can be expected that, at a constant load in the reactor, the lower the SRT, the higher the PHA content in the biomass. Given that conventional EBPR process is usually operated with SRT values between 10-20 d (which is adequate to promote PAO growth), it could be supposed that the long-sludge age generated would contain lower amount of PHA that would lead to a lower rate of biogas production. This issue could be addressed by optimizing the conventional EBPR configuration in order to generate waste activated sludge (WAS) with high levels of PHA; for example, operating EBPR process under short-SRT mode as in Chapter V and VI of this thesis. In this context PAO have the ability to store PHA under anaerobic conditions from organic substrates and, thus, they can be excellent candidates to being digested. However, in a conventional EBPR configuration, PAO use the internally stored PHA in the subsequent aerobic (or anoxic) phase as an energy source to drive cell growth and for poly-P and glycogen synthesis. Hence, if we want to benefit from the PAO ability to store PHA with a view to increasing biogas production, we need to maximise PHA production. Toward this end, the strategy of purging at the end of the anaerobic phase rather than at the end of the aerobic phase would help to increase PHA content in the biomass, although its impact on P-removal should also be studied.

The goal of this chapter is to evaluate the influence of SRT and anaerobic and aerobic environments on the PHA accumulation, in order to gain insight of the optimal conditions for harvesting the maximum amount of PHA present in the sludge generated in the EBPR

process. In addition, the biochemical methane potential (BMP) of the different types of sludge produced is evaluated.

## 7.2. Materials and methods

### 7.2.1. SBR operation

An EBPR- SBR system with a working volume of 10 L was used in order to develop sludge with different PHA content. The reactor was inoculated with sludge obtained from a pilot-plant using an $A^2O$ configuration at UAB facilities. The SBR was operated in four cycles of 6 h per day. Each cycle consisted of 120 min of anaerobic phase, 210 min of aerobic phase, 25 min of settling, and extraction of 5 L of supernatant during the last 5 min of the cycle. A volume of 5 L of synthetic medium (composition provided in chapter 3) was fed to the SBR during the first 5 min of the anaerobic phase, resulting in a hydraulic retention time (HRT) of 12 h. SRT was strictly monitored considering both the biomass in the effluent and in the reactor and controlled by manipulating the volume of sludge extracted. Different SRTs were studied (Table 1). The exact amount of sludge was removed by means of a controlled peristaltic pump at the end of the anaerobic phase of each cycle before turning off the stirrer. The SBR was mixed at 120 rpm during the anaerobic and aerobic phases. Dissolved oxygen (DO) was controlled between 2.5 and 3.5 mg DO/L by an on/off electro valve that allowed an aeration flow rate of 3 L/min in the "on" position. pH was controlled at 7.5 ± 0.1 by dosing 1M HCl or NaOH through peristaltic pumps. Temperature was controlled around 20 ºC by recirculating water from a thermostatic bath through the jacket of the reactor. Nitrogen gas was bubbled into the SBR during the anaerobic phase to ensure anaerobic conditions. Propionic acid was used as the carbon source with an initial concentration in the reactor of 226 mg COD/L.

### 7.2.2. Biochemical methane potential

The BMP experiments aimed to assess the amount of biogas produced by anaerobic digestion (AD) of sludge. Control experiments (i.e. without substrate) were included to correct BMP for residual methane production. The AD tests were carried out using identical 125 mL serum bottles, and all the experiments were performed in triplicate. The inoculum consisted of mesophilic AD sludge from an anaerobic digester of an urban WWTP. The AD

inoculum was degassed at 37 ºC for 3 days before its use. A substrate: inoculum (S/I) ratio of 1 was maintained in all the tests. The pH of each bottle was set at 7.0 at the start of the experiments and was left uncontrolled during AD. The bottles were closed tightly with butyl rubber septum and aluminium caps and were incubated under mesophilic conditions (37 ºC). Anaerobic conditions were achieved by purging the headspace of each bottle with $N_2$ for 2 min. The content of each bottle was mixed periodically. The gas production was estimated by measuring the pressure increase in the headspace with a gas pressure meter.

### 7.2.3. Chemical analysis

Activated sludge samples were withdrawn from the SBR to analyse EBPR performance. These samples were immediately filtered with a Millipore filter unit (0.22 µm pore size) for the analysis of P, VFA, PHA and glycogen. Analyses of phosphate in filtered samples were performed with a phosphate analyser (115 VAC PHOSPHAX sc, Hach-Lange) based on the Vanadomolybdate yellow method, where a two-beam photometer with LEDs measured the phosphate specific yellow colour. Propionate was measured by a gas chromatograph (GC Agilent Technologies 7820 A) equipped with a BP21 SGE column (30 m 0.25 mm 0.22 mm; length internal diameter film thickness) and a flame ionisation detector (FID). The solid-samples were fixed with formaldehyde immediately to stop the reaction and then lyophilised for further analysis of PHA and glycogen. Glycogen was analysed by a modification of the method proposed by Smolders et al. (1994). The concentration of glucose was measured using an YSI model 2700 Select Biochemistry Analyser (Yellow Springs Instrument, 167 Yellow Spring, Ohio, USA). PHA was extracted from lyophilised samples using hexane and butanol. Afterwards, PHA was determined with a GC (Agilent Technologies 7820A). As standards were used 3-hydroxybutyric acid and 3-hydroxyvaleric acid copolymer for polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) and 2-hydroxycaproic acid as standard for polyhydroxy-2-methylvalerate (PH2MV). PHA produced during the anaerobic phase was calculated as the sum of PHB, PH2MV and PHV. Finally, TSS and VSS were measured according to standard Methods (APHA, 1995).

In each AD test, biogas composition (i.e. $CH_4$, $CO_2$ and $H_2$) was determined by using a Perkin Elmer autosystem gas chromatograph equipped with a thermal conductivity detector (GC-TCD). Accumulated volumetric gas production was calculated from the pressure

increase in the headspace volume (125 ml) and expressed under standard conditions (25ºC, 1 atm).

## 7.3. RESULTS AND DISCUSSION

### 7.3.1. Performance of the EBPR-SBR system under different SRT

An SBR performing EBPR was continuously operated for 180 days at different SRTs in order to obtain sludge with different PHA content. Figures 1A and 1B show the variation of P removal efficiency, TSS, VSS and VSS/TSS ratio at different SRTs. The reactor was initially operated at SRT of 15 days (period I), reaching stable operation within the first 20 days. During this period, TSS and VSS concentrations, at the end of the aerobic phase, were 3115 ± 237 and 2177 ± 235 mg/L, respectively, resulting in an average VSS/TSS of 0.70. This value indicates high poly-P content suggesting a high fraction of PAO in the system. The average P removal efficiency during this period was 87% ± 4. In period II, the SRT was reduced to 10 days, which resulted in the decrease of TSS and VSS to 2348 ± 341 and 1708 ± 341 mg/L, respectively. In contrast a slight increase of VSS/TSS ratio was observed (0.74) indicating a decrease in the amount of poly-P stored in the sludge. This observation is in agreement with the slight decrease of P removal efficiency observed during this period (83% ± 7). A further reduction of SRT to 5 days (period III) provided a slight recovery of P removal (87% ± 7). In addition, lowering the SRT resulted in a significantly lower concentration of TSS and VSS (1466 ± 190 and 1093 ± 207 mg/L) resulting a VSS/TSS ratio of 0.74. Finally, the SRT was decreased to 3.5 days in period IV, leading to a deteriorated P removal performance. The sludge from this last period was not studied in this chapter due to the unstable operation with low PAO activity.

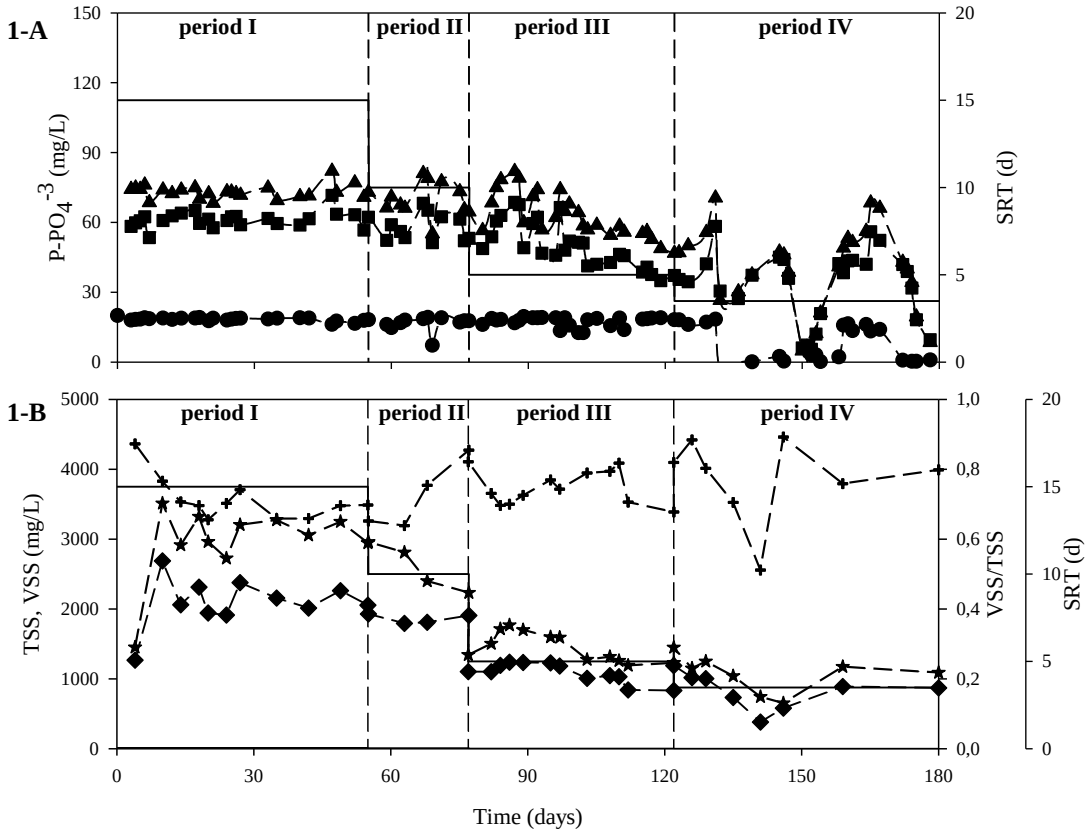


Figure 7.1. Evolution of the EBPR-system performance during the four experimental periods. a) SRT (solid line), P-release (■), P-uptake (▲), Net-P-removal (●) and b) VSS (♦), TSS (★) and VSS/TSS ratio (+) and SRT (solid line).

Three EBPR cycles were monitored including PHA in order to evaluate the effect of different SRTs (15, 10 and 5 d) (Figure 7.2). Under anaerobic conditions, propionic acid was taken up and PHA was produced, resulting in a fast P-release. Under aerobic conditions, PHA was utilized and P was taken up. The highest production of PHA was obtained with a SRT of 5 days, while the longest SRT (15 days) led to the lowest PHA formation per unit of biomass (Table 7.1). In summary, we observed that the system not only had the capacity to remove P in the range of applied SRTs (from 5 to 15 days) but also showed its potential to produce PHA.

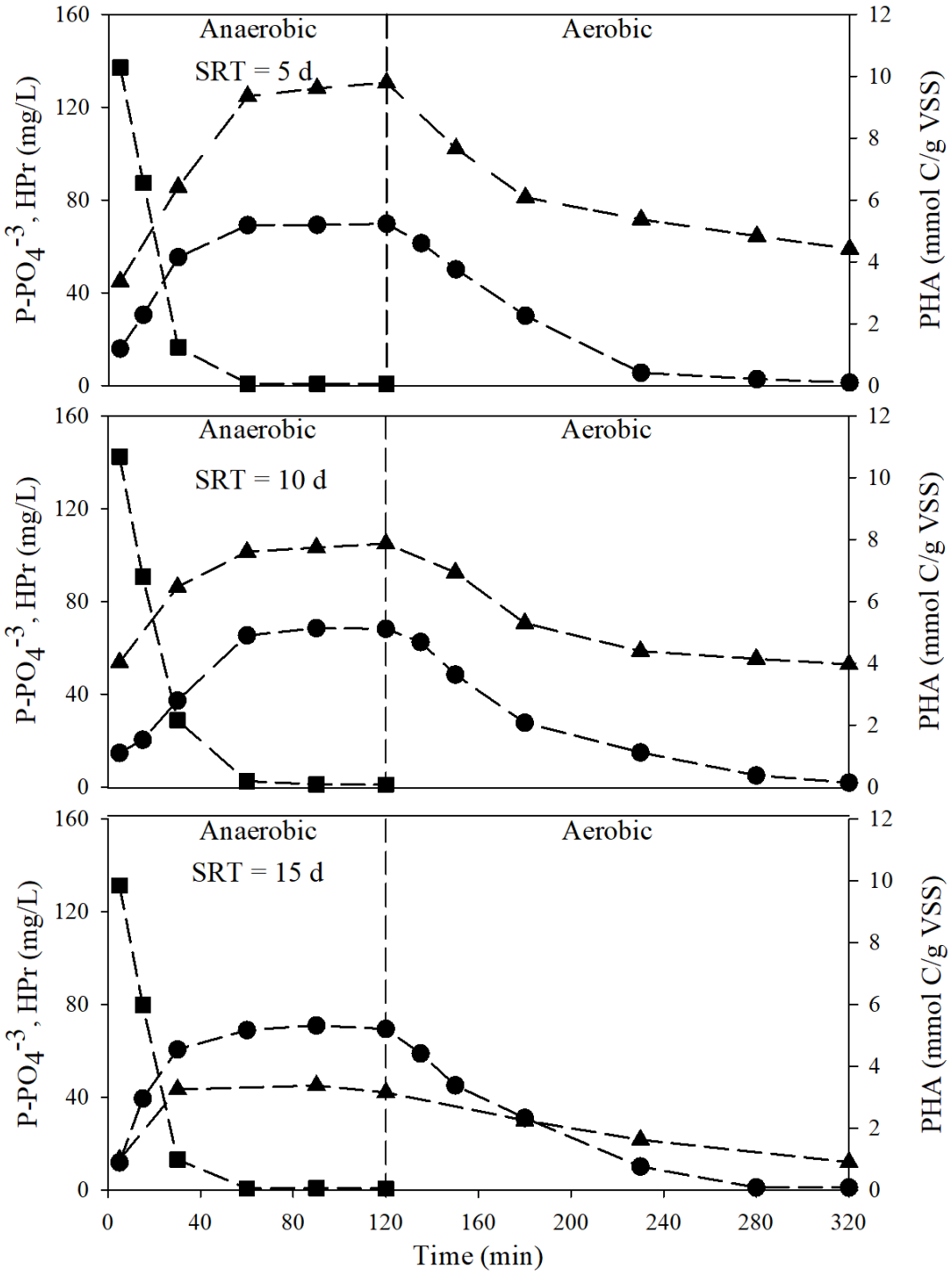


Figure 7.2. Monitored cycles for different SRTs. Evolution of propionic acid (■), P (●) and PHA (▲).

Table 7.1. Performance indexes in the monitored cycles: Ratio PHA produced vs. VFA uptake in the anaerobic phase, content and percentage of PHA in the sludge at the end of the anaerobic phase.

| **Period** | PHA/C (mol C/mol C) | PHA (mmol C/g VSS) | % PHA (g PHA/g VSS) |
|---|---|---|---|
| **I (SRT = 5 d)** | 0.91 | 9.80 | 18 |
| **II (SRT = 10 d)** | 0.64 | 7.89 | 15 |
| **III (SRT= 15 d)** | 0.60 | 3.26 | 8 |

### 7.3.2. Influence of SRT on the PHA content of the biomass

Table 7.2 shows the composition of the PHA measured at the end of the anaerobic phase at different SRTs. The fact that propionic was used as the sole carbon source led to the production of mainly PHV and PH2MV in all cases, which is in agreement with previous reports (Oehmen et al., 2005; Lopez-Vazquez et al., 2009; Pijuan et al., 2009).

Table 7.2. PHA composition measured at the end of anaerobic phase.

| **SRT (d)** | PHB | PH2MV | PHV | Total PHA | PHA composition (%HB: %H2MV: %HV, weight) |
|---|---|---|---|---|---|
| | (mmol C/g VSS) | | | | |
| **5** | 0.75 | 4.33 | 4.72 | 9.80 | 8: 44: 48 |
| **10** | 0.57 | 3.61 | 3.70 | 7.88 | 7: 46: 47 |
| **15** | 0.46 | 1.26 | 1.43 | 3.15 | 15: 40: 45 |

The maximum PHA content was 9.80 mmol C/g VSS (SRT = 5 days) which is equivalent to 18 % (g PHA/g VSS) of PHA in the biomass (Table 7.1). This value is far from the 80% recommended for an economically sustainable PHA production system (Lee et al., 1996; Takabatake et al., 2002), which is only achieved by systems that use pure cultures and specific carbon sources. The highest concentration values of PHA reported in different studies for both PAO and non-PAO systems focused in PHA accumulation are depicted in Table 7.3.

Table 7.3. PHA accumulation in activated sludge biomass obtained from different studies.

| **System** | % PHA content (g PHA/g VSS) | Reference |
|---|---|---|
| **EBPR-SBR** | 30 | Chua et al., 2003 |
| **Feast/famine regime SBR** | 36 | Montiel-Jarillo et al., 2017 |
| **EBPR-SBR** | 28.8 – 50 | Rodgers & Wu, 2010 |
| **Aerobic WWTP** | 29.5 | Takabatake et al., 2002 |
| **Feast/famine regime SBR** | 34.9 | Morgan-Sagastume et al., 2014 |

Also, from Table 7.1 it was found that the PHA content of the sludge decreased when the SRT increased. The highest PHA content of the sludge was obtained with the 5-day SRT, more than three times higher than with the 15-days SRT. These trends could be explained, as stated by Smolders et al. (1995), because the PHA content will be high if the substrate is anaerobically taken up at a low concentration of biomass, and low if there is a high biomass concentration in the reactor. The biomass concentration in the reactor depends on SRT. The VSS in period I (SRT= 15 days) was 2177 ± 235 mg/L, whereas in period II (SRT = 5 days) it was around 1093 ± 207 mg/L. Because of the difference in VSS concentrations, PAO at 5-days SRT had the opportunity to take up about 2 times more organic substrates and store them as PHA than at 15-days SRT. Salehizadeh and Van Loosdrecht (2004) state that the production and storage capacity of PHA in the activated sludge treating municipal wastewater is influenced by operating conditions, such as organic load, SRT and pH. In our study, the operation of the EBPR-SBR system was conducted under the same substrate, loading rate and pH (in all periods of operation). Therefore, we can assume that SRT was the main factor causing the variation of PHA concentration of the biomass in this work. In other words, these findings imply that the EBPR sludge has the potential to accumulate more PHA in EBPR systems operated at short SRTs. A similar behaviour was observed by Coats et al. (2007) with two non-EBPR mixed microbial consortia utilized for PHA production in SBR. They found that the biomass produced by the SBR operated at 4 days contained 28% more PHA than the biomass obtained from the SBR operated at 7 days of SRT. Similarly, Chua et al. (2003) observed that the sludge obtained at 3-days SRT could reach PHA content about 10% more than the sludge with a 10-days SRT

On the other hand, the PHA content of the sludge at the end of the anaerobic and aerobic phases was also measured (Figure 7.2). The sludge at the end of the anaerobic phase

had a higher PHA concentration compared to that obtained at the end of the aerobic phase for all the SRTs assayed. This was expected, since approximately 55% of the total PHA produced during the anaerobic phase was used in the aerobic phase for the formation of biomass, poly-P and glycogen.

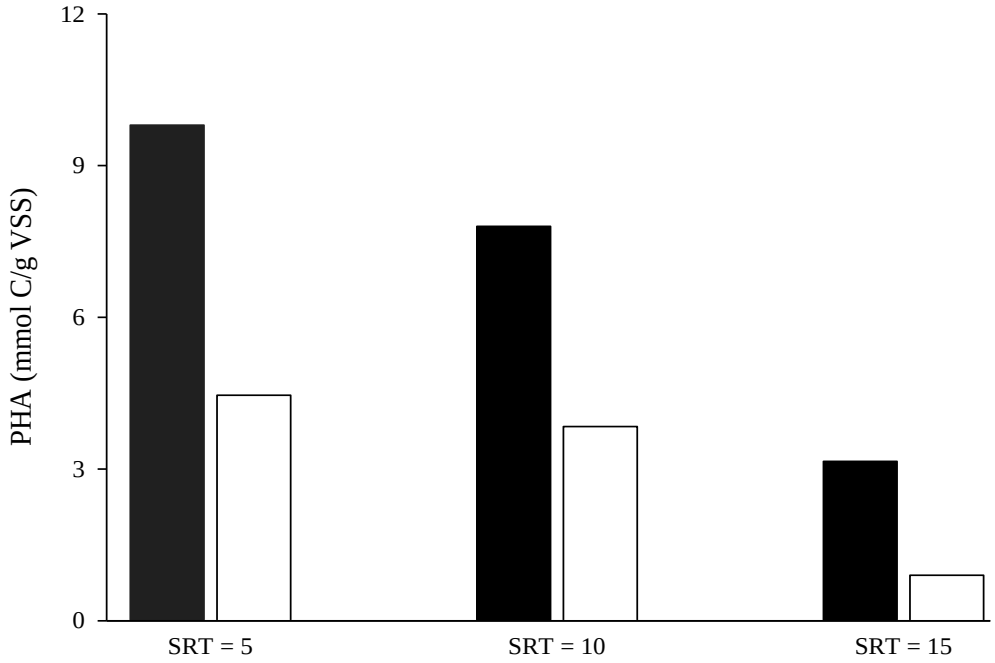


Figure 7.3. PHA content of the sludge obtained at different SRT. Black bars represent the content at the end of the anaerobic phase and white bars at the end of the aerobic phase.

### 7.3.3. Influence of PHA levels on methane production from bio-P biomass

The influence of PHA content on methane production from bio-P biomass was evaluated by a mesophilic (37 ºC) BMP test. For this purpose, six biomass samples with different PHA levels were tested (Table 7.4). Figure 7.4 shows the curve of cumulative methane production obtained over a 46 days period. Biomass A (the biomass with the highest PHA content) showed the highest methane production. When digesting this biomass (PHA of 9.80 mmol C/g VSS), the cumulative methane production was 401.5 ml $CH_4$/g VSS, 1.63 times higher than that obtained from biomass F (the biomass with the lowest PHA content). A similar behaviour was observed by Wang et al. (2015) who found that methane production from activated sludge with high PHA content was 1.46 times higher than that of low PHA content. According to Wang et al. (2016), promoting the enrichment of sludge with PHA would improve methane production during AD. This finding has increased the interest of

researchers in increasing PHA content in the biomass with the aim of improving methane production.

From Figure 7.3 it can be seen that the methane production of all biomass samples was very similar after 1 day of digestion. At the fifteenth day of digestion, the amount of methane from biomass A was significantly higher than that in the other EBPR-biomasses tested. In addition, it can be seen from Figure 3 that the methane production rate decreased after the first ten days. In the first ten days of the experiment, the methane production rate from biomass A was 26.20 ml $CH_4$/g VSS/d, which decreased to 3.55 ml $CH_4$/g VSS/d in the last days of experiment. This behaviour was also observed with biomass F. In this case, the methane production rate during the first ten days was 16.39 ml $CH_4$/g VSS/d and after that period decreased to 2.09 ml $CH_4$/g VSS/d. These results show two clear phases of methane production during anaerobic digestion of bio-P sludge. The high PHA content of biomass A increases the methane production rate by about 60% compared to biomass F during the first days of anaerobic digestion, indicating that higher PHA content could greatly improve the productivity of anaerobic digestion. These results are also consistent with Huda et al. (2016), who observed at 37 ºC that up to 76% of PHA accumulated in activated sludge was easily degraded within the first 8 days of PHA incubation.

Degradability is an important parameter related to the production of biogas. In this chapter is defined as the methane produced divided by the theoretical methane potential. Table 7.4 shows the degradability of each of the samples subjected to BMP tests. We can observe that the higher the PHA content in the biomass, the higher is its percentage of degradability. A similar trend was observed by Wang et al. (2015). They observed that the waste sludge containing 143 mg PHA/g VSS was 43% more degradable than the sludge containing 21 mg PHA/ g VSS.

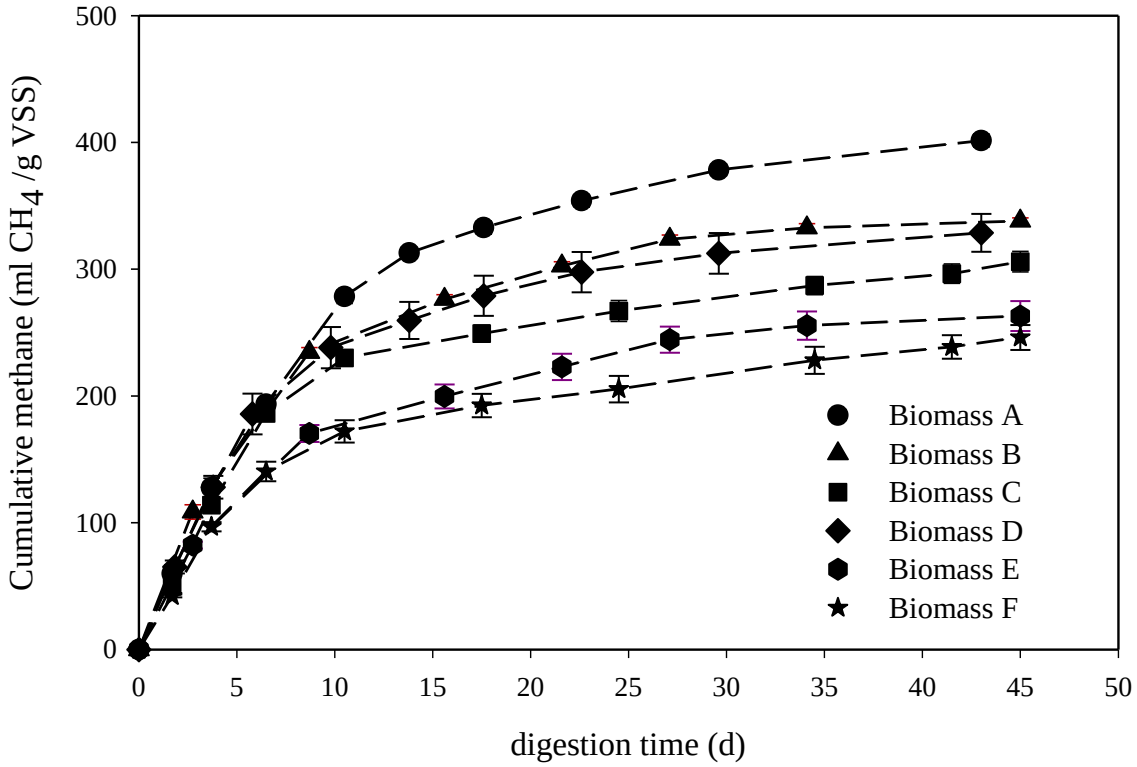


Figure 7.4. Methane production in EBPR sludge with different PHA content.

Table 7.4 shows the PHA content of the biomass samples used for the BMP test, as well as their methane production. It can be seen from Table 7.4 that PHA content in the biomasses ranged from 0.90 to 9.80 mmol C/g VSS. Comparing methane production from biomass A (401 mL $CH_4$/g VSS) and F (246 ml $CH_4$/g VSS), which represent the biomass with the highest and lowest levels of PHA, respectively, the methane production from biomass A was 63 % higher than the value for biomass F. However, this percentage is clearly reduced when comparing methane production obtained from sludge grown at the same SRT but extracted at different phase (anaerobic and aerobic). For example, the methane production from biomass A (obtained at the end of the anaerobic phase) shows 22% more methane production than biomass D (sludge obtained at the end of the aerobic phase). A similar pattern is observed when comparing the biomasses obtained from SRT of 10 and 15 days. Finally, when comparing the methane production of biomass from different SRTs but at the same phase (in this case anaerobic phase), for example biomass A and biomass C, we can observe that methane produced from the biomass A was 31 % higher than that produced from biomass C. In general, the higher the concentration of PHA in the biomass, the higher the production of methane. This fact is clearly observed in Figure 7.5, where experimental

BMP is represented as a function of PHA content. A clear correlation is observed for the six samples (BMP (mL $CH_4$/g VSS) = 240 + 15.3 * PHA (mmol C/g VSS), $R^2$=0.87), independently of SRT and the anaerobic or aerobic extraction.. Wang et al. (2015) showed that PHA has a higher BMP than protein and carbohydrate. During anaerobic digestion, 1 g of protein and carbohydrate can theoretically produce 0.59 and 0.45 L $CH_4$, respectively. In contrast 1 g of PHA can generate 0.65 L $CH_4$. Therefore, by promoting the enrichment of the sludge with PHA, methane production would be improved.

Table 7.4. PHA content of the sludge used in AD tests, as well as their degradability and methane production.

| | | Biomass | PHA content (mmol C/g VSS) | BMP (mL $CH_4$/g VSS) | Degradability (%) |
|---|---|---|---|---|---|
| Anaerobic sludge | | | | | |
| | 5 d | A | 9.80 | 401 | 81 |
| SRT | 10 d | B | 7.88 | 338 | 78 |
| | 15 d | C | 3.15 | 306 | 70 |
| Aerobic Sludge | | | | | |
| | 5 d | D | 4.46 | 329 | 73 |
| SRT | 10 d | E | 3.84 | 279 | 70 |
| | 15 d | F | 0.90 | 246 | 61 |

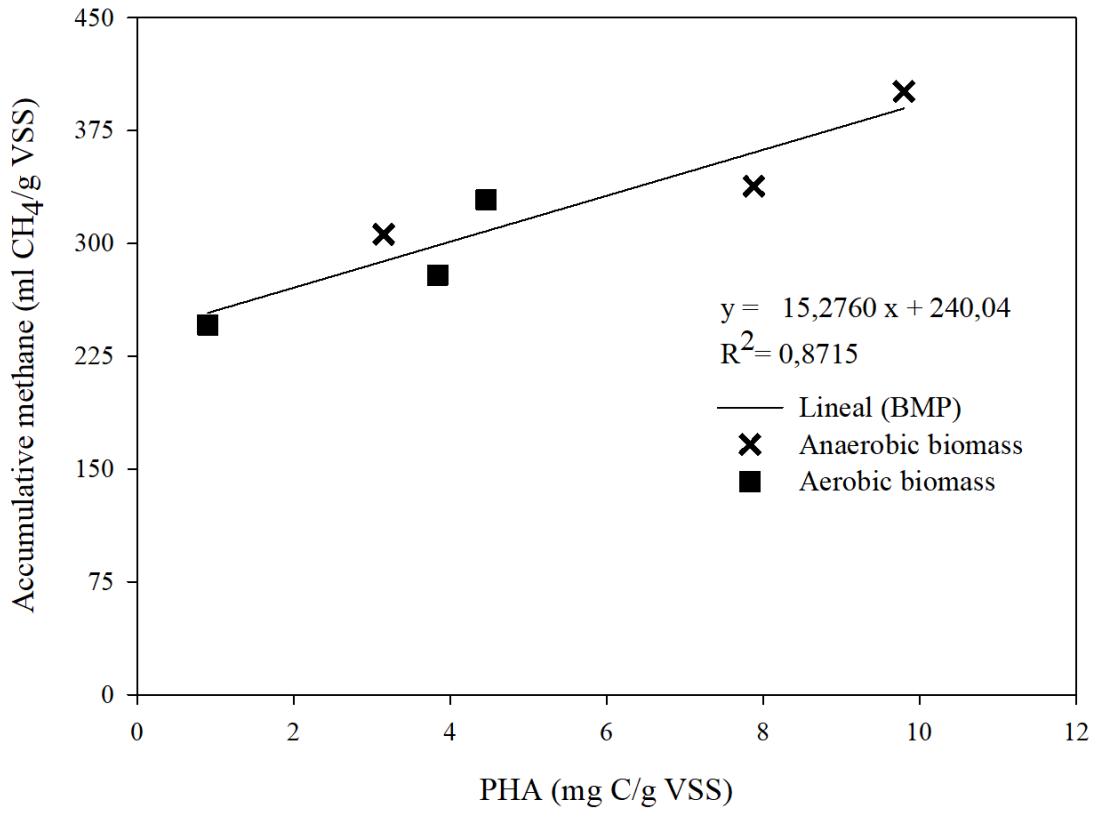


Figure 7.5. Linear correlation between BMP and PHA observed for the six samples

Although there are several operating conditions that influence the potential storage of PHA in sludge, existing studies on the enrichment of sludge in PHA for methane production have focused mainly on COD loading. Wang et al. (2015) studied the methane production from five sludge with different content of PHA obtained from five reactors operated with different COD concentration: 200, 400, 600, 800 and 1000 mg/L. They observed that with the increase of PHA in the sludge, the methane production levels increased. In our study, we used the ability of PAO (i.e. from an EBPR system) to store anaerobically organic matter as PHA (with consequent oxygen savings). In our study, the organic load remained constant and the PHA storing capacity of the sludge was mainly influenced by SRT and the phase at which it was harvested (aerobic or anaerobic extraction). Therefore, based on the above results we can say that this operational strategy (short SRT and anaerobic extraction) could potentially be useful to obtain sludge with a high PHA content. However, in opting for this strategy, the efficiency of P removal must not be compromised. Previous chapters have shown that the minimum value of SRT in which the EBPR can be maintained is 3.6 days at 25 ºC. Values below this SRT could be compromise the efficiency of EBPR. Regarding anaerobic purge extraction, Baeza et al. (2017) proposed a new EBPR-SBR configuration ($EBPR^2$) whose feasibility was evaluated by using modelling techniques. They showed that neither the stability of PAO population nor phosphorus removal efficiency were affected by extracting 4.3% of the total volume of anaerobic supernatant (for an SRT higher than 4 days), since P-removal was largely carried out by anaerobic extraction.

## 7.4. Conclusions

This chapter presents a general study on the influence of PHA content in bio-P biomass on methane production. Based on the findings of this study, the following conclusions can be drawn:

- EBPR activity with high P-removal performance can be maintained within the range of SRT of 5 to 15 days. However, reducing the SRT to 3.5 days resulted in the loss of EBPR activity of the system.
- The influence of the anaerobic phase and SRT can be used together as a strategy to obtain biomass with high levels of PHA. In this study, it was observed that the

PHA content of the biomass decreased from 9.80 to 3.15 mmol C/g VSS when the SRT increased from 5 to 15 days. The biomass harvested at the end of the anaerobic phase contained 45% more PHA than the biomass harvested at the end of the aerobic phase.

- Methane production was correlated with the PHA content, following a fairly approximate linear trend (BMP = 240 + 15.3 * PHA), independently of SRT and the anaerobic or aerobic extraction. BMP = 401 ml $CH_4$/g VSS was obtained with the biomass with the highest PHA content (9.80 mmol C/g VSS), while BMP = 246 ml $CH_4$/g VSS was obtained with the biomass with the lowest PHA content (0.90 mmol C/g VSS).

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# CHAPTER 8

# General conclusions

The main conclusions that can be drawn from this PhD thesis are:

- The long-term operation of a SBR-EBPR system oriented to obtain a P-enrich supernatant (suitable to be used in a P recovery process) is feasible. This system is a modification of the conventional EBPR configuration, which consist of adding a sedimentation and extraction phase at the end of the anaerobic period. The P recovery efficiencies achieved with this system were around 60%, i.e. more than half of the P could be extracted without a deleterious effect on EBPR activity. A key point for the success of this system is to extract the adequate amount of liquid, in this case 10% of the total work volume. However, when the volume of extraction was increased to 15 %, EBPR activity deteriorated.
- The conventional EBPR process can be operated with SRT values lower than those traditionally implemented at full scale systems (SRT = 10 days), this with the objective of improving its energy efficiency (integrating it in the A/B system). To ensure long-term permanence of PAO, which is related to the stability of biological P-removal, the minimal value of SRT must be around 4 days, since SRT values of less than 4 days lead to instability and/or loss of the EBPR activity. For example, when the system was operated at SRT = 3.6 days, EBPR functions could only be maintained for almost two weeks. The indicators that reflect the loss of EBPR activity are the low P removal efficiencies, the increase of the VSS/TSS ratio and the decrease of the P/C ratio. On the other hand, the lower the SRT, the higher the biomass yield.
- SRT and temperature are factors that are directly related, therefore, need to be taken into account while studying the feasibility of integrating the EBPR process to the A-stage system. We observed that EBPR could be maintained at a SRT of 5 days and at T of 20 ºC, achieving good P-removal efficiencies. However, when the temperature was lowered to 15 ºC, the EBPR performance was unstable. The situation worsened when the system was operated at 10 ºC. Although with the 5-day SRT it was possible to achieve an efficient EBPR process, temperatures below 20 ºC would imply increasing the SRT to values of up to 15 days in order to maintain this efficiency. This would complicate the integration of the EBPR process into the A-stage system.
- The biomass of short-SRT systems have a higher PHA concentration compared to that of long-SRT systems. Consequently, the higher PHA concentration in biomass the higher methane yield. For example, the biomass obtained from a SRT of 5 days

contained three time more PHA than the obtained from a SRT of 15 days. When these biomasses were anaerobically digested, it was observed that the highest production of methane was obtained with the biomass with the highest PHA content (SRT of 5 days). While, the lowest production of methane was obtained with the lowest PHA content (SRT of 15 days).

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| **A/O** | Anaerobic/aerobic configuration |
| **A2O** | Anaerobic/Anoxic/Aerobic configuration |
| **ATU** | Allylthiourea |
| **BMP** | Biochemical methane potential |
| **COD** | Chemical Oxygen Demand |
| **CAS** | Conventional Activated Sludge System |
| **DF1** | *Defluviicoccus vanus* cluster 1 |
| **DO** | Dissolved Oxygen |
| **DPAO** | Denitrifying Poly-phosphate Accumulating organisms |
| **EBPR** | Enhanced Biological Phosphorus Removal |
| **EDTA** | Ethylenediaminetetraacetic |
| **FID** | Flame Ionisation Detector |
| **FISH** | Fluorescence *in situ* Hybridization |
| **GAO** | Glycogen Accumulating Organism |
| **GC** | Gas Chromatography |
| **MAR** | Micro autoradiography |
| **HCl** | Hydrochloric Acid |
| **HRAS** | Hight Rate Activated Sludge System |
| **HRT** | Hydraulic retention time |
| **NaHCO3** | Sodium Hydrogen Carbonate |
| **NaOH** | Sodium Hydroxide |
| **PAO** | Phosphorus accumulating organism |
| **PAO I** | Accumulibacter cluster I |

| | |
|---|---|
| **PHA** | Polyhydroxyalkanoates |
| **PHB** | Poly-hydroxybutyrate |
| **PHV** | Poly-hydroxyvalerate |
| **PH2MV** | Poly-hydroxy-2-methylbutyrate |
| **PLC** | Program Logic Control |
| **Poly-P** | Poly-phosphate |
| **P-uptake** | Phosphorus uptake |
| **P-removal** | Phosphorus removal |
| **RNA** | Ribonucleic Acid |
| **SBR** | Sequencing batch reactor |
| **SRT** | Solid retention time |
| **TSS** | Total suspended solids |
| **UCT** | University of Cape Town |
| **VFA** | Volatile Fatty Acid |
| **VSS** | Volatile suspended solids |
| **WWTP** | Wastewater treatment plant |
| **Yobs** | Observed Yield |