LEUPHANA
UNIVERSITÄT LÜNEBURG

DOCTORAL THESIS

Doctorate in Natural Sciences - Dr. rer. nat.

"PHOSPHORUS DESORPTION AND SORPTION PROCESSES IN ACTIVATED SLUDGE SAMPLES WITH CAPACITY TO PERFORM EBPR PROCESSES"

DATE: 17 DECEMBER 2014

REVIEWERS:

1. Prof. Dr. Brigitte Urban
   Professor of Biology and Soil Science
   Institute of Ecology (IE)
   Leuphana University

2. Prof. Dr.-Ing. Artur Mennerich
   Vice Dean
   Faculty of Civil and Enviromental Engineering
   Ostfalia – University of Applied Sciences

3. Dr.-Ing. habil. Christine Helmer-Madhok
   Research Associate
   Workgroup Water and Environment
   Hannover University

AUTHOR: Patricia Elizabeth Minaya Bedón

To Elva, Daniel, Andrés and Justino

ACKNOWLEDGMENTS

First, I want to thank God for the faith and strength.

I would like to express my deepest gratitude to my advisor, Prof. Dr. Brigitte Urban for the opportunity to perform this research under her supervision and her willingness to support me in all aspects.

The present study was performed in the installations of the Bau-Wasser-Boden Fakultät – Ostfalia Universität – Campus Suderburg, under the co-supervision by Prof. Dr.-Ing. Artur Mennerich. I would like to express my warmest thanks to the entire SiWaWi team, to Prof. Mennerich for the freedom and support he granted me, to Dipl.-Geol. Ingeborg Joost, Dipl.-Ing. Pamela Holweg, Patrick Stohri and Dipl.-Ing. Sacha Ogassa, for the friendship and the continuous support. I will take you always with me, my “Lab Family”

I wish to express my thanks to Dr. Manfred Schütze – ifak Magdeburg, for his continuous interest in my research work, for the interesting discussions and fruitful comments on my thesis.

To Karin Dziarski for her continued support, interest in my work and friendship. Her guide was invaluable for me and other doctoral students.

The analysis in the chemical laboratory would not have been possible without the assistance of Sabine Henschel and Kerstin Lammers. I would want to thank them for this valuable support.

I would also like to thank to Dipl.-Ing. Katharina Mey from the Labor für Material design und Werkstoffzuverlässigkeit - Hochschule Osnabrück, for providing me with the facilities being required to perform the electron microscopy analysis. I would want to thank her for the interest and participation in my research study.

Last but not least, to my parents for having unchanging faith in me, to my big family and friends for giving me a reason to laugh and make the work more bearable, to Elena, Pepe, Carmencita, Emilio, Silvia, Cecilia y Rosa. To my friends in Germany for the patience and friendship, to Vanessa, Yan Yan, Maria and Christian.

ZUSAMMENFASSUNG

Die Entfernung von Phosphor aus Abwasser bleibt ein Forschungsthema, das in Zukunft nur an Wichtigkeit gewinnen kann. Das wird durch die Umweltauswirkungen der Eutrophierung und den Verlust eines essentiellen Nährstoffes für die Nahrungsmittelproduktion dessen Knappheit immer offensichtlicher wird, immer deutlicher. Auf Kläranlagen werden heute hauptsächlich zwei Techniken verwendet um Phosphor zu entfernen, biologisch aktive Verfahren wie das Enhanced Biological Phosphorus Removal (EBPR) Verfahren und Fällungstechniken unter Verwendung von Metallsalzen. Bei beiden Methoden gibt es gegenwärtig Schwierigkeiten wie z.B. die Instabilität des EBPR Prozesses wegen des Mangels an Wissen über die Grundlagen des Stoffwechselprozesses. Bei der Verwendung von Fällungsmitteln kommt es zu vielen Nachteilen im Zusammenhang mit der Nachbehandlung des Schlammes, bei der Entsorgung kommt es zu dem Verlust der Schlammmassen und damit auch des Phosphors aus dem Nährstoffkreislauf.

Die vorliegende Arbeit entstand also aus dem Bedarf nach einer nachhaltigen Methode der Phosphorentfernung aus Abwasser bzw. des Überschussschlammes der in der Siedlungswasserwirtschaft anfällt. Beim Sorptionsprozess kam Belebtschlamm als Substrat zum Einsatz. Das erste Ziel der Untersuchungen war verschiedene Bakteriengemeinschaften mit erhöhtem Potential für effiziente EBPR Prozesse zu identifizieren und herzustellen. Anschließend war das nächste Ziel die Beurteilung der Leistungsfähigkeit der Bakterienmassen den Phosphor zu sorbieren und zu desorbieren. Schließlich fokussierte sich die Arbeit auf das dritte Ziel, die Bewertung der Möglichkeiten die Sorptionskapazität für Phosphor verschiedener Schlämme zu erhöhen. Die verschiedenen Belebtschlämme wurden dabei vorherigen Ionenaustauschverfahren unter Verwendung von Kaliumchlorid, Calciumchlorid und Magnesiumchlorid Lösungen ausgesetzt.

Die verschiedenen Bakteriengemeinschaften die in der Lage sind EBPR Prozesse zu entwickeln, wurden in sequentiellen Batch-Reaktoren mit: Trockensubstanz-Konzentrationen von 1-4 g/l, hydraulischer Verweilzeit (HRT) von 24 Stunden und einer mittleren Verweilzeit (MCRT) von 15 Tagen betrieben. Die Dauer der Betriebszyklen betrug 8 Stunden und die Betriebsparameter der Reaktoren wurden auf Basis verschiedener Zulaufkonzentrationen variiert. Der Zulauf variiert entsprechend der verwendeten Kohlenstoffquelle (Glucose, Mischung von Volatile Fatty Acids und Propionsäure) und entsprechend unterschiedlicher C:N:P Anteile (18.1: 1.9: 1; 37: 2.5: 1; 45.2: 4: 1). Unter diesen Bedingungen wurden zwei große Bakteriengemeinschaften erhalten, eine gemischte Gemeinschaft aus verschiedenen Morphotypen (bakterielle

Gemeinschaft EBPR) und eine bakterielle Gemeinschaft dominiert von dem Morphotyp "Tetrad Forming Organisms" (TFO). Mit Neisser und PHA Färbetechniken wurde es möglich in beiden bakteriellen Gemeinschaften die typischen Speicherpolymere für bereits bekannte EBPR Systeme zu identifizieren. Darüber hinaus wurde bestätigt dass es sich um den erwarteten EBPR Prozess in beiden Bakteriengemeinschaften handelt in dem beiden Bakteriengemeinschaften eine gute Phosphor Entfernung von 90% nachgewiesen werden konnte.

Nach der Bestätigung des ordnungsgemäßen Funktionieren des EBPR Prozesses in den Belebtschlammproben wurden Phosphor Desorptionsprozesse mit Hilfe der Batch Equilibrium Methode eingeführt. So, wurde jeder Schlammprobe in einem Verfahren zur Vor-Stabilisierung eine Lösung aus $CaCl_2$ und $MgCl_2$ zugegeben, um anschließend die Desorptionsphase zu starten, wobei als Elutionsmittel Zitronensäure, Schwefelsäure und Chlorwasserstoffsäure benutzt wurden bis die Desorption bei pH-Werten von 2, 3, 4 und 5 erreichet wurde. Zusätzlich wurde ein Ionenaustauscher als Elutionsmittel (KCl) mit Konzentrationen von 20, 30, 40 und 50 mmol/l in Mischflüssigkeit eingesetzt. Die besten Ergebnisse von Phosphordesorption wurden unter Verwendung von Zitronensäure als Elutionsmittel erhalten, bei einem pH-Wert von 3 und mit der EBPR Bakteriengemeinschaft. Nach Abschluss der Desorption wurden die Schlammproben wieder stabilisiert um später als Sorbens für Sorptionsverfahren eingesetzt werden zu können.

Für die Sorptionsversuche wurde ein spezifisches synthetisches Abwasser mit einer gegebenen Konzentration von Phosphor als Adsorbat verwendet. Die Ergebnisse zeigten positive Sorptionsraten in den Reaktoren, die Gegenstand eines früheren Desorptionsverfahrens unter Verwendung von Kaliumchlorid als Elutionsmittel mit einer Konzentration von 30 mmol/l waren. Auch, die Reaktoren die unter Verwendung sauer Elutionsmittel ein Desorptionsverfahren durchliefen, zeigten während der Experimente in der wässrigen Phase ein erhöhtes Potential Phosphor aufzunehmen. Die Schlammproben mit den höchsten positiven Sorptionsraten wurden in der bakteriellen Gemeinschaft erreicht die TFO's bilden. Im Vergleich zu dem Belebtschlamm der kein vorheriges Desorptionsverfahren durchlief, konnte eine erhöhte Fähigkeit zur Phosphorbindung festgestellt werden, sie betrug während der Sorptionsphase in den Schlammproben der TFO-bakteriellen Gemeinschaft bis zu 45,7%, während die EBPR bakterielle Gemeinschaft ihre phosphorbindende Fähigkeit bis zu 21.7% erhöhen konnte. Beide Male wurde Kaliumchlorid als Elutionsmittel in dem vorangegangenen Desorptionsversuch benutzt.

Das Ergebnis dieser Forschung ist, dass es möglich ist, die Phosphorspeicherkapazität von Belebtschlamm zu erhöhen wenn dieser spezifische Anforderungen erfüllt. Diese Anforderungen werden wie folgt zusammengefasst: Die Schlammmasse muss in der Lage sein, EBPR Prozesse zu entwickeln, auch muss der Schlamm aus einem Belebungsbecken-System kommen, weil die Schlammflocken unter den Umgebungsbedingungen des Reaktors stabilisiert worden sind. Schließlich muss der Belebtschlamm aus einem Verfahren kommen, bei dem man für die Phosphorentfernung keine Metallsalze verwendet.

Die erhöhte Phosphorstoffspeicherkapazität der Belebtschlammmassen in Verbindung mit der Möglichkeit den Phosphor aus dem Belebtschlamm über Rücklösung in die wässrige Phase wieder gewinnen zu können, bietet großes Potential in der Zukunft einen in der Abwasserwirtschaft geschlossenen Phosphorkreislauf zu entwickeln und so den Verlust des wichtigen Nährstoffes nachhaltig zu verhindern.

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A%	Phosphorus sorption percentage
A/O	Two – stage high rate PHOREDOX system
A_2O	Three – stage BARDENPHO system
AcCoA	Acetate acetyl CoA
Anammox	Anaerobic Ammonium Oxidation
BOD_5	Biochemical Oxygen Demand
c(S)	Total concentration of Volatile Fatty Acids (VFA) in mmol/l
C:N:P	Carbon : Nitrogen : Phosphorus ratio
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EBPR	Enhanced biological phosphorus removal
ED	Entner-Doudoroff pathway
EDX	Energy Dispersive X-ray Spectroscopy
EMP	Embden-Meyerhoff-Parmas pathway
F/M	Food to Microorganism Ratio
$FADH_2$	Reduce Flavin Adenine Dinucleotide
GAO	Glycogen Accumulating Organism
GL	Gigaliter
HRT	Hydraulic retention time
IFDC	The International Fertilizer Development Center
K_d	Distribution coefficient
K_{des}	Apparent desorption coefficient
LPO	Lactic Acid Producing Organisms
MCRT	Mean Cell Residence Time
Mg	Megagram
MLVSS	Mixed Liquor Volatile Suspended Solids
Mt	Megatonne

$NADH_2$	Reduce Nicotinamide Adenine Dinucleotide
NMR	Nuclear Magnetic Resonance
OLR	Organic Loading Rate (mg COD/l.cycle)
PAO	Polyphosphate Accumulating Organisms
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PMF	Proton Motive Force
P_{tot}	Total phosphorus concentration
RAS	Return Activated Sludge
RBCOD	Readily Biodegradable COD
R-D-N	Regeneration-Denitrification-Nitrification process
SBR	Sequencing Batch Reactor
SEM	Scanning Electron Microscopy
SHARON	Single Reactor System for High Rate Ammonium Removal over Nitrite
SLR	Sludge Loading Rate
SRT	Sludge Retention Time
SVI	Sludge Volume Index
TCA	Tricarboxylic Acid Cycle
TFO	Tetrad F**o**rming Organisms
TFO	Tetrad Forming Organism
TS	Total Solids
TSS	Total suspended solids
UCT system	University of Cape Town System
VFA	Volatile Fatty Acids
VFAc	VFA composition
VSS	Volatile Suspended Solids
WWTPs	Wastewater Treatment Plants

CHAPTER 1: Introduction

Phosphorus from mined phosphate rock is a crucial resource in the world today. Agricultural activities all around the world depend on the industrial production of synthetic fertilizers and, therefore, human food production relies on mined phosphorous (Cordell & White, 2011). According to various scholars (Tweeten, 1989; Fixen, 2009; Smit et al., 2009), phosphate rock will be depleted quite soon, estimations for depletion ranging from 2050 to 2100. However, notwithstanding this very negative prospect, human use of phosphorus remains extremely inefficient, due to significant loss of phosphorus during mining extraction, fertilizer production, and crops and livestock production. Cordell et al., (2009) estimates that approximately the loss of phosphorous along the route from mining to food is so large that 80% of the mined phosphorus rock will never be consumed as human food. Similarly, according to Naidu et al., (2012), only 20% of the phosphorus supplied to agriculture is transferred into agricultural products. In addition to the urgent attention that the high risk of phosphorus scarcity demands, the environmental problems caused by phosphorus loss in effluents, which seriously affects the stability and equilibrium of the natural water bodies, is also a very large problem. Furthermore, eutrophication due phosphorous in water bodies may adversely affect potable water treatment making this process more difficult and more expensive.

The nutrient removal processes used in the wastewater treatment plants (WWTP) may represent an accessible and suitable possibility for avoiding discharge of effluents with high phosphorus concentration into natural water bodies. At the same time, these processes may offer the possibility of significant phosphorus recovery. According to Cordell et al., (2009) and Rittmann et al., (2011), approximately 15% of mined phosphorus passes through WWTPs. Thus, WWTPs represent a phosphorus source that has a high potential to meet the agricultural demand for fertilizers. The methods currently applied in WWTPs to remove phosphorus are focused on biological processes such as enhanced biological phosphorus removal (EBPR) and phosphorus precipitation using metallic salts. The EBPR process can be considered as an environmentally and economically optimal process for phosphorus removal since it is based only on a metabolic process. The EBPR process is able, through the development of a specific bacterial community, to uptake more phosphorus than needed to cover the bacterial growth process. According to Stensel (1991), approximately 1.5 - 2% of the sludge dry mass in activated sludge consists of phosphorus that was assimilated for normal bacterial growth requirements, but in EBPR bacterial communities 3 - 6% of the sludge dry mass may be phosphorus. This

bacterial community, called the phosphorus accumulating organism (PAO), is responsible for the excess phosphorus uptake that creates the possibility of treating effluents to have a total phosphorus concentration as low as 0.1 mg/l. However, despite the benefits of the EBPR process, this system can become unstable and it may unpredictably lose its phosphorus removal performance, a possibility which remains unexplained in the literature (Seviour & Nielsen, 2010). Phosphorus precipitation using metallic salts is a complementary method used to compensate possible bad functioning of the EBPR process. Thus, EBPR-WWTPs also often utilize phosphorus precipitation methods using compounds of calcium, magnesium and iron (Tchobanoglous, Burton, & Stensel, 2003). However, phosphorous precipitation using metallic salts is not an unproblematic solution.

The use of metallic salts in phosphorus removal processes in WWTPs have several drawbacks. These include the costs of the chemical compounds that must be used, the high amount of sludge produced, the damage to subsequent bacterial growth because of shortage of phosphorus, and the reduction of the biological treatment capacity due to the increase of the inorganic fraction among others (van Haandel & van der Lubbe, 2012). A further important disadvantage of chemical precipitation relates to the reuse of the sludge mass in agricultural processes since, after phosphorus precipitation as a salt, the availability of the phosphorous as fertilizer is limited (Donnert & Salecker, 1999).

On the other hand, the phosphorus removed in an EBPR process not only corresponds to the phosphorus uptake through metabolic process but also to the sorption process into the exopolymeric substances (EPS) of the floc. According to Cloete & Oosthuizen (2001), 30% of the phosphorus content in the sludge biomass corresponds to the EPS media. Additionally, several investigations have recorded that, although the tetrad forming organisms (TFO) commonly present in the EBPR bacterial community does not store intracellular polyphosphate, their cell envelope (EPS) has recorded a positive staining reaction for polyphosphate in some cases (Cech & Hartman, 1990; Blackall, et al., 1997; Sudiana, Mino, Satoh, & Matsuo, 1998).

The present research is interested in the sorption process in activated sludge masses for the phosphorus removal process of wastewater. To date, information about the sorption process in activated sludge is limited, and almost completely non-existent for specific bacterial communities.

This work does take into account more generalized information such as, for example: the structure and composition of the EPS and the strengths involved in the sorption

process on activated sludge samples (Müller, 2006; Grupo de Investigación de Recursos Hídricos - IUPA, 2009), and also the factors involved in the ion exchange processes (Grupo de investigación de recursos hídricos – IUPA, 2009). With this information it was possible to develop an idea of the chemical structure of the exposed surfaces in the floc and about the floc's superficial charges. Additionally, investigations on the role of calcium ions in floc stability have shown that even small changes in calcium concentration may result in the desorption of organic molecules and the disintegration of the floc (Keiding & Nielsen, 1997).

Estuarine ecosystems are useful to study the ion exchange dynamic and the effects that a substrate could show after facing constant processes of ion exchange. This research therefore also looks at these processes in an estuarine ecosystem. In an estuarine ecosystem, the constant ion exchange of Ca^{+2} and Na^{+} between the sediment (substrate) and the aqueous phase during the high tide phase may slightly destabilize the chemical structure of the sediment surface because of the loss of the calcium ion. Subsequently, during the low tide phase, the sediment surface structure can stabilize again due to the ion exchange process between the calcium bicarbonate facies of the aqueous media and the sodium ion of the sediment surface. In this research, it is assumed that this constant ion exchange may promote the release of active sites in the sediment surface and that these free active sites increase the sorption capacity of the substrate. Research by Wang & Li, (2010) into the phosphorus desorption-sorption capacity of different sediments recorded that estuarine sediment showed the highest phosphorus sorption capacity. The possibility of phosphorus precipitation was also taken into account by Wang & Li, but the estuarine sediment's concentrations of calcium carbonate, iron and aluminum were among the lowest of all sediments.

On the basis of such data, this research proposes that the sorption capacity of the activated sludge may be influenced by the type of bacterial community in the sludge biomass and by sludge environmental conditions such as the pH, aeration and stirring conditions, which can directly affect floc stability. Additionally, it is proposed that higher sorption capacity might be observed in substrates that undergo constant processes of ion exchange with loss and recovery of the calcium ion. Therefore, the possibility of subjecting the sludge samples to similar variations of ion exchange was investigated, but using the potassium ion instead of the sodium ion as the exchangeable cation for the calcium ion.

To test these hypotheses, the first objective was to obtain different bacterial communities able to perform good EBPR processes. To achieve this aim several SBR

reactors, at lab scale, were installed and fed with synthetic wastewater, varying the carbon source used across the different reactors. These carbon sources were glucose, mixed VFA and propionic acid. Additionally, activated sludge samples from the Steinhorst and Lüneburg WWTPs were used. As a secondary objective during this stage, the EBPR stability and performance was evaluated according to the different carbon source and the different C:N:P ratios in the influent.

Subsequently, if good EBPR processes were confirmed in the activated sludge samples, the second objective was to evaluate their phosphorus desorption and sorption capacity using different eluents such as organic and mineral acids and one ion exchanger (KCl solution) during the desorption process. Then, the sludge samples from the desorption process underwent a re-stabilization process using a solution of $CaCl_2$ and $MgCl_2$. Once the sludge samples were re-stabilized, these were used as substrates in the subsequent sorption process and a synthetic wastewater volume containing a specific concentration of phosphorus (adsorbate) was used as contact solution.

The third objective was the evaluation of the influence that the previous desorption process using potassium chloride as eluent might have on the phosphorus sorption capacity of the activated sludge samples. Therefore, after finishing the desorption–stabilization–sorption process, the total phosphorus content in the sludge masses and in the aqueous phase was assessed, enabling a comparison of which sludge samples had increased their phosphorus sequestration capacity at the end of the sorption process compared to their initial phosphorus concentrations and in relation to the control reactors.

The possibility of increasing the phosphorus sequestration capacity of the activated sludge by biological means is of great interest. The benefits of increasing the capacity of the sludge to retain phosphorus are related to the possibility of reusing these sludge masses (which are free of metallic salts) as a nutrient source in agriculture and, additionally, to the possibility of reducing the cost of the treatment, disposal and incineration processes due to the high phosphorus concentration and low sludge volumes.

CHAPTER 2: Objectives

The information concerning the phosphorus sorption capacity of the activated sludge is not abundant or even non-existent for specific bacterial communities.

There are some factors that may influence the sorption capacity of the activated sludge biomasses. Among these factors the physicochemical characteristics of the influent and the wastewater treatment applied would determine the bacterial community in the activated sludge. In turn, this investigation proposes that the type of bacterial community and the environmental conditions of the activated sludge masses would influence their phosphorus sorption capacity.

To test these hypotheses the following objectives were determined:

1. First objective: To obtain different EBPR bacterial communities according to the use of different synthetic wastewater. The variations of the wastewater composition are related to the carbon source and to the C: N: P ratio used. Additionally, other activated sludge samples from WWTPs are evaluated.

   To achieve the first objective, SBR lab scale reactors were installed. The experimental process was developed in several phases with the following secondary objectives:

   1.1 Secondary objective 1: To obtain effluents with high concentration of volatile fatty acids (VFA) through the use of one SBR anaerobic reactor using glucose as carbon source.

   1.2 Secondary objective 2: To confirm the presence of the EBPR process in the different activated sludge samples using staining techniques and the EBPR performance results.

   1.3 Secondary objective 3: To assess the influence of the different carbon sources and C:N:P ratios in the influent on the EBPR performance.

2. Second objective: To assess the phosphorus desorption and sorption capacity of the activated sludge samples.

   To assess the phosphorus desorption capacity of the activated sludge samples, different types of eluents were used with determined concentrations of total suspended solids (TSS) in batch reactors. Subsequently, each sludge sample underwent a re-stabilization process using a $CaCl_2$ and $MgCl_2$ solution. Finally, the re-stabilized activated sludge samples were used as substrate in sorption

processes where the synthetic wastewater contained a specific concentration of phosphorus considered as the adsorbate.

3. Third objective: To assess the influence of a previous desorption process using potassium chloride as eluent on the phosphorus sorption capacity of the activated sludge samples.

3.1 Secondary objective 4: To determine if the phosphorus sorption capacity of the activated sludge samples are influenced by the bacterial community structure and/or by the previous environmental conditions that the sludge samples faced. The previous environmental conditions of the activated sludge samples are determined by:

- The potassium chloride concentration used during the desorption process.
- The environmental conditions as pH, aeration and stirring conditions that the activated sludge samples faced in their origin reactors.

CHAPTER 3: State of Knowledge

3.1 Phosphorus in aquatic systems

Phosphorus (Atomic mass = 30.974) is a chemical multivalent element which because of its high reactive condition is not be found free in nature (Canadian Council of Ministers of the Environment, 2004). The fundamental reserves of phosphorus are located in the Earth's crust in the form of, for example apatite, a mineral contained in phosphate rock. Phosphorus becomes available for plants through rock weathering or through extraction of volcanic ash. Subsequently, the phosphorus would be leached and dumped into the sea where part of the phosphorus would settle to the bottom to form rocks, from which, it will take millions of years to its phosphorus content reach again the surface of the earth (Filippelli, 2002).

Phosphorus is an essential component of the organisms. Organisms contains about 0.3% of dry weight as phosphorus (Horne & Goldman, 1994) that forms part of the nucleic acids (DNA and RNA); ATP the principal stock of chemical energy; phospholipids and other organic molecules. As an example, in plants a phosphorus proportion of 0.2% (Miraj, Shah, & Arif, 2013) of dry mass is mentioned while in animals this proportion can increase up to 1% (Vitti & Kebreab, 2010). The little amount of phosphorus present in the organisms is not proportional to its importance since when comparing phosphorus with the other macromolecules in living organisms, phosphorus is the least abundant but almost always the first to limit biological productivity (Wetzel, 2001)

3.2 Forms of phosphorus in aquatic systems

It can be generalized that unimpacted water bodies contain low phosphorus concentrations that may range from < 1 ug/l in ultra-oligotrophic waters, to >200 ug/l in highly eutrophic waters with an average between 10 and 50 ug/l as total phosphorus (Wetzel, 2001).

The phosphorus in aquatic systems is present in two principal forms: Organic and inorganic phosphorus. More in detail, inorganic phosphorus is not associated with a carbon based molecule while the organic phosphorus can be subdivided in particulate organic phosphorus and dissolved organic phosphorus (Canadian Council of Ministers of the Environment, 2004). Among all these forms of phosphorus, the most significant inorganic phosphorus form is present as orthophosphate ions (PO_4^{3-}) and is the only

form of soluble inorganic phosphorus directly utilized by aquatic primary producers. However, in fresh water up to 95% of the phosphorus is present as organic phosphate and included or adsorbed as part of inorganic matter or organic dead matter.

Because of anthropogenic activities, the input of phosphorus into water bodies may increase and this fact may adversely affect the aquatic ecosystems (Chambers, et al., 2001). When a water body receives an input of phosphorus that exceeds the normal phosphorus concentration the first response to this addition is the increase of plants and algae productivity causing an eutrophication problem. Generally, the source of this excess of phosphorus input are the secondary wastewater treatment methods because the increase amount of the urbanized human population. For example, the use of polyphosphate-based detergents has contributed to the problem (Reynolds and Davies, 2001), the industrial effluents and runoff from fertilizers and manure spread on agricultural areas (Ansari *et al.*, 2011).

3.3 Problems with phosphorus excess in aquatic systems

Through the eutrophication the decomposition of the primary producers consume the dissolved oxygen (DO) and because the oxygen depletion the aerobic organisms will decrease in concentration. Simultaneously, as the death rate of the aerobic organisms increase the oxygen consumption will increase so far than the oxygen concentration becomes limiting. At this point the water bodies would change their condition from aerobic to anaerobic. These environmental changes may cause adverse effects such as the emergence of a dominant organism therefore the biodiversity index would decrease. Additionally, the replacement of indigenous organisms for other more tolerant and the high amount of organic matter in decomposition would increase the amount of sediments and simultaneously the turbidity (Mason, 1991). Finally, under anaerobic conditions the bloom development of cyanobacteria is reported and at the same time the risk of toxic compounds that may cause severe effects to the organisms (Ansari, Singh Gill, Lanza, & Rast, 2011).

Directly related to human quality life, the eutrophication may affect adversely the potable water treatment making this process more expensive and difficult. The recreational use of eutrophic water is affected also because taste, health or odor problems (Van Horn, et al., 1991) and some species of fishing interest could be displaced from their ecosystems (Mason, 1991).

On the other hand, phosphorus as chemical element is not considered toxic for aquatic organisms as well as for us in concentrations and forms normally found in natural environments therefore the limitations for this element may not be determined as for example for heavy metals. However, the discharge of effluents to water bodies has generally specific limitations of phosphorus concentration trying to minimize the adverse effects aforementioned.

3.4 The problem of phosphorus starvation

Humans are highly dependent today on phosphorus from mined phosphate rock. Although a plentiful source of highly concentrated phosphorus apparently exists, phosphorus is still being a non-renewable resource. Phosphate rock as the principal source of phosphorus for the fertilizer manufacturing has a renewable cycle between the lithosphere and hydrosphere that takes a long time of “millions of years” therefore is considered non-renewable. The economical importance of phosphorus is better understood when is taken into account that the phosphorus cannot be obtained by synthetic elaboration and that there is no substitute for phosphorus in crop growth, therefore in food production.

The phosphorus consumption has increased further after the Second World War with the industrial production of fertilizers, such a manner that fertilizer use increased six-fold between 1950 and 2000 (International Fertilizer Industry Association - IFA, 2008).

The principal use of the phosphate rock resources is in the agricultural fertilizer industry. The natural biochemical cycle, which recycles phosphorus back to the soil via dead plant matter is severely injured by the mechanisms used in the Industrial Agriculture since they remove all the crops (P source) without leaving residues for the possibility to phosphorus recovery naturally, therefore the continuous need of phosphorus rich fertilizer is understandable. Additionally other consequences of mobilizing excess nutrients into the environment become evident in the past decades as the eutrophication problems in natural water bodies (Barnard, Elimination of eutrophication through resource recovery, 2009).

In this chapter the actual and future scarcity of phosphorus is analyzed. To determine a scarcity problem it is necessary to determine the total amount of resource available to be used. There are some problems to determine the remaining time of phosphate rock availability from natural sources for meeting the future demand. According to Cordell & White, (2011) in the earth crust should be around 4,000,000,000,000,000 tons P, thus, geochemically this element may not be considered in scarcity situation. The phosphorus scarcity problem is related to the depletion of high-concentration

phosphate rock reserves and the economic and energetic barriers of their exploitation. The International Fertilizer Development Center (IFDC) estimate the phosphate rock reserves in 60 tons x 10^9 (U.S.Billion) as product (Van Kauwenbergh, 2010) and the US Geological Survey taking into account this last estimation updated their reserves for Morocco from 5600 to 51000 Mt of phosphate rock (85% of the phosphate rock reserves) considering that this estimations are preliminary (Van Kauwenbergh, 2010; Global Phosphorus Research Initiative - GPRI, 2010).

3.4.1 Mismanagement of the phosphorus resource

As was already mentioned the phosphate rock reserves are the principal source of phosphorus but because the extremely inefficient use of the resource the losses of it are giving a final amount of phosphorus consumed as food of up 3 x 10^6 tones p/year (Cordell & White, 2011).

Phosphorus loss begins already during mining and fertilizer production where approximately 30 – 40% of phosphorus can be lost during extraction and primary processing (Cordell & White, 2011). Meanwhile, much of the phosphorus used in cropping and livestock systems will remain in the soil and in manure respectively. Additionally, according to Cordell, Drangert, & White, (2009) approximately 80% of mineral bond phosphorus rock will never be consumed as human food and according to (Naidu, Lamb, Bolan, & Gawandar, 2012) only 20% of the phosphorus supplied to agriculture is transferred into agricultural products, of which, only 8% of this would be found in municipal wastewaters. The phosphorus loss from wastewater is proportionally low to other areas compared to mining and agricultural activities but the phosphorus recovery from a WWTP is possible and accessible. For some countries with evident water scarcity the reuse of wastewater provides an option of water and nutrients for agricultural uses. As examples of the possibility to cover the phosphorus agricultural demand with the phosphorus recovered from a WWTP, the Australia and New Zealand cases are presented. In Australia, approximately 3300 GL of domestic effluent are discharged into surface waters per year. The total mass of phosphorus contained in this effluent is 5 x 10^6 Mg P. In comparison the total phosphorus consumption of fertilizers is 0.28 x 10^6 Mg P thus the amount of phosphorus contained in the domestic effluent would be able to cover the agricultural demand 20 times. Meanwhile, in New Zealand yearly approximately 550 GL of domestic effluent is discharged into surface waters and the total mass of phosphorus contained in this effluent is 0.4 x 10^6 Mg P. Similarly to the last case, the total phosphorus consume by fertilizers is 0.04 x 10^6 Mg P, thus the amount of phosphorus contained in the domestic effluent would cover also

the agricultural demand 10 times. These calculations are very simple but to recover the phosphorus contained in the effluents it is a continuous matter of investigation that means to improve the wastewater treatment performance. However, these calculations present the possibility to cover the agricultural phosphorus demand of some countries by recycling the phosphorus from the WWT systems (Naidu, Lamb, Bolan, & Gawandar, 2012)

The demand of phosphorus is now stabilized in Europe and North America because decades of fertilizer over-applications to soils but the demand in developing countries and emerging economies would still increase thus the supply of high grade phosphate rock would be constrained in the future. By dividing the total phosphate rock reserves by the average annual consumption the lifetime of the phosphorus reserves could be estimated. According to Tweeten, (1989); Fixen, (2009) and Smit, Bindraban, Schröder, Conijn, & van der Meer, (2009) the estimated year of phosphorus depletion would be around 2050 and 2100 assuming in some cases an increase in demand or taking into account the current rate of extraction.

The evident reason for the increase of the phosphorus demand lays in the increasing population growth (approximately 9 billion in 2050), but there are other reasons as the change in the diet preference around the world towards more meat consumption which require more phosphorus fertilizers per capita (FAO, 2006; Cordell, Drangert, & White, 2009) and the increasing demand of crops to be used in biofuels production (International Fertilizer Industry Association - IFA, 2008).

3.4.2 Management and technology approaches to phosphorus recovery

To face the complex problem of phosphorus scarcity an integrated approach should be considered. This approach may include diversifying the source of phosphorus by investigating the production of renewable phosphorus fertilizers and the phosphorus recovery from all sources of loss (manure, wastewater, crop and food residues). Additionally, the reduction of the phosphorus demand by increasing the efficiency of the phosphorus use in mining, agricultural and food chain should be considered.

As already mentioned one of the main phosphorus sources that can cause eutrophication problems in water bodies is the phosphorus content in the urban waste water. Therefore, the environmental impact and the loss of phosphorus would be considerable. According to Cordell, Drangert, & White, (2009) and Rittmann, Mayer,

Westerhoff, & Edwards, (2011), approximately 15% of mined P is estimated to flow through WWTPs (7 % solids, 8% discharged).

To recover the phosphorus from the wastewater the development of new systems and the improvement of the already existing phosphorus recovering mechanisms would be necessary as well. The phosphorus recovery process from a WWTP is performed actually using some technological designs that are based in phosphorus precipitation, use of chemicals and thermal process principally. These technologies can be applied considering different locations in the wastewater treatment system, thus, can be applied in the watery phase, the dewatered sewage sludge phase and the sewage sludge ash phase. Table 1 shows some of these technologies and their efficiency of phosphorus recovery.

Table 3.1 Technologies available for phosphorus recovery. Adapted from the work of Schick, Kratz, Adam, & Schnug, (2009)

Point of P recovery	Volume or mass to treat	Suitable for application	Percentage of P recovery	Technology applied	Specific process
Watery phase Effluent	Very high volume	Not efficient	-	Precipitation	Process Berliner Wasserbetrieb Air Prex (Heinzmann, 2008)
Process water	Much lower volume	Applicable	30 – 45% from P imput	Crystallization	DHV-Crystalactor The Ostara Pearl
Dewatered or dried sewage sludge	Reduced mass to be treated	Applicable	>90% of P influent (10 g P/kg DM)	Wet chemical	Seaborne / Gifhorn Process.
				Crystallization	CSH-Process Darmstadt
				Thermal	Mephrec process
Sewage sludge ash (SSA)	Reduced mass to be treated	Applicable	>90% after monoincine-ration (64 g/kg)	Wet chemical	Sephos process PASH process
				Thermal	BAM/AshDec processElectro thermal P

There are some requirements that the phosphorus obtained from a recovery process should fulfill before usage. Some of the requirements can difficult the reuse of the phosphorus and among these the following chemical characteristics of the phosphorus recovered are the most important as the low phosphorus content in relation to the high amount of heavy metals, the reactivity problems in the wet chemical processes, the

chemical quality stability of the phosphorus and the prove of its agronomic production efficacy. Other requirements are related to the economical viability of the technology applied and the environmental acceptability of this technology. These last two requirements can be limiting factors for the reuse of phosphorus (Schick, Kratz, Adam, & Schnug, 2009)

3.5 Biological wastewater treatment for phosphorus removal

3.5.1 Phosphorus removal from wastewater using activated sludge

The biological phosphate removal was first mentioned by Srinath, Sastry, & Pillai, (1959) in a wastewater treatment process where they observed that the sludge from some treatment plant recorded a higher uptake of phosphorus than the normal for cell growth during the aeration phase. To demonstrate that this higher uptake of phosphorus was a biological process the researchers used a metabolism inhibitor. Later this process was called enhanced biological phosphorus removal (EBPR). Subsequently, Barnard, (1976) introduced the anaerobic/aerobic cycling in the activated sludge system known as Phoredox principle.

The activated sludge methodology consists to obtaining a bacterial community in the form of sludge from a wastewater treatment plant, which is responsible for removing (through metabolic, physicochemical and other processes) the nutrients (carbon, nitrogen and phosphorus) and other substances and compounds present in the influent wastewater. The aim of this treatment is to obtain an effluent with a concentration of these substances which meet the discharge or reuse requirements of the regulations.

The EBPR process, purely metabolic, promotes the development of a special bacterial community that will be able to include in their cytoplasm a greater amount of phosphorus in the form of polyphosphate than other bacterial communities (Stensel, 1991; Mino, Van Loosdrecht, & Heijnen, 1998). The ability to achieve a constant and stable phosphorus removal performance of this community is one of the objectives of the current scientific community, as this process has variations in performance of removal which have not been clarified so far (Jenkins & Hermanowicz, 1991; Neethling et al., 2005)

3.5.2 Wastewater quality – A challenge for wastewater treatment.

The activated sludge systems should be strong. The composition and amount of the influent wastewater represents a challenge for the bacterial community as they need to face different chemical compounds as bioactive farmaceuticals (Ternes, 1998), surfactants (Kraigher et al., 2008), endocrine compounds (Lozada, et al., 2004) and phthalate esters used as plasticizer (Marttinen et al., 2003). Some of this chemical compounds would be adsorbed and/or degraded (Lozada et al., 2004) but other, because their toxic condition, would not be degraded (Byrns, 2001).

These toxic chemicals could affect seriously the performance of the WWTP (Seviour & Nielsen, 2010) therefore monitoring the presence of these compounds is of interest. For example some technologies were developed to identify the presence of stress proteins and to identify an increase in the level of the precursor 16S rRNA in cells exposed to primary effluents. The presence of this precursor would indicate a damage caused by chemicals in the influent or from metabolic products of less hazardous substrates (Stroot & Oerther, 2003). The use of commercial kits that employ specific bacterial strains are able to predict the effect of influent toxic chemicals from industrial wastes in the WWTPs but the frequency of its use is unclear. Other compounds as endocrine disrupters (degradation products of alkylphenol polyethoxylate, natural and synthetic oestrogens) although do not represent a direct problem for the activated sludge process they may affect seriously the organisms development.

After the activated sludge treatment, low but biologically active levels of endocrine disrupters could often be found in the effluent therefore, additional treatment processes as nanofiltration and reverse osmosis would be required to improve the removal efficiency of these chemicals (Ternes et al., 2003).

Heavy metals may represent another challenge to the activated sludge bacterial community as they can be present in domestic and industrial wastewater and in the specific cases of chromium, cadmium, mercury and nickel they can affect their adsorption to the sludge mass and the general performance of the plant (Dilek, Gokcay, & Yetis, 1998; Vankova, Kupec, & Hoffmann, 1999; Yetis, Demirer, & Gokcay, 1999). Additionally, their biocidal effects may affect negatively the respiration process and the nitrification rates (Madoni, Davoli, & Guglielmi, 1999).

3.5.3 Wastewater treatment plants configurations

The next section was prepared considering the publications of Seviour & Nielsen, (2010), and Wiesmann, Choi, & Dombrowski,(2007).

The basic design of the activated sludge plants changed little over the preceding 100 years; the only innovation is the incorporation of more sophisticated instrumentation for the in situ monitoring of the activated sludge process. The aerobic reactor still been a rectangular basin with air diffusers located submersed on it, in the clarifier the solid phase will be separated from the supernatant and this settled sludge will be recycled to inoculate the incoming wastewater (Tchobanoglous, Burton, & Stensel, 2003). Initially the activated sludge system was designed to remove carbonaceous material from domestic wastewater which additionally contained organic compounds and to produce effluents with a BOD_5 concentration low enough to be discharged into natural water bodies. Subsequently, because ammonia (NH_3) was recorded to be toxic for fish its content in the effluent had to be reduced also and the WWTPs were re-designed to improve the nitrification process. Later, concerning about the eutrophication problems in natural water bodies, it became necessary to re-design the WWTP to remove nitrogen and phosphorus by biological means.

The actual designs of the WWTPs comprise the addition of some reactors with the aim to provide different environmental conditions that are suitable for specific bacterial communities. For example, the incorporation of anoxic zones with high concentrations of nitrate are appropriate for denitrification processes and the anaerobic zones provide the appropriate environment to the development of PAO microorganisms since they would not need to compete with other bacterial groups for the carbon source.

The real situation about the phosphorus removal in wastewater treatment plants could be summarized when taking into account that the regulations about the phosphorus content in the effluents are achieved in most countries by applying the technology of chemical phosphorus precipitation with metallic salts and not by improving the design of the biological processes (Seviour & Nielsen, 2010).

Actually, most activated sludge systems run continuously but they can be differentiated from each other from their mixing regimes (Gray, 1990; Grady, Daigger, & Lim, 1999; Tchobanoglous, Burton, & Stensel, 2003). These can be the plug flow or completely mixed systems.

The plug flow system, because of the absence of agitation becomes a problem of irregular load distribution therefore the oxygen demand would be not balanced (Figure

3.1). Some modifications to the plug flow system as the “tapered aeration system” and “the step aeration” were applied trying to distribute the oxygen more efficiently.

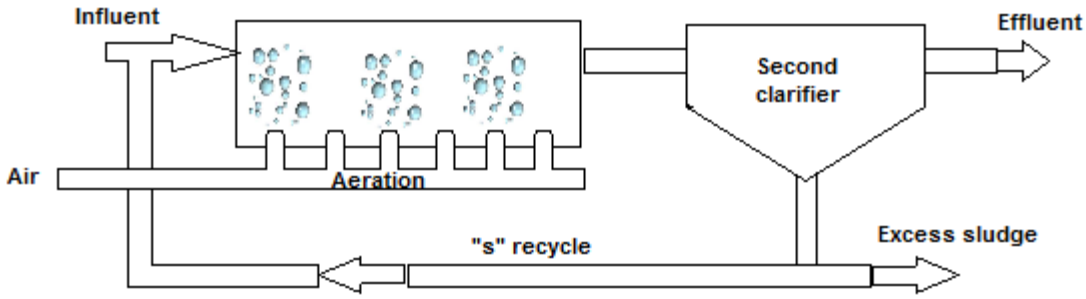

Figure 3.1: General design of the plug flow activated sludge system. The return activated sludge (RAS) is mentioned as “s” recycle.

The completely mixing system performs by using a square or circular reactor where the influent wastewater and the RAS are putting in contact rapidly distributing homogeneously the organic load and the incoming oxygen (Figure 3.2). Subsequently a contact stabilization process was developed by locating a small aerobic reactor which would receive directly the influent and the RAS during one hour (Gujer & Jenkins, 1975 a,b). The removal of the ready biodegradable organic substrate was recorded but not the removal from the slowly degraded particulate matter (Gray, 1989).

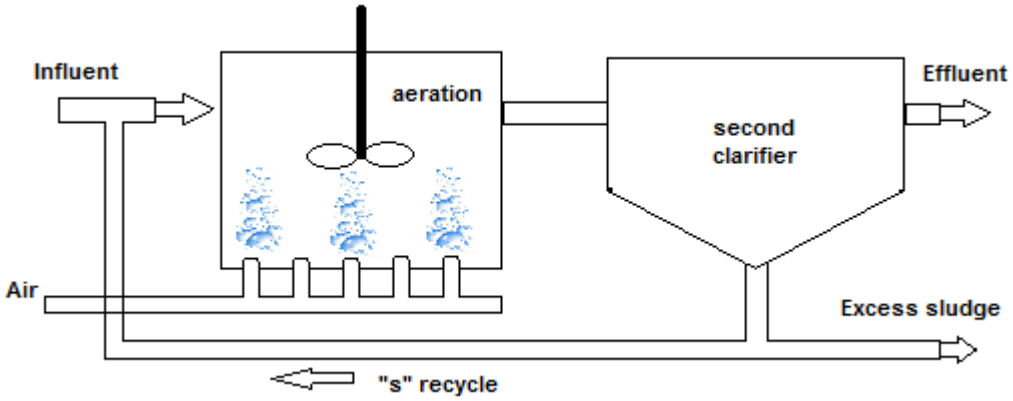

Figure 3.2: General design of the completely mixing activated sludge system.

The extended aeration system consists of a shallow earthen oval ditch with an aeration system which provides also circular mixing (Seviour & Nielsen, 2010). These reactors can operate with low loadings, high suspended solids levels, long aeration periods and sludge ages with which it is possible to achieve a complete oxidation and a better sludge stabilization (Gray, 1989, 1990; Eckenfelder & Grau, 1992; Wanner, 1994). The principal characteristic of this design is that close to the aerator the oxygen

concentration is high allowing nitrification processes while in a distance the anoxic conditions would be appropriated for denitrification processes. The addition of a separate clarifier allowed the system to treat influents with higher loads (e.g. carrousel oxidation ditch).

3.5.4 Wastewater treatment plants configurated for nitrogen removal

Only a little part of the total nitrogen in the influent is removed through sludge production, growth process and wasting. When anoxic and aerobic zones are incorporated in a plant the denitrification and nitrification process may be achieved (Robertson & Kuenen, 1992; Bryan, 1993; Grady, Daigger, & Lim, 1999; Tchobanoglous, Burton, & Stensel, 2003). To achieve nitrification and denitrification processes it is necessary first to count with an aerobic zone for the nitrification process where the chemolithoautotrophic nitrifying bacteria are able to use the CO_2 as a carbon source and the oxygen to oxidize inorganic nitrogen components to obtain energy. As principal genera the *Nitrosomonas* are responsible for the oxidation of ammonium to nitrite (nitritification process) and the *Nitrobacter* for the oxidation of nitrite to nitrate (nitratification process) (Wiesmann, Choi, & Dombrowski, 2007). Further, it is necessary to create an anoxic zone where the nitrate and nitrite may act as electron acceptors for the chemoorganoheterotrophic denitrifying organisms and the organic substrates may act as electron donors of ATP (Adenosine Triphosphate) production. Therefore, the nitrite and nitrate are reduced to gases as NO, N_2O or N_2 (Wiesmann, Choi, & Dombrowski, 2007). All of these requirements were transferred to specific WWTP designs trying to achieve efficient nitrification-denitrification processes. It should be noted here that the denitrification process is performed for a wide group of bacteria and not only under anoxic conditions (Robertson & Kuenen, 1992). Among the wastewater treatment plants configurations designed to remove nitrogen, Wuhrmann, (1957) proposed the addition of an aerobic zone preceding the anoxic zone and also the addition of a complementary carbon source (e.g. methanol) to the anoxic zone (Figure 3.3).

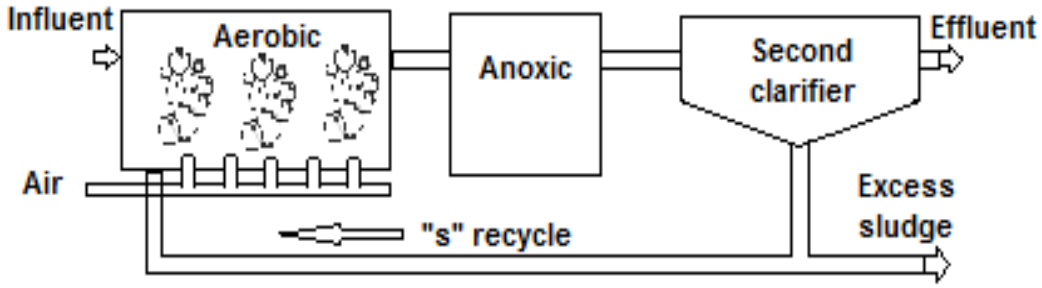

Figure 3.3: The Wuhrmann (1957) activated sludge configuration for nitrogen removal.

Ludzack & Ettinger, (1962) placed an anoxic reactor in front of the aerobic reactor to receive the incoming wastewater. Because most of the organic material was consumed in the anoxic reactor, the remaining organic material available to heterotrophic bacteria in the aerobic zone is lower. Therefore the heterotrophic community was reduced and the competition for the dissolved oxygen also, and hence the nitrification process was increased (Tchobanoglous, Burton, & Stensel, 2003). The mixing of anoxic and aerobic zones was achieved using aerators, but controlling the mixing process was not always possible (Bryan, 1993). Further, the separation of the anoxic and aerobic zones and the recycling of the aerobic and the second clarifier sludge to the anoxic reactor was considered by Barnard (1975 a, 1975 b, 1976). This modification was called modified Ludzack-Ettinger process and can not remove totally the nitrate content as the recirculation from the aerobic reactor may bring back some nitrate (Bryan, 1993).

Later, Barnard designed a four stage system called BARDENPHO PROCESS (Figure 3.4) which consists of an anoxic reactor followed by an aerobic reactor and subsequently a second anoxic reactor followed by a re-aeration reactor. The recirculation masses were taken from the aerobic and the second clarifier reactors to the first anoxic reactor. In the second anoxic and the re-aeration reactors almost all the nitrate may be removed. This system is able to remove also phosphorus.

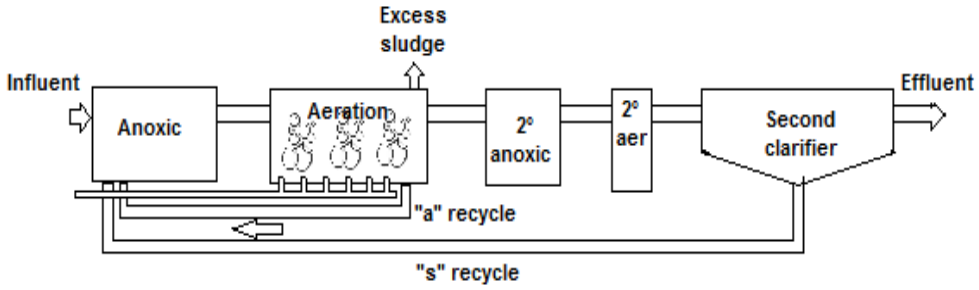

Figure 3.4: The BARDENPHO PROCESS activated sludge configuration for nitrogen removal.

Trying to overcome the limitations of the previous nitrogen removal process the R-D-N process (Regeneration-Denitrification-Nitrification) was developed by Kos et al. (1992).

A contact stabilization reactor for regeneration was added at the beginning of the system (Figure 3.5) where the RAS was re-aerated. With this addition the sludge age of the aeration phase was prolonged and the volume of the nitrification reactor was reduced.

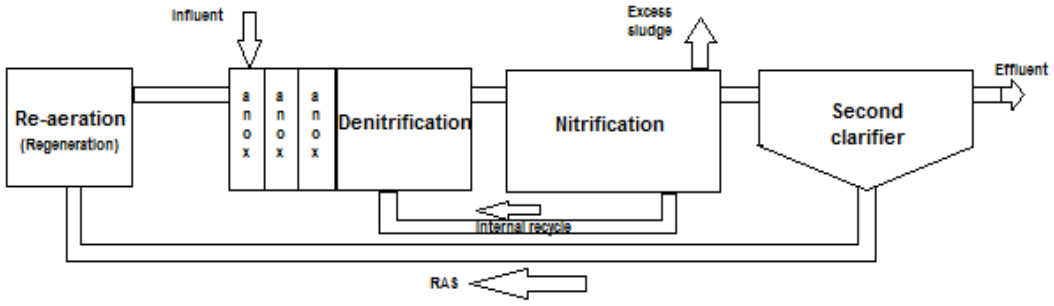

Figure 3.5: The R-D-N PROCESS for nitrogen removal.

Lately, as a result of a joint effort between engineers and microbiologists some new methods were developed for nitrogen removal. The SHARON (Single Reactor System for High Rate Ammonium Removal over Nitrite) was designed to remove nitrogen over nitrite in concentrated wastewater (Van Dongen, Jetten, & van Loosdrecht, 2001). This process is known for not to retain sludge, since the growth and washout of sludge are equilibrated. Additionally, it is important to maintain a low pH or low oxygen concentrations to inhibit nitrite oxidation (Van Kempen et al., 2001). Other strategy to limit the nitrite oxidation is to operate at temperatures higher than 25 °C which promotes the fast growing of *Nitrosomonas* (regeneration time = 8 – 36 hours) in comparison to *Nitrobacter* (regeneration time = 12 – 60 hours) which can be purged to the system.

This process needs less aeration, when nitrite is the end product searched. Therefore, only a partial nitrification occurs and a lower amount of organic substrate would be required since only nitrite would need to be reduced to dinitrogen gas (Schmidt, et al., 2003; Seviour & Nielsen, 2010).

The ANAMMOX (Anaerobic Ammonium Oxidation) process reduces the nitrite to dinitrogen gas under anoxic conditions, where the ammonium (NH_4^+) is the electron donor (Mulder et al., 1995; Helmer, et al., 2001). This process is performed by certain chemolithoautotrophic bacteria that belong to the order planctomycetes. These organisms are autotrophic and converting nitrite into nitrogen gas without using organic carbon since the electron donor would be the ammonium. The low sludge production, the reduction of the energy requirements during aeration (60%), the reduction in the use of chemicals for neutralization and the considerable reduction of the CO_2 emissions makes this process of a great interest since the cost reduction compared to

conventional nitrogen removal systems is considerable (Van Dongen, Jetten, & van Loosdrecht, 2001)

3.5.5 Configuration of wastewater treatment plants for phosphorus removal

3.5.5.1 Metabolic processes in the EBPR system – Metabolic models

Later some of the designs mentioned above were modified to enable the removal of nitrogen and phosphorus simultaneously.

The phosphorus required for bacterial growth and energy source accounts for up to 2% of the total bacterial mass. Meanwhile, through the in excess biological phosphorus removal, some bacterial groups are able to store up to 6% of its dry mass as phosphorus (Stensel, 1991). This in excess phosphorus removal was called Enhanced Biological Phosphorus Removal (EBPR) and its operational requirements started to be elucidated thanks to the efforts of Barnard (Barnard, 1994; Barnard, 1998; Wentzel et al., 1991 a,b). In general terms these requirements can be summarized as follows:

- An initial anaerobic zone which receives the raw wastewater (feed stage). This stage would be appropriated for the development of the polyphosphate accumulating organisms (PAO) since these microorganisms would be able to uptake the carbon source (principally as VFA) under anaerobic conditions, hence, the competition with the heterotrophic community for carbon sources would be limited.
- The aerobic heterotrophic bacteria are not the only competitor of PAO for carbon sources. In presence of nitrate, during the no aerated phase, the denitrifier community is able, as well, to use the VFA as electron donors; therefore, controling the presence of nitrate in the anaerobic zone is necessary.
- Subsequently, it is necessary to count with an aerobic zone (famine stage) where the phosphorus is assimilated as intracellular polyphosphate chains. The amount of phosphorus uptaken during the aerobic phase exceeds the amount of phosphorus released during the anaerobic phase. The anaerobic – aerobic cycles should be alternated to promote the development of PAO (Mino, Van Loosdrecht, & Heijnen, 1998; Barnard & Abraham, 2006).

The application of the EBPR process in real scale was performed empirically (Mino, Van Loosdrecht, & Heijnen, 1998; Tchobanoglous, Burton, & Stensel, 2003). Later, the metabolic processes involved in the EBPR process began to be understood as the microbiological conditions were becoming clearer. Some metabolic models were

developed trying to explain the biochemical dynamic in the EBPR process. These models are based on measurements of chemical transformations in mixed liquor (Seviour & Nielsen, 2010) and on microscopic techniques as staining techniques (Serafim, et al., 2002). Additionally, it is assumed that a single PAO population, with homogeneous metabolic behavior, performs the EBPR metabolic process (Seviour & Nielsen, 2010). However, most part of the research in EBPR metabolism were developed using mixed bacterial communities. Even though, some investigators mentioned the use of enriched PAO sludge; this term would mean that a considerable percentage of these biomasses were recorded as PAO but the complete composition of the biomass stills being a mixed community.

Traditionally the EBPR biochemical behavior was described as a process that should perform the following biochemical transformations:

Table 3.2: Biochemical transformations described for the anaerobic and aerobic phases in an EBPR process (Mino, Van Loosdrecht, & Heijnen, 1998; Seviour, Mino, & Onuki, 2003; Oehmen et al., 2007).

Anaerobic phase	Aerobic phase
• The short chain VFA in mixed liquor are rapidly uptaken and converted into PHA. The specific composition of the PHA depends on the composition of the VFA (e.g. if acetate is the VFA available, the PHA obtained will be the poly-β-hydroxybutyrate - PHB). • The intracellular polyphosphate concentration decreases and the orthophosphate concentration in the aqueous phase increases. • Other intracellular storage polymers are degraded (e.g. glycogen).	• The intracellular PHA concentration decreases and the biomass growth rate increases. • The intracellular polyphosphate concentration increases as the orthophosphate concentration in the aqueous phase decreases. • The storage of intracellular polymers is replenished.

Several models of EBPR metabolism have been proposed based on the biochemical transformations above mentioned (Table 3.2) and two of them are the most widely accepted. These models consider acetate as the ideal carbon source.

a. COMEAU-WENTZEL Model

The Comeau (Comeau et al., 1986) - Wentzel (Wentzel et al., 1986) model was the first model proposed to explain the EBPR chemical transformations. In summary, this model suggests that during the anaerobic phase in presence of a carbon source the intracellular polyphosphate is degraded to provide ATP which will be used to synthesize the energized form of acetate acetyl CoA (Ac CoA) and will reestablish also the proton motive force (PMF) consumed during the substrate transport. A part of the Ac CoA will be oxidized using the route of the Tricarboxylic Acid Cycle (TCA) and hence the NAD will be reduced to NADH. The reducing power of the NADH should be enough to convert the Ac CoA into PHB. Because of the degradation of the ATP, some orthophosphate will be released to the aqueous phase and some cations as calcium (Ca^{2+}) and magnesium (Mg^{2+}) will be also co-transported with the orthophosphate (Seviour & Nielsen, 2010). Subsequently, during the aerobic phase (Phamine phase where almost all carbon source was consumed) the stored PHA is oxidized using the TCA route and this oxidation process will generate the PMF to produce ATP, and this ATP will be used to replenish the intracellular polyphosphate stores.

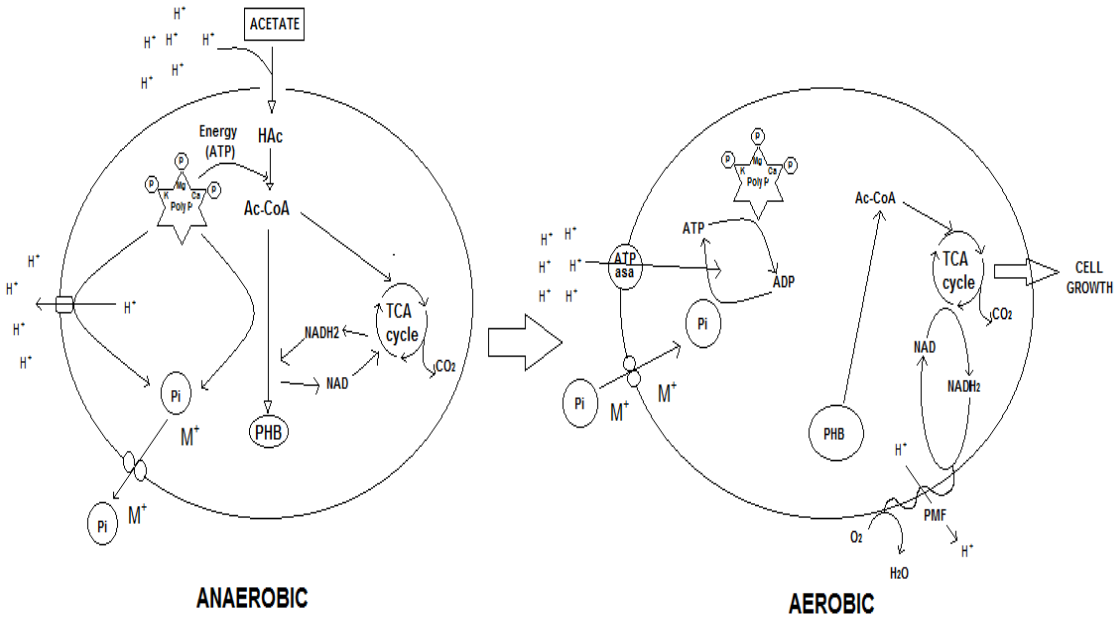

Figure 3.6: EBPR metabolism according to the Wentzel-Comeau model. (Redraw from Seviour & Nielsen, 2010).

There are some inconsistencies in this model, such as, for example, that the amount of $NADH_2$ produced will not be enough to produce the PHA mentioned, since the PHAs are higher reduced than acetate. Other inconsistency lays on the fact that the $FADH_2$ (Reduce Flavin Adenine Dinucleotide) will be accumulated in the TCA cycle during the

anaerobic phase from the oxidation of succinate to fumarate. Because during this phase there is no oxygen available as electron acceptor, the accumulation of $FADH_2$ will block the TCA cycle and also the PHA storage. Research by Garcia-Martin, et al., (2006) on metagenomic analysis of a PAO enriched sludge sample suggested how the bacterial metabolism overcomes this $FADH_2$ accumulation during the anaerobic phase. In this research, the anaerobic oxidation of $FADH_2$ was proposed using quinones as electron acceptors; these reduced quinones would transfer the electrons to NAD $(P)^+$. This last process would be catalyzed by a novel cytochrome b/b6 but its role needs to be confirmed.

b. MINO Model

As mentioned above, in the stoichiometric balance, the amount of reducing power (NADH) obtained from the oxidation of acetate through the TCA route would not be enough to produce the PHA stored. Therefore, other source of this reducing power should exist.

Mino et al., (1987) proposed that the reducing power not only would come from the acetate oxidation but also from the anaerobic degradation of the intracellular glycogen. Part of the glycogen would be oxidized using the TCA route to CO_2 and other part to AcCoA. Further research (Arun, Mino, & Matso, 1988) supported the Mino model when observed that intracellular carbohydrates were consumed during the anaerobic acetate uptake and PHA production. Apparently, these carbohydrates would be degraded and converted to AcCoA by the Embden-Meyerhoff-Parmas (EMP) pathway and from this reaction the NADH would be obtained. Research by Hesselmann et al., (2000) proposed that the glycogen degradation occurs using the Entner-Doudoroff (ED) pathway but these results are not consistent with the genomic analysis proposed by Garcia-Martin et al., (2006).

Other contribution of the Mino model refers to the energy source required for the EBPR process (Figure 3.7). According to the Mino model, the energy required during the anaerobic phase has several sources. As proposed, not only the degradation of the stored polyphosphate will provide the energy required, but also the glycogen catabolism is proposed as a source of ATP for the substrate assimilation, PHA synthesis and cell maintenance. With these two modifications the Mino model seems to reflect more accurately the biochemical processes that occur in the EBPR process (Mino et al., 1998; Seviour, Mino, & Onuki, 2003).

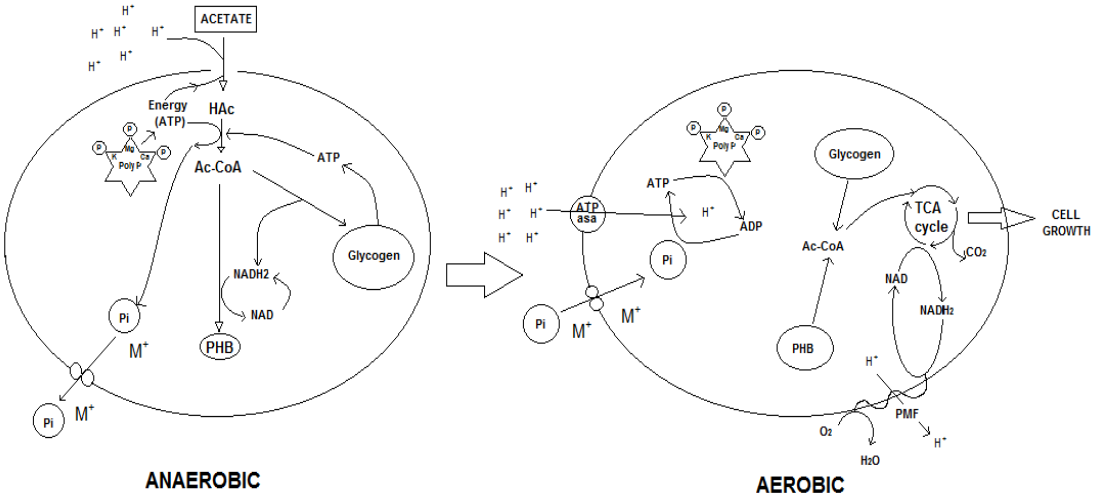

Figure 3.7: EBPR metabolism according to the Mino model. (Redraw from Seviour & Nielsen, 2010).

Further research supported the Mino model. Research by Satoh et al., (1992), and Smolders et al., (1994 a) reported that the anaerobic transformation of acetate, PHB, glycogen and CO_2 fitted with the stoichiometric balance proposed in the Mino model. As mentioned above the Wentzel-Comeau model proposed that the acetate oxidation provided the NADH reducing power, but research by Bordacs & Chiesa, (1989) observed that the acetate was not oxidized to CO_2 through the full TCA route when used radiolabeled acetate as carbon source (Seviour & Nielsen, 2010). Additionally, Kong, Nielsen, & Nielsen, (2004) using metabolic inhibitors and a PAO model strain observed that the NADH obtained during the anaerobic phase came from a glycolisis process and not from a TCA cycle. Meanwhile, according to Pereira et al., (1996) and Hesselmann et al., (2000) the glycogen degradation alone would not be able to provide enough NADH reducing power to produce the PHA synthesis during the anaerobic phase, therefore it seems that both metabolic processes i.e. acetate-TCA cycle and glycogen-anaerobic degradation will provide the reducing power required for the synthesis of PHA. This assumption was confirmed later on a full scale EBPR research (Pijuan et al., 2008).

3.5.6 Wastewater treatment plants configurations for EBPR processes

The metabolic models mentioned above are the most broadly accepted to explain the biochemical procedures that occur in an EBPR process and are reflected in the requirements for a good functioning of an EBPR system (Table 3.2). However, these requirements were discovered empirically and on them are based most part of the full-

scale EBPR plants configurations (Mino et al., 1998; Tchobanoglous, Burton, & Stensel, 2003).

Barnard (1994) modified the BARDENPHO or PHOREDOX systems and created the FIVE STAGE PHOREDOX process where an additional anaerobic reactor or zone was added to the initial part of the plant (Figure 3.8). The recycled amount of nitrate in the RAS is minimized since the “a-recycle” is taking the possible product of nitrification back to the anoxic zone. Meanwhile, the “s-recycle” is taking back to the anaerobic reactor the RAS from the second clarifier where to recycle considerable amounts of nitrate will not be possible. In consequence, the anaerobic zone will provide an appropriate environment to the PAO group.

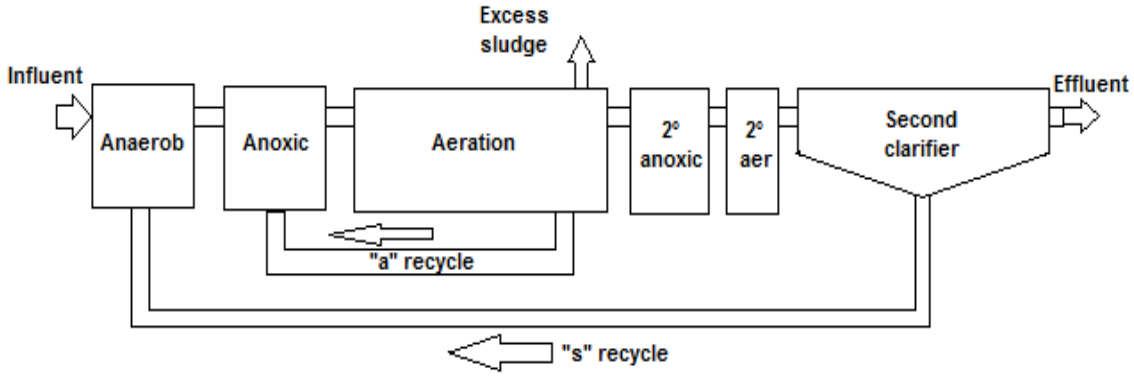

Figure 3.8: The FIVE STAGE PHOREDOX process.

To simplify the FIVE STAGE PHOREDOX system, the size of the first anoxic reactor was increased and also the denitrification efficiency; as result, the secondary anoxic and re-aeration phases were not necessary (Bryan, 1993). This process was called the THREE-STAGE PHOREDOX. On this basis other modifications were developed as the THREE-STAGE BARDENPHO system also called A_2O process (Barnard, 1994; Oehmen et al., 2007). This system tends to have a long sludge age while the TWO-STAGE HIGH RATE PHOREDOX, also called A/O, operates with a shorter sludge age (Figure 3.9).

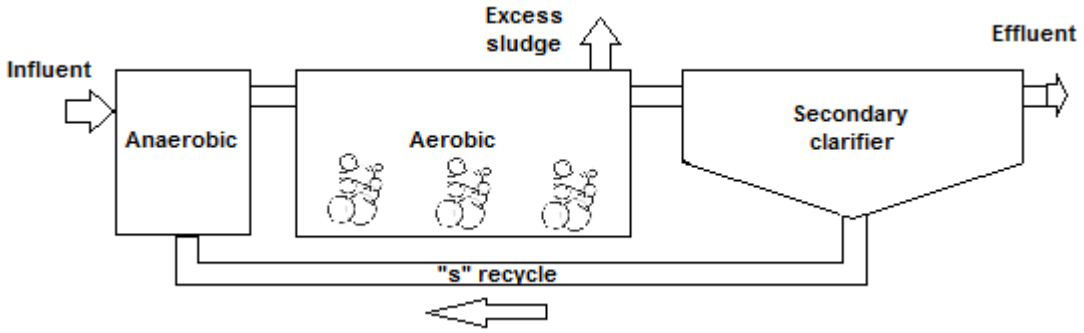

Figure 3.9: The TWO-STAGE HIGH RATE PHOREDOX process or A/O process.

Further modification to the Phoredox system is the JOHANNESBURG PROCESS (Barker & Dold, 1996). This system is similar to the A_2O configuration with the addition of an anoxic zone/reactor previous to the anaerobic phase with high biomass concentration which intent to ensure a complete denitrification. As shown in Figure 3.10, the influent is discharged always in the anaerobic zone/reactor and the second and first anoxic zones are receiving the RAS from the second clarifier and from the aeration reactor, respectively. Thus, the nitrate produced in these reactors will be denitrified in the anoxic zones. Apparently, this plant design operates successfully in several countries (Barnard, 1994).

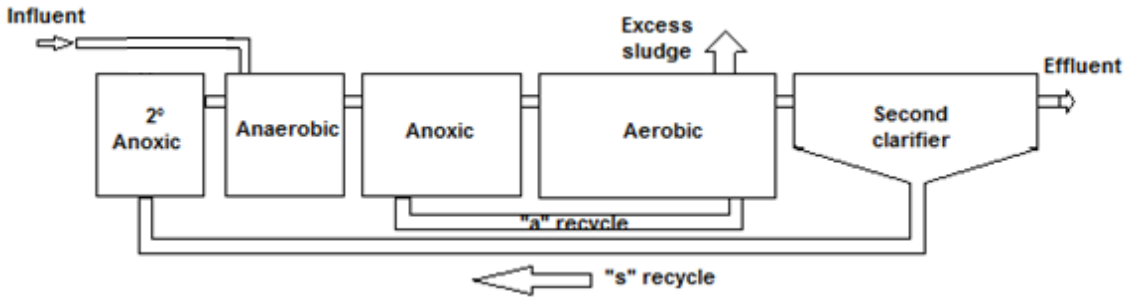

Figure 3.10: The JOHANNESBURG process.

Other modification of the Phoredox system was developed at Cape Town University and is known as the UCT SYSTEM (Wentzel et al., 1991b; Bryan, 1993). As shown in Figure 3.11, the two RAS recirculate from the second clarifier and the aeration reactor to the anoxic zone/reactor. Additionally, mixed liquor from the anoxic reactor is recirculated to the anaerobic reactor ensuring the complete denitrification in the anoxic phase and the absence of nitrate in the anaerobic phase (Oehmen et al., 2007).

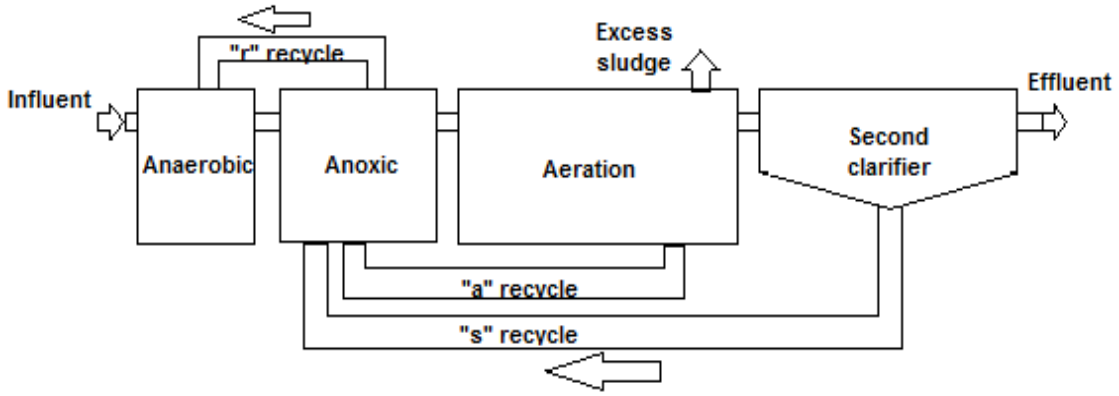

Figure 3.11: The UCT system.

Later, to improve the denitrification process the anoxic zone was divided into two separated reactors with different dimensions where the larger received RAS from the aerobic reactor and the little anoxic reactor received the RAS from the second clarifier. This system was called MODIFIED UCT and apparently operates worldwide successfully (Seviour & Nielsen, 2010).

The WWTPs configurations presented so far were developed with one main objective: to avoid the presence of nitrate during the anaerobic feed phase but in summary they are modifications of the PHOREDOX – BARDENPHO initial configurations. Because the performance of the EBPR process in real scale is not stable, the use of chemical precipitation processes as back up to the system is necessary. Apparently, the EBPR process stability depends on several factors and not only on the presence of nitrate during the anaerobic phase. Once the nitrate presence during the anaerobic phase is avoided it is possible that the EBPR process is still having problems. Moreover, the loss of EBPR performance in many plants can occur without explanations (Seviour & Nielsen, 2010). The instability of the EBPR process in real scale is the result of the lack of knowledge about the biochemical processes that occur in the activated sludge which strictly depend on the characteristics of the bacterial communities.

3.6 Wastewater composition and operational parameters required to achieve good EBPR processes.

To summarize the requirements needed to achieve a good EBPR performance the work of Mulkerrins et al., (2004) was taken into account.

The first group of requirements is related to the wastewater composition and is summarized as follows:

- The influent flow and the loading rate of wastewater should be maintained as much as possible stable (Shehab et al., 1996).
- The VFA content in the influent should be maintained within ranges as mentioned by Satoh et al., (1994) who worked with a biofilm system using a COD concentration in the influent of 400 mg/l in the form of acetate. In this research the phosphorus uptake was improved but when the acetate concentration increased up to 600 mg/l the P-uptake started to decrease.
- The control of the F/M ratio. Research by Chuang et al., (1998) recorded high P-uptake potential when low COD-SS ratios were used. In contrast, using a high COD-SS ratio, in the same research, a low potential of P-uptake was recorded. Additionally, the risk of having problems with filamentous bacteria increased when the influent contains high F/M ratios (Furumai et al., 1999).
- The relation between the carbon concentration and the phosphorus concentration in the influent is of major importance. As Stensel & Barnard, (1992) mentioned, a BOD_5/Ptot ratio lower than 20 and a COD/Ptot ratio lower than 40 will not guarantee an appropriate performance of the EBPR process.

An agreement has been reached that the use of VFA as carbon source improves the EBPR performance (Comeau et al., 1996; Ruel et al., 2002), but, it is also accepted that the VFAs are not the only substrates to achieve efficient EBPR performance. Other substrates as glucose or peptone have been used with similar successful results of EBPR (Carucci et al., 1999).

Additionally, the adequate concentration of cations as magnesium and potassium in the influent wastewater seems to stabilize the intracellular polyphosphate chains (Rickard & McClintock, 1992). Even more, according to Romanski et al., (1997) in a polyphosphate chain each phosphate group is stabilized by a positive charge, and to release a phosphate molecule a cationic charge (K^+ or Mg^{+2}) is required. Research by Randall et al., (1992) mentioned that generally an excess of potassium and magnesium in municipal wastewater may be reported, therefore, limitation problems of these ions are improbable. However, sometimes the WWTPs can face potassium shortage and according to Brdjanovic et al., (1996), the consequence of this scarcity would be the absence of phosphorus removal, the decrease in the intracellular polyphosphate stores, and the adverse effect on the acetate uptake and the anaerobic phosphorus release after several days of potassium scarcity.

The temperature in activated sludge systems is not only related to the phosphorus removal performance but to all metabolic processes, gas transference and sludge sedimentation characteristics (Metcalf & Eddy, 1991). According to Metcalf & Eddy, (1991), the bacterial growth rate will increase to the double for each 10°C of temperature rises. With regard to the optimal temperature for EBPR processes, the references are contradictory. For example, McClintock et al., (1993) and Converti et al., (1995) reported improvements of the EBPR performance with temperatures ranging between 20 and 37°C, while Viconneau et al., (1985) and Florentz et al., (1987) have reported improvements with temperatures between 5 and 15°C. Additionally, Panswad et al., (2003) suggested that PAO bacteria would be mesophilic (optimal temperature around 20°C) or even psychrophilic. On the other hand, several studies supported the adverse effects of low temperatures on EBPR processes. Apparently, low temperatures between 5 and 10°C would be adverse for the oxygen consumption rate (Brdjanovic et al., 1997), and the phosphorus release during the anaerobic phase may also decrease with low temperatures (Converti et al., 1995). Other adverse effects, not related directly to the PAO metabolism but affecting the development of the activated sludge community, would be the detriment of the denitrification process at low temperatures resulting in nitrate accumulation in the return sludge (Helmer & Kunst, 1998). Additionally, Knoop & Kunst, (1998) reported that low temperature are appropriate for the development of *Microthrix parvicella* since the optimal temperature for this filamentous bacterium would range between 12°C and 15°C.

Another challenge for the EBPR-WWTPs is the proliferation of filamentous bacteria that can adversely affect the settlement characteristics of the activated sludge. An indicator of settleability is the Sludge Volume Index (SVI). Stable settlement processes are recorded with SVI values lower than 150 ml/g (Kunst et al., 2000). The filamentous bulking is a common problem faced in WWTPs. According to Blackbeard et al., (1988) in South Africa approximately 80% of the WWTPs with biological nutrient removal processes have faced bulking problems, and Eikelboom et al., (1998) concluded that because the addition of the P-removal process to the WWTPs, the percentage of plants with high SVI problems increased to the double. Additionally, Chang et al., (1996) reported that it was not possible to ensure low SVI values (69 – 370 ml/g) with influents with high COD/P ratios, but the phosphorus removal performance was up to 75%. This last study highlighted that the excess of filamentous bacteria was directly related to the sludge settlement capacity but not to the phosphorus removal capacity. Additionally, it is well known, that *Microthrix parvicella* is a common filamentous bacteria in nutrient removal plants (Eikelboom et al., 1998; Wagner & Loy, 2002). This microorganism was

recorded to predominate during the colder winter and early spring (Eikelboom et al., 1998).

Other factors that may be mentioned are directly related to the wastewater characteristics as the pH, the chemical composition of the wastewater and the flow and strength fluctuations (Metcalf & Eddy, 1991). The low F/M ratios are also reported as a risk condition for the appearance of bulking problems (Rossetti et al., 1994).

Furthermore, the oxygen demand of an activated sludge system depends on the nutrient removal process that this system performs. For carbon oxidation and nitrification processes, an activated sludge system requires dissolved oxygen levels greater than 2 mg/l (Louzeiro et al., 2002). Research by Shehab et al., (1996) mentioned that in EBPR systems the dissolved oxygen concentration in the aerobic phase should be maintained between 3 and 4 mg/l, and Mulkerrins, Dobson, & Colleran, (2004) mentioned that dissolved oxygen concentrations greater than 4 mg/l seems not to improve the EBPR performance. Additionally, Brdjanovic et al., (1998) recorded that an excess of aeration adversely affect the EBPR performance resulting in the decrease of the PHB production. According to Scruggs & Randall, (1998), the development of some filamentous bacteria such as Type 1863 is promoted by low dissolved oxygen concentrations (0.1 mg/l or less), and in contrast, the growth of *Nocardia* was enhanced according to the increase of the dissolved oxygen concentrations from 1 to 5 mg/l.

On the other hand, the phosphorus content in wastewater may influence the development of PAO and GAO bacterial communities. Research by Sudiana et al., (1999) reported that low phosphorus loadings would not be adequate for the PAO development as it may result in the establishment of the GAO community. This latter result was supported by Liu et al., (1998) who used low P/TOC ratios in the influent and recorded, as a consequence, that the growth of PAO was suppressed. In contrast, when high P/TOC feeding ratios were used the PAO growth seems to improve as some research reported. Research by Converti et al., (1993), used wastewater with a phosphate concentration of 70 mg/l, obtaining phosphorus removal percentage of more than 90%. Additionally, research by Comeau et al., (1996) used a phosphorus concentration of 60 mg/l in SBR at lab scale, obtaining a phosphorus removal percentage of 88% during 193 days of operation time. On average, the domestic wastewater contains a phosphorus media concentration of 8 mg/l (Metcalf & Eddy, 1995), and as Sudiana et al., (1999) mentioned, a mixed bacterial community of PAO and GAO may be obtained with this concentration. Additionally, efficient EBPR processes are commonly achieved with this phosphorus concentration. Research by

Randall et al., (1997) recorded that after increasing the phosphorus concentration up to 20 mg/l in the influent, a considerable increase in the phosphorus removal performance was observed.

Several investigations reported different pH ranges as optimal for specific processes in the removal of biological nutrients (Table 3.3).

Table 3.3: Optimal pH values proposed for nitrification, denitrification, acetate uptake and phosphorus release processes in activated sludge systems

Process	pH optimal	pH limit	Comments
Nitrification	7.5 - 9	6	Eckenfelder and Grau, (1992); Surampalli et al., (1997)
Denitrification	7 - 8	7	pH 7 would be the limit when nitrite concentration of 250 mg/l is reported. Metcalf & Eddy, (1991); Sanchez et al., (1998); Glass & Silverstein, (1998)
Acetate uptake and phosphorus release	7	Acid or alkaline	Alkaline pHs have a negative effect for acetate uptake but positive effect for P release. Converti et al., (1995); Liu et al., (1996).

It is important to note here, that a research by Bond et al., (1998) suggested that no control of the pH may result in a better phosphorus removal in SBR reactors.

Other operational parameters also influence the EBPR performance. The sludge retention time (SRT) can vary between seasons in biological nutrient removal plants. For example, in western Canada the SRT is ranging between 9 and 13 days in winter and between 5 and 7 days in summer (Oldham & Rabinowitz, 2002). Additionally, the SRT depends on the sludge loading rate (SLR) or the F/M ratio. For example, with low F/M ratios and long SRT, effluents with good quality may be obtained (Metcalf & Eddy, 1991).

To design biological nutrient removal plants, the F/M ratios for carbon oxidation, nitrification and denitrification processes are ranging between 0.03 and 0.06 kgBOD_5/kg MLVSS/day (Crites & Tchobanoglous, 1998). As mentioned above, low F/M ratios seem to promote the development of filamentous bacteria resulting in bulking problems (Rossetti et al., 1994). Additionally, Knoop & Kunst, (1998) reported that when the F/M ratio increased from 0.1 to 0.2 kg COD/kg MLVSS/day the development of *Microthrix parvicella* was prevented even at low environmental temperatures. Other reason to vary the SRT is the type of wastewater treatment that depends on the characteristics of the influent wastewater. Mamais & Jenkins, (1992) observed efficient EBPR performances with SRT greater than 2.9 days. Additionally, Chang et al., (1996) recorded that with a SRT of 10 days the best phosphorus removal rates were obtained. Similar results were obtained in a research by Choi et al., (1996) where the best phosphorus removal performance was observed with an SRT of 10 days in comparison to 5 and 20 days. In WWTPs with simultaneously removal process of phosphorus and nitrogen, the right SRT should be carefully calculated to avoid problems as nitrification. When a long SRT is applied the opportunity for the development of nitrificants are higher (generation time = 60 h) and, as a result, the nitrate levels may increase (Furumai et al., 1999). However, if the goal of the wastewater treatment is to remove phosphorus, the SRT should not be so high, thus, the nitrification process could be minimized. Research by Kargi & Uygur, (2002) recorded optimal COD, nitrogen (NH_4-N, NO_3-N) and phosphate (PO_4-P) removal with a SRT of 10 days.

3.7 Performance loss in EBPR processes

Although the operational parameters and wastewater composition may be appropriate, the EBPR systems can suddenly lose the performance without an explanation, and to recover the removal performance is not always possible (Seviour & Nielsen, 2010). This chapter attempts to draw the attention to some basic assumptions of the experimental design and to new approaches in EBPR research that may give some explanations about the bad functioning of the EBPR process.

As mentioned above, the EBPR metabolic models were developed assuming that the PAO bacterial community was a single population and additionally, that the EBPR process is performed following an only metabolic process (Seviour & Nielsen, 2010). If this assumption would be right, the metabolic behavior of EBPR bacterial communities in real scale should fit with the Comeau-Wentzel model or with the Mino model; however, several inconsistencies to these models are observed in lab and real scale. Traditionally, it has been assumed that the amount of phosphorus released during the

anaerobic phase was directly related to the phosphorus uptake during the aerobic phase (Wentzel et al., 1986; Comeau et al., 1986; Mino et al., 1987). Research by Helmer & Kunst, (1998) mentioned that this relation was evident with environmental temperatures between 15°C and 20°C, but with temperatures of 10°C this relation was not so close and with 5°C no correlation between the phosphorus release during the anaerobic phase and the phosphorus uptake during the aerobic phase was observed.

Some factors as the presence of nitrate during the anaerobic phase can adversely affect the phosphorus release (Furumai et al., 1999). Other factor is the presence of oxygen or other oxidizing agents during the anaerobic phase that may affect the redox potential and as a result impair the process of phosphorus release (Mulkerrins et al., 2004).

On the other hand, several investigations reported that working with glucose as carbon source, good EBPR performance were achieved, but the carbon consumed was not related to the amount of phosphorus released (Carucci et al., 1994; Jeon & Park, 2000). Other research using laboratory scale reactors reported that the relation between the anaerobic phosphorus release and the acetate assimilation was not constant (Seviour et al., 2003). Research by Mino et al., (1998) suggested that this relation may depend on the pH of the mixed liquor that affects the energy demand for the acetate transport. Additionally, Jeon & Park, (2000) proposed an EBPR metabolic pathway using glucose as only carbon source. In this research the participation of two bacterial communities in the EBPR process may explain the inconsistence observed about the amount of phosphorus released during the anaerobic phase and the amount of glucose consumed. According to this research the lactic acid producing organisms (LPO) would be able to uptake directly the glucose during the anaerobic phase and subsequently they would release lactate to the aqueous phase. Then, the PAO bacteria would uptake the lactate, previously released by the LPO, and in consequence would release an amount of orthophosphate that would not fit with the amount of glucose initially consumed.

However, all these metabolic explanations proposed are not well supported because the lack of the respective experimental biochemical data. Some research using Nuclear Magnetic Resonance (NMR) attempts to follow the metabolic flux in mixed communities in presence of different substrates under specific conditions, but even this methodology is not able to explain the metabolic behavior of the different bacterial groups involved in the EBPR process since the structure and the metabolic relations between them are unknown (Seviour et al., 2003).

Additionally, research by Pijuan et al., (2005) reported other inconsistency to the metabolic models, when EBPR activity was observed even during the addition of acetate in the aerobic phase. As mentioned in the metabolic models, to achieve a high phosphorus removal performance, the separation of an electron donor zone (anaerobic feed stage) from the electron acceptor zone (aerobic famine stage) is necessary. Under anaerobic conditions the PAO bacteria would be able to uptake the RBCOD while other competitors (mostly heterotrophic microorganisms) would not be able to metabolize the substrate since the oxidative phosphorylation would be unable to proceed. Additionally, Pijuan et al., (2005) mentioned that the electron donor was present during the aerobic phase and, despite it, substantial phosphorus release and acetate uptake were observed. In this case, the phosphorus uptake was recorded once the carbon source was consumed (Seviour & Nielsen, 2010). Research by Ahn et al., (2007) observed a stable phosphorus removal performance using continuous aeration, but feeding acetate and phosphorus in different moments. Apparently, PAO microorganisms would be able to assimilate the substrate and to release phosphorus under aerobic conditions, but the amount of dissolved oxygen provided in the continuous aeration phase should be mentioned. In the research of Pijuan et al., (2005), the dissolved oxygen concentrations in mixed liquor were maintained only above 2 mg/l and in the research of Ahn et al., (2007) the dissolved oxygen concentration was approximately 1 mg/l. Other research suggested that when the COD influent is relative high (60 - 120 mg/l) and the dissolved oxygen concentration in mixed liquor is insufficient (less than 4 mg/l), only the surface of the floc would have aerobic conditions (Li & Bishop, 2004). Research by De Kreuk & van Loosdrecht, (2004), and Lemaire et al., (2008) recorded the presence of PAO in granular aerobic sludge; this could mean that, the anaerobic conditions inside the floc would be appropriated for the growth of PAO.

It is necessary to clarify whether the separation of the electron donor and acceptor zones is indispensable for PAO to uptake phosphorus or if this separation only would provide PAO with a metabolic advantage to substrate uptake (Seviour & Nielsen, 2010).

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CHAPTER 4: Material and methods

The main objectives of this research have been presented in Chapter 2. To achieve these objectives the research was subdivided into five investigations. Figure 4.1 shows the principal objectives and a general description of the experimental procedure.

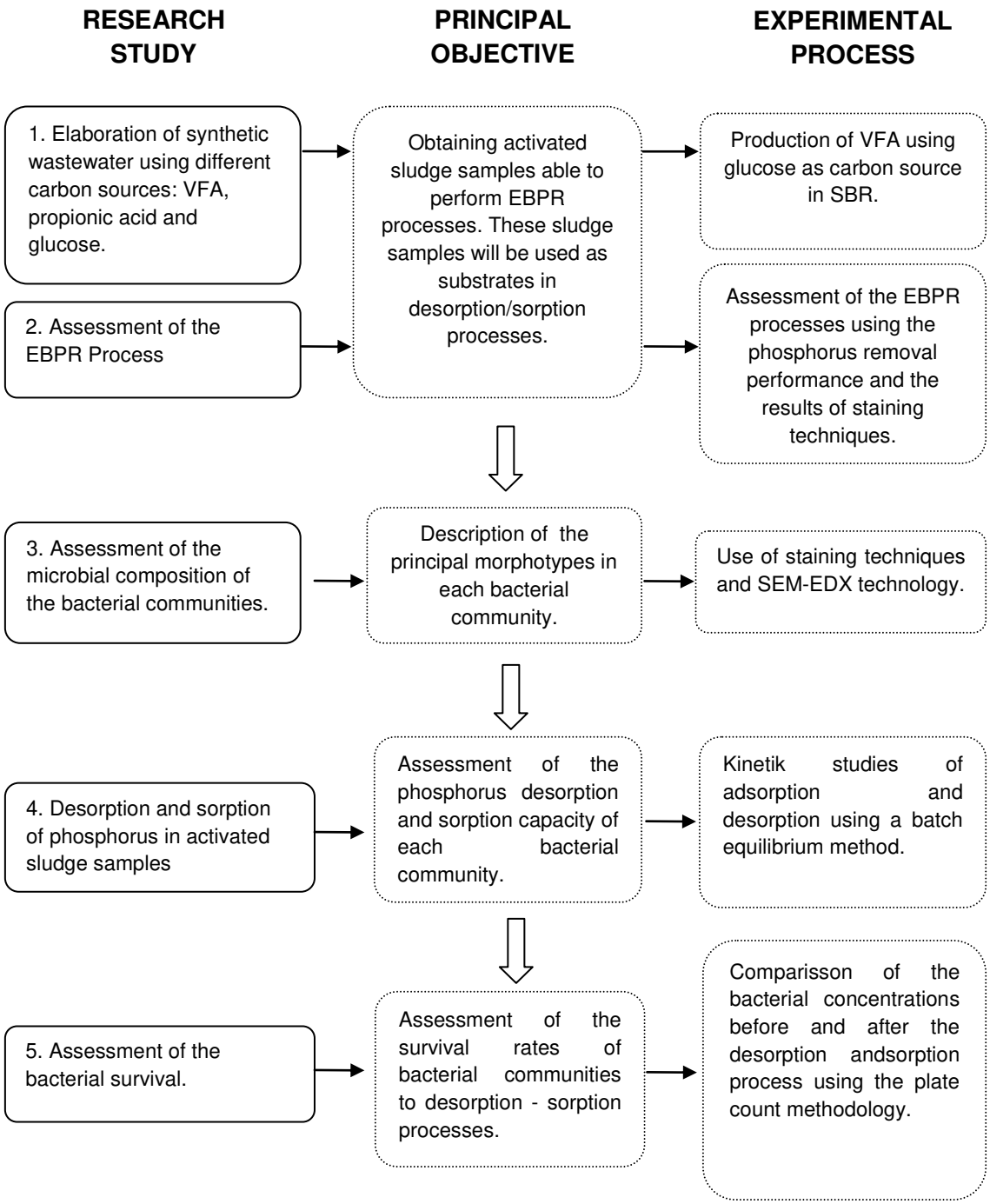

Figure 4.1: Flowchart of the principal objectives and experimental processes in this research.

According to the different objectives pursued, the methodoly used in each research differs from each other. However, general methodological procedures were used repeatedly. These general methodological procedures are described below.

4.1 Chemical analysis

4.1.1 Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5)

a. COD measurement:

The standard method corresponds to the DIN 38409-H41-1 Norm (Deutsches Institut für Normung, 1980). This methodology can be used in water samples that contain a COD concentration between 15 and 300 mg/l. The restriction of this method lies in the chloride concentration of the sample that should be less than 1 g/l.

Oxidable substances in aqueous samples are oxidized using potassium dichromate solution ($K_2Cr_2O_7$). The potassium dichromate, which was not consumed, is titrated with standard iron (II) ammonium sulphate using orthophenanthroline ferrous complex (ferroin) as redox indicator.

b. BOD_5 measurement:

The standard method corresponds to the DIN 38409-H51 (Deutsches Institut für Normung, 1987 a). This methodology can be used in water samples with COD concentrations higher than 50 mg/l where is necessary to apply a previous dilution process. The DIN 38409-H52 (Deutsches Institut für Normung, 1987 b) is used for diluted samples where the oxygen content is measured directly without using a nitrification inhibitor.

BOD measures the amount of oxygen consumed because of microbiological decomposition (oxidation) of organic material in water. The amount of oxygen consumed, through this oxidation process in 5 days, is measured at a constant temperature of 20°C in the dark. When the water sample has a high COD concentration, it is necessary to

suppress the possibility of oxygen consumption through nitrification process. To do this, the N-allylthiourea nitrification inhibitor is used.

4.1.2 Nitrogenous compounds:

a. Total nitrogen (TN) concentration:

For total nitrogen measurements, the commercial fast test Dr. Lange LCK 238 was used from the Hach-Lange company. This method can be applied in water and wastewater samples which contain a total nitrogen concentration between 5 and 40 mg/l.

The methodology corresponds to the dimethylphenol method previous oxidation. The nitrogen contained in organic and inorganic molecules is previously oxidized using peroxodisulphate to produce nitrate ions. Nitrate ions react with the reagent 2.6-dimethylphenol forming 4-nitro-2.6-dimethylphenol into an aqueous acid solution of sulfuric and phosphoric acid. After the reaction time, total nitrogen concentration can be measured using a spectrophotometer.

b. Nitrate (NO_3^-) and Nitrite (NO_2^-) concentration:

For semi-quantitative measurement of nitrate and nitrite concentrations in water samples, the Quantofix Nitrate/Nitrite test strips from the company Macherey–Nagel were used. The strips are composed of plastic plates with bonded reaction zones that contain the chemical reagents.

The basic chemical reaction is the diazotization of the sulfanilamide with nitrite or nitrate in an acid media. The color of the reaction is varying between white and red. Water samples should have a nitrate concentration ranging between 10 and 500 mg/l and nitrite concentration ranging between 1 and 80 mg/l. The results of nitrate and nitrite concentrations follow a scale shows in Table 4.1.

Table 4.1: Scale of nitrate and nitrite concentrations in water samples recorded by the Quantofix Nitrate/Nitrite test strips (Macherey-Nagel Company)

Nitrate concentration (mg/l)	Nitrite concentration (mg/l)
0, 10, 25, 50, 100, 250, 500	0, 1, 5, 10, 20, 40, 80

c. Ammonia-nitrogen (NH_4^+-N) concentration:

The standard method corresponds to the DIN 38406 - E5 Norm (Deutsches Institut für Normung, 1983). This methodology can be applied in water, wastewater and sludge samples with ammonium-N concentrations between 0.03 and 1 mg/l. If the sample contains a high concentration of suspended solids, a previous filtration process is recommended using a millipore membrane of 0.45 µm pore size. The initial pH of the sample should lie between 5 and 8.

In presence of natriumpentacyanonitrosylferrat (2-) (Nitroprussidnatrium) and with a pH of approximately 12.6, the ammonium ions react with hypochlorite and salicylat ions taking a bluish color. The color variation is measured using a spectrophotometer.

4.1.3 Total phosphorus concentration (Ptot)

The standard method corresponds to the DIN EN ISO 6878 (Deutsches Institut für Normung, 2004). This Norm describes the methodology to measure the orthophosphate and total phosphorus after an oxidation process using peroxodisulphate. This method is applicable to water samples and wastewater. Without dilution the water sample should have a phosphorus concentration between 0.005 and 0.8 mg/l.

For total phosphorus analysis, if the water sample contains other phosphorus compounds, different to orthophosphate, the water sample should be previously oxidized by boiling with peroxodisulphate. Subsequently, orthophosphate ions will react with molybdate and antimony ions in an acidic solution to produce an antimony phosphomolybdate complex. When this complex is reduced with ascorbic acid, the result is the molybdenum blue complex which can be measured using a spectrophotometer.

4.1.4 Volatile fatty acids (VFA) concentration by titration and volatile fatty acids composition by gas chromatography.

a. Volatile Fatty Acids concentration (VFA)

The method used is described in the DIN 38414-S19 (Deutsches Institut für Normung, 1999). The distillation apparatus used is described in the DIN 38414-19:1999-12.

5 ml of phosphoric acid were added to 100 ml water sample. Subsequently, the first distillation process, of 40 minutes in length, started. Then, the sample faced a second reflow heating process during 10 minutes, to finally, be titrated with sodium hydroxide (NaOH = 0.1 mol/l) using phenolphthalein as indicator.

The same procedure was applied with a deionized water sample of 100 ml which represented the control.

The results were calculated in millimoles per liter (mmol/l) of volatile organic acids according to the Formula 4.1.

$$c(S) = \frac{cNaOH*(V_1 - V_0)}{V_p} * 1000 \quad \text{..... (4.1)}$$

where:

- $c(S)$: Total concentration of Volatile Fatty Acids -VFA (mmol/l)
- V_1: Volume of sodium hydroxide used in the titration of the sample (ml)
- V_0: Volume of sodium hydroxide used in the titration of the control (ml)
- V_P: Sample volume (ml)
- $_C$(NaOH): 0,1 mol/l

b. Volatile Fatty Acids composition (VFAc)

The composition of VFAs was determined by Gas Chromatography (GC) methodology. This analysis was performed in the Stadtenwässerung Braunschweig Laboratory (Niedersachen, Germany). The results were recorded as Acetic, Propionic, Butiric, Iso-Butiric, Iso-Valeric, and Valeric Acids in mg/l.

4.1.5. pH, dissolved oxygen (DO) and temperature

The pH, dissolved oxygen and temperature were monitored using multimeters WTW Multi 3430.

4.2 Volumetric analysis

4.2.1 Sludge Volume Index (SVI)

The standard method is described in the DIN 38414-S10 (Deutsches Institut für Normung, 1981). SVI is an indicator of sludge settleability. By definition, it corresponds to the volume that a gram of suspended solids occupy after 30 minutes of settling. To perform this measurement, a representative sample of mixed liquor of one liter should be taken and subsequently, the sludge volume accumulated after 30 minutes of settling should be measured.

To calculate the SVI, the sludge volume achieved should be divided by the total suspended solids concentration contained therein (Formula 4.2).

$$SVI = \frac{V_S}{TSS} \quad \text{..... (4.2)}$$

where:

- SVI: Sludge Volume Index (ml/g)
- V_s: Sludge volume after 30 minutes of settling (ml/l)
- TSS: Total Suspended Solids in mixed liquor (g/l)

The following conditions should be taken into account:

- If the sludge volume, after 30 minutes of settling, is greater than 250 ml/l, a previous dilution should be performed.
- The temperature difference between the sample and the environment should not be more than 2°C, to avoid the formation of air bubbles because of convection phenomena.
- For samples with high stringiness conditions, the sample can be diluted to a sludge volume of 100 ml/l.
- When episodes of low loads (F/M <0.15 kg/kg.d) and nitrification process in the aeration basin occur simultaneously, floating sludge during the settling phase might be observed. This phenomenon occurs because small bubbles of nitrogen, from the denitrification process, are released within the floc and attached to the outer surface of the floc. In this case, the sample should rest for two to three hours, to subsequently, being stirred and let stand it an additional 30 minutes to continue with the SVI measurement.

4.3 Microscopic analysis

The methodology used is described in Betriebsprobleme auf Kläranlagen (Kunst et al., 2000) and in Methods for the Examination and Characterization of Activated Sludge Community (Seviour & Nielsen, 2010a).

4.3.1. Floc morphology

Samples observations were performed with fresh samples according to the following procedure:

1. The sludge sample should be homogenized gently without intense agitation since the floc may be fragmented.
2. Place a drop of the sludge sample on the slide. The slide should be previously cleaned with ethanol before being placed on the microscope stage.
3. Place over the sample a cover slip supporting it first with the hand and dropping gently to the opposite, thus avoiding the appearance of large air bubbles between the slide and the cover slip.
4. Place a piece of laboratory blotting paper on the slide slip surface, doing a bit of pressure with the fingertips until decreasing the height of the film sample between the slide and the cover slip.
5. Microscopic observations were performed using the microscope objectives 10x, considering the structural characteristics of the floc.
6. The floc shape was classified according to the scale given by Jenkins et al., (1986).

4.3.2 Determination of the relationship between the SVI and the sludge stringiness with the aid of crystal violet staining.

Crystal violet is a cationic dye which colored through the metachromasia method. This dye interacts with sulphate and phosphate groups in nucleic acids and polysaccharides.

Preparation of reagent:

- Solution A: Crystal Violet (Merck 9218) dissolved at 0.2% in distilled water.

Procedure:

1. Clean the slide with ethanol and place a drop of sludge, previously homogenized, on the slide. With the eyedropper spread the sample over the entire surface of the slide and allow to air dry.
2. Add one drop of solution A directly on the dry extension.
3. Immediately after, cover the preparation with a cover slip and then, pressing the surface thereof with a pen.
4. Wipe excess solution with a laboratory absorbent paper.
5. Observe the sample at 100x magnification - dark field.

Floc masses will stain purple while, filamentous organisms and free microorganisms will stain yellowish orange color. When comparing the observations with the respective reference photos (Kunst et al., 2000) it is possible to determine the extent of SVI related to the relevant degree of stringiness (Table 4.2).

Table 4.2: Experimental values linking the SVI-relevant stringiness and the SVI values (Kunst et al., 2000)

SVI -Relevant stringiness (-)	Sludge Volume Index (SVI) (ml/g)
0 – 1, 2 and 3	< 150
4 and 5	> 150
6 and 6 – 7	> 200
7	Foam alone

4.3.3 PHA staining technique

Sudan black is a lipophilic stain dissolvable in fatty materials. Therefore, this stain is capable to identify the storage of intracellular PHA. However, sudan black stain is not specific for PHA.

A positive reaction for the PHA staining technique includes intracellular blue-black granules on a clear or slightly colored background (Bartholomew, 1981; Murray et al., 1994; Seviour & Nielsen, 2010a).

Reagents:

- Solution A: Sudan black B (IV) 0.3% in Ethanol 60%.
- Solution B: Safranin O 0.5% dissolved in deionized water.

Procedure:

1. Prepare an air-dried smear.
2. Add A solution direct in the dried smear trying to cover the entire sample. The reaction shall be allowed to continue for 12 minutes. Add extra stain if the slide begins to dry.
3. Discard the excess of A solution and carefully rinse the slide with water.
4. Add B solution direct in the smear trying to cover the entire sample. Reaction time 10 - 15 seconds.
5. Discard the excess of B solution and rinse the slide with water. Leave to air-dry. Take care about the stain rest in the other side of the slide and when necessary, remove it with ethanol.

The stained smear should be observed at 1000X magnification with a bright-field objective lens under oil immersion.

4.3.4 Neisser staining technique

Active stain in Neisser staining technique is the methylene blue. Because of its cationic nature, methylene blue may link the anionic sites in polyphosphate chains giving to them a characteristic lilac color.

A positive reaction for Neisser staining technique includes intracellular black-purple granules distinguishable between them if the intracellular phosphorus concentration is not so high. In case that the intracellular phosphorus concentration is high, for example at the end of the aerobic phase, the whole cell could be stained in black-purple color (Lindrea et al., 1999).

There are certain contradictions about the positive reaction of Neisser staining technique. For example, for some investigators the intracellular black-purple granules are not considered a positive reaction, but the whole cell stained in grey blue (Jenkins et al., 1993).

Reagents:

- Solution A: Methylene blue (0.1 g) in acetic acid glacial (5 ml), ethanol 95% (5 ml) and 100 ml deionized water.
- Solution B: Crystal violet 10% w/v in 95% ethanol (3.3 ml), ethanol 95% (6.7 ml) and 100 ml deionized water.
- Solution C: Chrysoidin Y 1% w/v (33.3 ml) in deionized water (66.7 ml)

Procedure:

1. Prepare an air-dried smear.
2. Mix two volumes of A solution with one volume of B solution. This new stain can be stored for maximal four weeks. Add this solution direct in the dried smear trying to cover the entire sample. Reaction time 10 - 15 seconds.
3. Discard the excess of solution and carefully rinse the slide with water.
4. Add C solution direct in the smear trying to cover the entire sample. Reaction time 45 seconds.
5. Discard the excess of C solution and rinse the slide with water. Leave to air-dry.

The stained smear should be observed at 1000X magnification with a bright-field objective lens under oil immersion.

4.4 Gravimetric analysis

4.4.1 Total Solids (TS)

Total solids concentration is determined as the total dry matter contained in a sludge sample, which means, the sum of suspended and dissolved solids.

Procedure:

1. Ignite and evaporate a crucible of 100 ml for 1 hour in the muffle furnace.
2. Allow the crucible to cool to room temperature, weigh and store it until use. Save the crucible weight data (TS_B).
3. The sludge sample should be at room temperature. Place 100 ml of the well mixed sample into the crucible.
4. Evaporate to dryness in the oven at 103 °C – 105 °C during 24 hours.

5. Repeat the cycle of drying, cooling and weighing until a constant weight is achieved.
6. Record the final weight (TS_A) and calculate the total solids concentration (Formula 4.3)

$$TS = \frac{(TS_A - TS_B)}{S_V} * 1000 \quad \text{.....(4.3)}$$

where:

- TS: Total solids (mg/l)
- TS_A: Weight of the dried sludge and the crucible (mg)
- TS_B: Weight of the crucible (mg)
- S_V: Volume of the sludge sample (ml)

4.4.2. Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

Total Suspended Solids concentration was determined using the methodology described in the DIN 38414-S2 (Deutsches Institut für Normung, 1985). Similarly, Volatile Suspended Solids concentration was determined using the methodology described in the DIN 38414-S3 (Deutsches Institut für Normung, 1985a). These methods can be applied in sludge and sediments.

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CHAPTER 5: The production of volatile fatty acids in an SBR reactor using glucose as a carbon source

5.1 Introduction

One of the products obtained from the fermentation processes of domestic and industrial wastewaters are volatile fatty acids (VFA).

As is well known, VFA have been identified as the carbon sources most readily integrated in the metabolic processes of the activated sludge bacterial community. Specifically, VFA are easily assimilated by the microorganisms involved in phosphorus removal from wastewater through the EBPR process (Randall & Khouri, 1998; Pitman, 1999; Ruel et al., 2002).

Thus, there is significant interest in also promoting VFA production in wastewater when phosphorus removal by biological means is being pursued. In this regard, some WWTPs have included phases in their systems that promote the fermentation processes that produce VFA (Maharaj & Elefsiniotis, 2001).

The most suitable operational parameters to achieve good VFA production from wastewater have long been studied. Various research has focused on showing the influence on VFA production of operational parameters such as Hydraulic Retention Time (HRT), Organic Loading Rate (OLR), pH, temperature and COD concentration in influent. However, several inconsistencies regarding these influences have been observed (Dinopoulou et al., 1987; Alkaya & Demirer, 2010).

According other research, for specific requirements such as the EBPR process, obtaining high concentrations of specific VFA, as acetic acid, was the most suitable. Therefore, in this research, the effects of the operational parameters on the VFA composition were also evaluated (Dinopoulou et al., 1987; Albuquerque et al., 2007; Silva et al., 2013).

Previous investigations (Breure & van Andel, 1984; Dohányos et al., 1985) used different substrates to assess VFA production and they concluded that the VFA composition was specific to the type of substrate. Considering these contributions, comparisons of results about the influence of operational parameters on VFA production should only be made between studies that used similar fermentation substrates.

The present research aims to achieve a stable VFA production at a high rate from synthetic wastewater. To achieve this goal, operational parameters related to the biomass concentration, such as TSS and the amount of substrate available for the biomass (F/M ratio), were taken into account. Meanwhile, other parameters were maintained at constant values such as HRT (12 hours), influent COD (600 mg/l) and OLR (400 mgCOD/l.cycle).

In turn, the bacterial community in this investigation corresponds to an acidogenic community maintained at an appropriate HRT which allowed the development of these organisms and prevented the development of their competitors.

5.2 Material and methods

5.2.1 Synthetic wastewater composition.

The synthetic wastewater was elaborated with the composition shown in Table 5.1. The synthetic wastewater was composed of three groups of chemical components:

- Carbon source: α-D(+)-Glucose Monohydrat (Carl Roth-Germany) and Peptone from casein (Merck –Germany)
- Nutrients: KH_2PO_4-P (Merck-Germany) and NH_4Cl-N (Carl Roth-Germany)
- Other (sources of Mg, Cu, Ca, Mn, Zn, Cl, S)

Table 5.1: Composition of the synthetic wastewater for VFA production.

Components	Initial concentration (mg/l)
Glucose	589.5
P (KH_2PO_4)	7
N (NH_4Cl)	30
Mg ($MgCl_2$)	10
Cu ($CuSO_4$)	0.1
Ca ($CaCl_2$)	5
Mn ($MnSO_4$)	0.1
Zn ($ZnCl_2$)	0.1
Pepton	10
C:N:P	35:3.8:1

5.2.2 Inoculum

The microbial community used as inoculum came from the aeration tank of the activated sludge system of the Uelzen WWTP (Niedersachsen - Germany).

5.2.3 Location:

The experimental process was performed at the laboratories of the Civil and Environmental Engineering Faculty of the Ostfalia University - Campus Suderburg (Niedersachsen, Germany) from March 2011 to June 2013.

5.2.4 Reactor design and operational parameters

The experiment was conducted in a polyethylene glass tank which worked as a sequential batch reactor (SBR) with working volume of 30 liters. The agitation system and the charge and discharge pumps were controlled by digital timers Theben TR 644 top2 RC.

The reactor was operated in 3 cycles of 8 hours per day. Each cycle consisted of 56 minutes of filling, 6 hours of anaerobic reaction, 35 minutes of settling, 10 minutes of drawing and 19 minutes of idle phase.

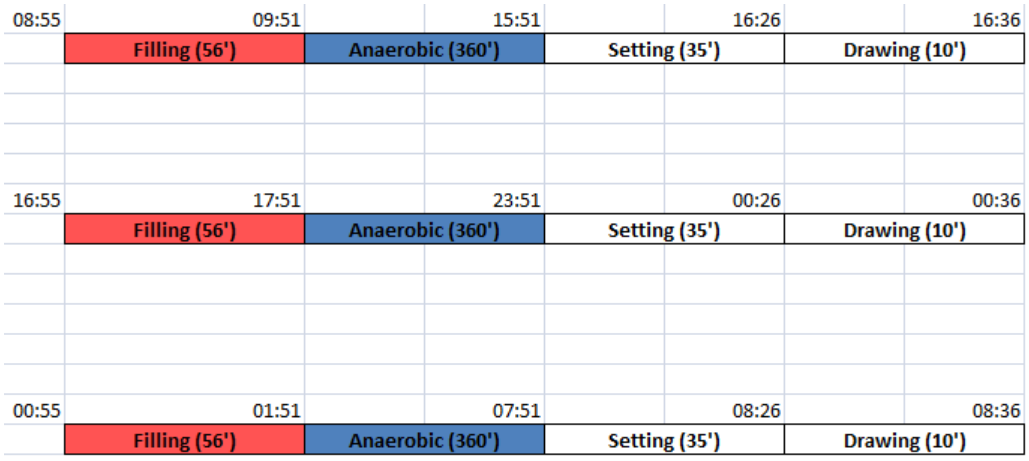

Figure 5.1: Time distribution in each cycle of operation during fermentation process

At the beginning of each cycle, a volume of 20 liter of synthetic wastewater was added during the filling phase, resulting in a HRT of 12 hours. The Mean Cell Retention Time (MCRT) or Sludge Retention Time (SRT) in mixed liquor was 4.9 days to maintain a TSS of 1000 mg/l; 7.4 days to maintain a TSS of 1500 mg/l and 9.9 days to maintain a TSS of 2000 mg/l. The excess sludge was removed during the anaerobic phase each

day. The F/M ratio was ranging from 0.059 $gBOD_5$/gTSS.cycle to 0.165 $gBOD_5$/gTSS.cycle (Table 5.2).

Since the operating temperature was not controlled, it was varying between 18°C to 23°C depending on the season.

The biomass concentration (TSS) and the F/M ratio changed during time to show its influence on the VFA production.

Table 5.2: Variation of the operational parameters during the experimental process of VFA production.

Parameter	Unit	Value 03.11-12.11	Value 01.12-05.12	Value 06.12-11.12	Value 12.12-02.13	Value 03.13-04.13	Value 05.13-06.13
COD influent	mg/l	600	600	60	600	600	600
Total Volum (Vt)	l	35	35	35	35	35	35
Filled volum (Vf)	l	30	30	30	30	30	30
Total suspended solids (TSS)	mg/l	2000	1500	1000	2000	1500	1000
Volatile suspended solids (VSS)	mg/l	1810	1250	865	1700	1275	832
Sludge volume index (SVI)	ml/g	110	95	97	100	100	85
Hydraulic retention time (HRT)	h	12	12	12	12	12	12
Mean cell retention time (θc)	d	9.9	7.4	4.9	9.9	7.4	4.9

5.2.5 Sampling

To determine the VFA composition and the production capacity of VFA within an operating cycle, three samples were taken of about 300 ml each of mixed liquor , at the end of the second, fourth and sixth hour of the anaerobic phase. The mixed liquor samples were allowed to decant and the supernatants were considered the final samples. Additionally, a sample of the effluent of about 250 ml was taken as well. The

samples were taken in transparent polyethylene bottles of 250 ml and filled to the brim to be subsequently stored at 4°C during 24 hours according to the DIN 38414-19 (Deutsches Institut für Normung, 1999), pending analysis.

5.2.6 Samples analysis

The samples were collected, preserved, stored, and analyzed following the guidelines described in the relevant standard DIN Norms of the German Institute for Standardization. The DIN Norms used in the experimental processes are shown in Table 5.3.

Table 5.3: DIN Norms used for collecting, preserving, storing, and analysis of the samples during the experimental process of VFA production.

Parameter	Sampling and analysis	Preservation and storage
COD	DIN 38409-H41-1	DIN EN ISO 5667 – 3 (A21) (Deutsches Institut für Normung, 2004)
TSS	DIN 38414 – S2	
VSS	DIN 38414 – S3	
Organic acids	DIN 38414 – S19	

5.2.7 Stable operational conditions

Broadly speaking, two main factors affect the productivity and stability of the acidogenic phase in anaerobic reactors. These factors are the operational parameters and the characteristics of the organic substrate used in fermentation processess.

Based on previous research, the main operational parameters affecting the productivity of the acidogenic phase in anaerobic reactors are HRT, temperature (T°) and COD concentration in the influent (Dinopoulou et al., 1987; Banerjee, 1997). Other investigators included parameters as organic loading rate (OLR) and pH (Breure and van Andel, 1984; Donányos, 1985).

Previous investigations on the most suitable operational parameters to optimize the productivity of the acidogenic phase were considered. From these investigations, the operational parameters shown in Table 5.4 were controlled in an attempt to show the

influence of the TSS and F/M ratio in mixed liquor on the VFA production, acidification degree (α) and acidogenic potential.

Table 5.4: Constant operational parameters used in the fermentation reactor.

Operational parameter	Unit	Value
pH	----------	* 4.5 - 5.5
HRT	hours	12
Temperature (T°)	°C	* 18 - 23
COD influent	mg/l	600
OLR	mgCOD/l.cycle	400

* Values obtained without controlling the operational parameters.

Research by Dinopoulou et al., (1987) showed the influence of the HRT on the acidogenic phase productivity of organic substrates, reporting that HRT and temperature had the strongest influences on the degree of acidification and acidification potential.

Choosing the right HRT to enhance the VFA productivity was of great importance since it was necessary to minimize the development of the methanogenic bacterial community, as this bacterial group consumes the VFA produced. Sun et al. (2010) suggested that the use of short HRT would not allow the extension of the substrate degradation process, fact confirmed through the low methane production. In turn, Alkaya (2010) evaluated the VFA production using sugar beet wastewater as fermentation substrate and COD influent of 6621 mg/l, obtaining the highest VFA production with HRT of 2 days. Subsequently, the VFA production decreased and the methane production increased significantly when the HRT was extended to 4 days.

Research by Dinopoulous et al. (1987) used beef extract as fermentation substrate and initial COD concentration of 3 g/l, reporting a critical HRT between 6 and 8 hours. With HRT lower than the mentioned, the acidification degree (α) decreased markedly and with higher HRT the acidification degree reached values that did not vary significantly when increasing the HRT.

In the present research, to avoid the VFA consumption at the end of the acidogenic phase by the methanogenic community, the HRT was reduced to 12 hours. Previously, a research by Elefsiniotis & Oldham, (1994) evaluated the VFA production during the acidogenic phase using primary sludge as substrate. The results showed that the VFA production decreased if the HRT was increased to more than 12 hours.

Moreover, a previous research maintained constant the HRT and varied the OLR from 9.4 gCOD/l.d to 72 gCOD/l.d, observing no significant effect on the acidification degree (α) which remained at 56% (Dinopoulous et al., 1987). However, Alkaya and Demirer (2010) evaluated the VFA production using sugar beet wastewater as fermentation substrate and a constant HRT, recording that when increasing the OLR also observed an increase in the VFA production.

With the objective of evaluating the VFA production, this research used synthetic wastewater as substrate with a medium concentration of 600 mg/l COD (Metcalf and Eddy, 1998). With this COD concentration OLR values of 0.1667gDBO5/l.cycle or 0.4 gCOD/l.cycle were obtained, and these values are within the appropiated range for sequential batch reactors (Metcalf and Eddy, 1998).

There is a general consensus about the influence of temperature on acidogenic processes of organic substrates fermenters. Research by Maharaj and Elefsiniotis (2001) observed a decrease of the VFA production rate when the operating temperature decreased from 25°C to 8°C, using as fermentation substrate primary sludge supplemented with starch. However, it was also mentioned that the acidogenic process did not stop even when the lowest temperatures were achieved. In turn, Yuan, et al. (2011) mentioned the drop of the primary sludge fermentation rate, when the temperature decreased from 24°C to 14°C, showing a VFA composition that was unchanged despite the temperature drop. In this latter study, even with 4°C, VFA production was observed. Additionally, Maharaj and Elefsiniotis (2001) suggested that temperature variation (between 8 and 25°C) did not affect the VSS concentration, since this parameter was stable throughout the experimental process.

In the present study, the VFA production was evaluated at room temperature (18°C - 23°C).

pH values, recorded during acidogenic phases in fermentation reactors, vary considerably between investigations. There are contradictions regarding which pH will be the most suitable to increase the VFA productivity. Research by Alkaya and Demirer (2010), reported that the highest VFA production was obtained at a pH of 5.3 (tests without additional external alkalinity) using a mix of sugar beet wastewater and pulp in a 1:1 ratio and with HRT of two days. In this latter research, the highest VFA production was recorded with the lowest pH values. In turn, Silva et al. (2013) reached stable VFA production using as fermentation substrates, cheese whey, sugarcane molasses and OFMSN (organic fraction of municipal solid wastes) separately, recording pHs of 4.4, 4.5 and 5.6, respectively. Despite the low pH values reported by Silva et al. (2013), a

research by Dinopoulou et al. (1987), using beef extract as fermentation substrate, and an HRT of 8 hours, reported high production of VFA (0.829 g/l) with neutral pH.

In the present research, pH values between 4.5 and 5.5 were recorded, using synthetic wastewater with glucose as fermentation substrate, and HRT of 12 hours. The pH values were recorded under steady state conditions. The steady State conditions were reached when a stable VFA production was recorded.

None of the above investigations consider the biomass as an influencing variable on the productivity of the acidogenic phase. In this investigation, keeping constant the operational parameters already mentioned (Table 5.4), the influence of operational parameters directly related to biomass concentration (TSS and F/M rate) was assessed.

5.3 Results and discussion

5.3.1 VFA production within an operational cycle

Initially the influent (Table 5.1) had a COD concentration of 600 mg/l, resulting in a C:N:P ratio of 35:3.8:1.

The initial concentration of TSS was 1000 mg/l, which was then given an adaptation period of 2 months. In each cycle of operation, a specific volume of effluent was discharged, allowing an HRT of 12 hours to be maintained and, therefore, minimizing the development of the methanogenic bacterial community. Additionally, to avoid the development of undesirable microorganisms, a TSS concentration between 1 and 2 g/l was maintained.

The VFA production increased gradually as the bacterial community developed. After 4 months of operation, a VFA production of more than 200 mg/l was recorded. With an anaerobic phase of 6 hours, a mixed liquor sample was taken every two hours and then analyzed for total VFA concentration. Figure 5.2 shows the production of organic acids within a cycle of operation. In this figure, each reactor is labelled by its TSS concentration (g/l) in mixed liquor.

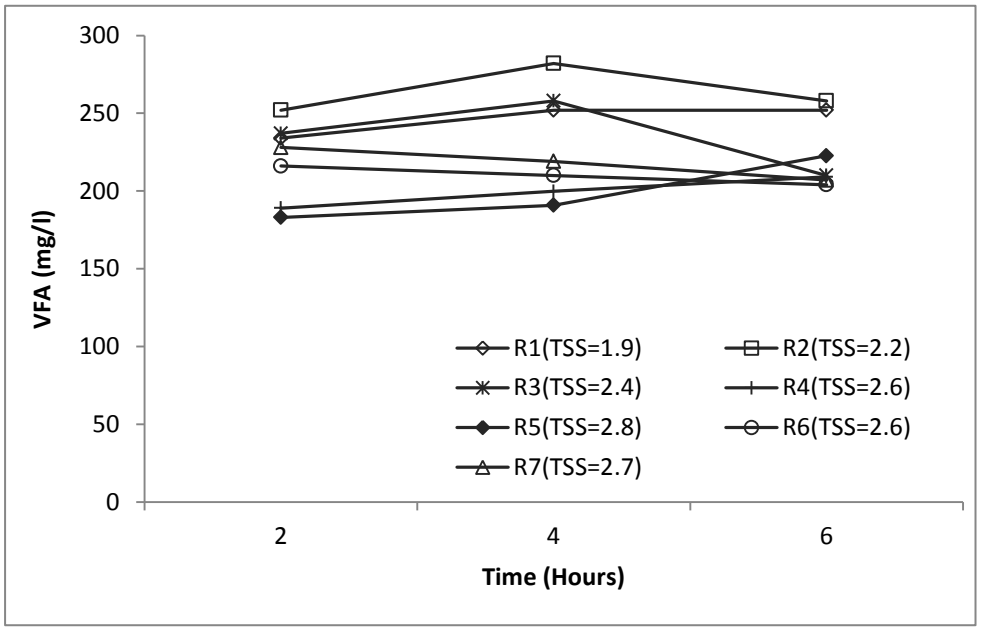

Figure 5.2: VFA production within cycles of operation related to the TSS concentration

VFA concentrations ranged between 175 mg/l and 282 mg/l. In general, it can be seen that once the TSS concentration increases above 2 g/l, the VFA production decreases.

The highest VFA concentrations were recorded during the fourth hour of the anaerobic phase. These results correspond to the lowest TSS concentrations, which ranged between 1.9 g/l and 2.4 g/l (Figure 5.2), and also to the highest F/M ratios, which ranged between 0.069 $gBOD_5$/gTSS.cycle and 0.09 $gBOD_5$/gTSS.cycle (Figure 5.3). At the end of the sixth hour of operation, the VFA concentration decreased gradually, probably because of the consumption of organic acids as a carbon source.

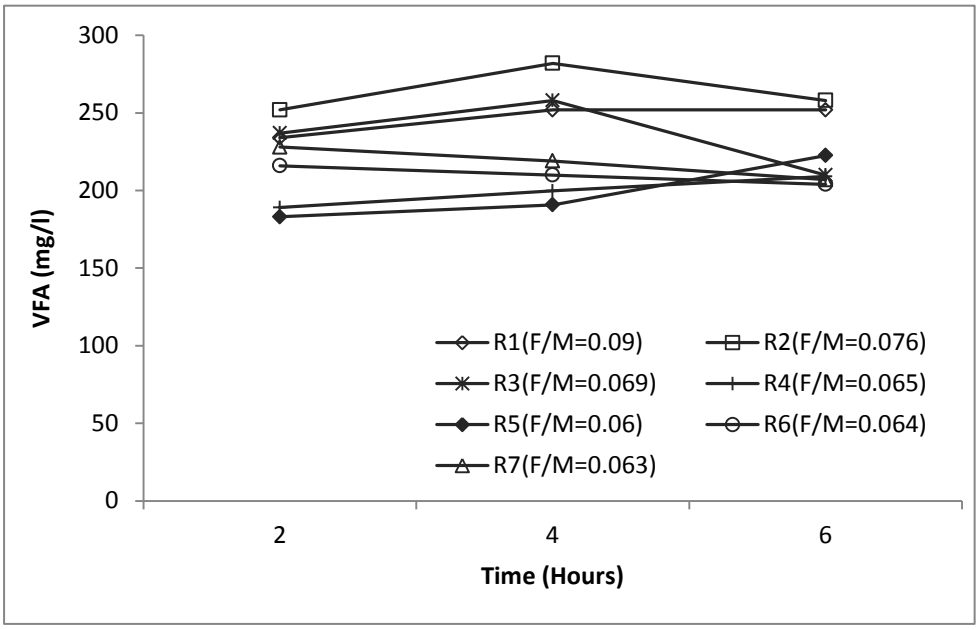

Figure 5.3: VFA production within cycles of operation related to the F/M ratio (gBOD5/gTSS.cycle).

5.3.2. VFA production according to the TSS concentration (g/l) and the F/M ratio ($gBOD_5$/gTSS.cycle)

To show the influence of the TSS concentration on VFA productivity, samples of mixed liquor were taken at the end of the sixth hour of the anaerobic phase in reactors with different TSS concentrations, which ranged between 1 g/l and 2.8 g/l. The VFA concentrations varied between 204 mg/l and 328 mg/l. The highest VFA concentrations were obtained with a TSS concentration of approximately 1 g/l, and as the biomass concentration increased thereafter, the amount of VFA decreased (Figure 5.4). Likewise, the VFA production was directly related to the F/M ratio in mixed liquor, obtaining a VFA concentration of 319 mg/l when one of the highest F/M ratios (0.165 $gBOD_5$/gTSS.cycle) was used.

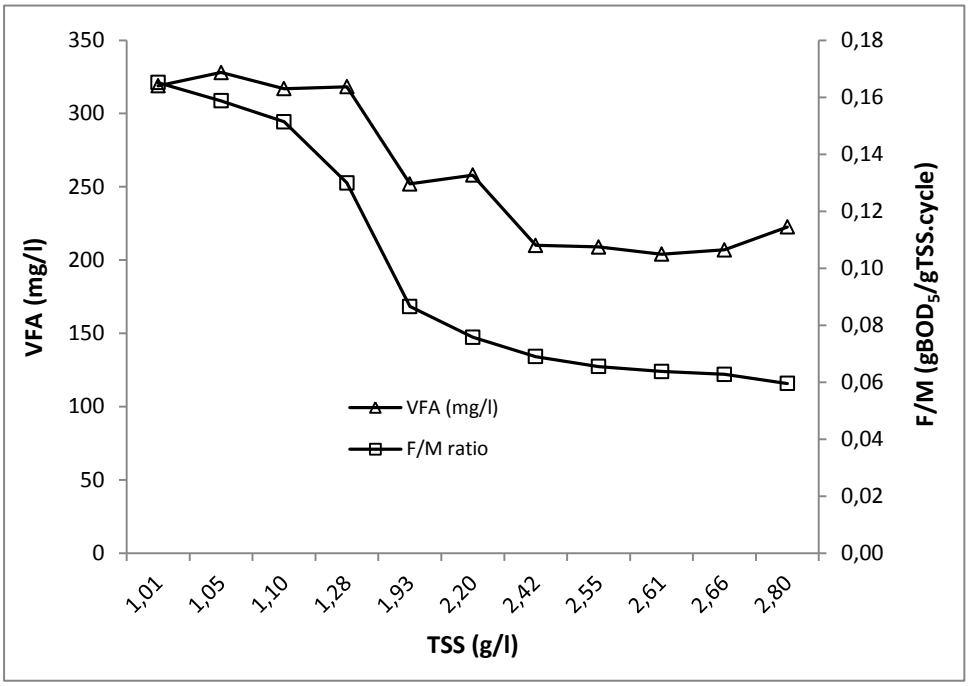

Figure 5.4: Total VFA production at the end of the anaerobic phase against the TSS concentration and the F/M ratio.

The high VFA production obtained with low TSS concentrations could be because the VFA produced might be consumed as a carbon source by the same biomass; this VFA consumption would increase as the TSS concentration increases. Additionally, it is unlikely that the VFA consumption can be attributed to the methanogenic bacteria community since the HRT (12 hours) was calculated to avoid this risk.

The use of low biomass concentrations in fermentation processes is mentioned in research by Skalsky & Daigger, (1995). They suggested that VSS concentrations of less than 10000 mg/l could improve the fermentation performance. In the light of this suggestion, research by Banerjee (1997) and Maharaj and Elefsiniotis (2001) used TSS concentrations of approximately 5000 mg/l. These investigations were conducted using sludge (primary and excess sludge) and industrial wastewater as fermentation substrate. Additionally, Cuevas et al. (1997), using domestic wastewater enriched with molasses as fermentation substrate, TSS concentration of 900 mg/l, COD influent of 500 mg/l, F/M ratio of 1.35 gCODtotal/gTSS.d and HRT of 8 hours, obtained a maximum VFA production of 280 mg/l. In this investigation, the maximum VFA concentration obtained was 328 mg/l, using a TSS concentration of 1.05 g/l, COD influent of 600 mg/l, F/M ratio of 0.381 gCODtotal/gTSS.cycle and HRT of 12 hours.

5.3.3. Influence of the TSS concentration and the F/M ratio on the acidogenic potential (gVFA/gCOD fed) and on the degree of acidification -α (%)

The degree of acidification (α) and the acidogenic potential were used as indicators of stability of the acidification process. The degree of acidification can be defined as the degree of acidogenic success, comparing the amount of solubilised material which was converted to volatile fatty acids (Maharaj, 1999).

The degree of acidification is calculated according to (Alkaya & Demirer, 2010):

$$\% \text{ Acidification degree } (\alpha) = (S_f / S_i) * 100 \text{ …….. } (5.1)$$

where:

- S_i: Initial substrate concentration as COD (mg/l)
- S_f: Net production of VFA, expressed as COD-equivalent (mg/l)

To convert the VFA concentration (mg/l) to COD-equivalent (mg/l), the theoretical equivalences (Table 5.5) reported by Yuan et al. (2011) were used.

Table 5.5: COD-theoretical equivalences for Volatile Fatty Acids according to Yuan et al. (2011)

	C	H	O	
VFA	x	y	z	mgCOD/mgCxHyOz
Acetic acid	2	4	2	1.07
Propionic acid	3	6	2	1.51
Butyric acid	4	8	2	1.82
Isobutyric acid	4	8	2	1.82
Valeric acid	5	10	2	2.04
Isovaleric acid	5	10	2	2.04
Caproic acid	6	12	2	2.21
Isocaproic acid	6	12	2	2.21
Heptanoic acid	7	14	2	2.34

The acidogenic potential is defined as the maximum VFA concentration obtained at the end of an anaerobic fermentation process (Ruel et al., 2002). This calculation does not consider the metabolic participation of the methanogenic bacterial community.

The results of the degree of acidification and acidogenic potential in relation to the TSS concentration and the F/M ratio are shown in Figure 5.5. These results correspond to VFA concentrations (mg/l) obtained at the end of the anaerobic phase. As shown in Figure 5.5, the lowest TSS concentration (1.05 g/l) and highest F/M ratio (0.159 $gBOD_5$/gTSS.cycle) corresponded to the highest values of degree of acidification (53.1%) and acidogenic potential (0.722 gVFA/gCOD).

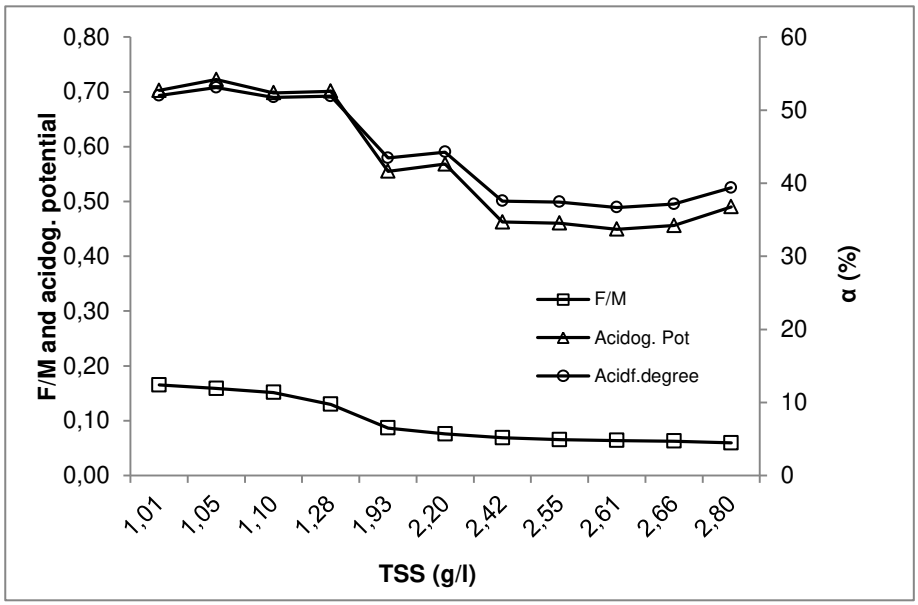

Figure 5.5: Degree of acidification (α-%) and acidogenic potential (gVFA/gCOD) against the TSS concentration (g/l) and the F/M ratio (gBOD5/gTSS.cycle) in mixed liquor.

Additionally, the highest acidogenic potential values (> 0.5 gVFA/gCOD) were obtained with TSS concentrations of less than 2.4 g/l. After this, the acidogenic potential decreased to 0.463 gVFA/gCOD when a TSS concentration of 2.42 g/l was used. Research by Silva et al. (2013) using organic waste as fermentation substrates (cheese whey, sugarcane molasses and organic fraction of municipal solid wastes-OFMSW), VSS concentration of 2 g/l and influent COD concentration of 8000 mg/l, resulted in degress of acidification which varied between 31% and 39.8% and acidogenic potential values between 0.3 and 0.4 gVFA/gCODfed. These results are consistent with the results obtained in the present research using a TSS concentration of 2.2 g/l.

In turn, Dinopoulou et al. (1987) obtained acidification degrees between 30% and 60%, using beef extract as fermentation substrate, HRT of 6 hours, COD influent of 3 g/l and very low TSS concentrations (144 - 164 mg/l). Comparing the results of Silva et al. (2013) and Dinopoulou et al. (1987) to the results here, it is possible to estimate that in both previous investigations the F/M ratios were quite high. However, the present study

also used lower TSS concentrations, which produced better values of degree of acidification.

Arroja et al. (2012) reported significant differences for the degree of acidification obtained with two different bacterial communities (acidogenic biomass and conventional mixed anaerobic biomass). This research used sugarcane molasses as fermentation substrate, HRT of 12 hour and OLRs which were gradually increased up to approximately 35 gCOD/l.d. The degree of acidification obtained by the acidogenic community was 28%, while the conventional mixed anaerobic biomass obtained a maximum degree of acidification of 14%.

In the present research, the high degrees of acidification obtained are strongly related to low TSS concentrations (1 g/l) and to F/M ratios of approximately 0.16 $gBOD_5$/gTSS.d. Additionally, the acidogenic bacterial community, selected from the HRT, was a crucial factor in obtaining these results. This acidogenic bacterial community had a typical creamy white color due to the low concentration of sulphate in the synthetic wastewater and to suppression of the sulphate-reduction activity due to the operational pH between 4.5 and 5.5 (Liu & Fang, 2002). These conditions were not suitable for the development of the sulfate-reducing bacterial community (Herbert et al., 2002).

5.3.4 Influence of TSS concentration and F/M ratio on VFA composition.

Several investigations have tried to determine the factors that influence VFA composition in the acidogenic process of organic substrates. These investigations evaluated the influence of operational parameters such as HRT, OLR, pH, temperature, COD influent and type of organic substrate used.

The influence of HRT on VFA composition is not clear and different investigations have produced different results. Cohen et al. (1984) reported that HRT has a significant influence on VFA composition, while Breure and Van Andel (1984) stated that this influence was negligible. Meanwhile, Dinopoulou et al. (1987), using meat extract as fermentation substrate, reported that the proportion of acetic acid increased when the HRT and influent COD increased simultaneously. Additionally, in this study the proportion of propionic acid decreased as the HRT increased. However, the authors mentioned that in no case could a quantitative relationship be statistically derived.

On the other hand, research by Dinopoulou et al. (1987) evaluated the influence of temperature on the VFA composition. This research reported that a gradual increase of

temperature from 25°C to 40°C did not significantly influence the composition of the VFA. Only a slight decrease in the percentage of propionic acid was observed as the temperature increased, and in turn, a slight increase in the proportion of acetic acid.

The results of the analysis of the spectra of the acids in the present research are shown below (Table 5.6).

Table 5.6: VFA composition in supernatant at the end of the anaerobic phase of the fermentation process.

Acetic acid (mg/l)	Propionic acid (mg/l)	Butyric acid (mg/l)	VFA Total* (mg/l)
180	17	46	259
200	63	36	318
220	33	78	351
240	28	69	354
210	34	92	358

* VFA total also includes acids present in low concentrations such as Valeric acid and/or Isovaleric acid.

Figure 5.6 shows the influence of the increase of the F/M ratio and the TSS concentration on the VFA composition at the end of the acidogenic phase. It can be seen that as the F/M ratio increased the VFA production increased also. Similar results were reported in section 5.3.2. However, the VFA composition in each sample did not vary significantly. Generally, acetic acid (58.7% - 69.5%) was the dominant VFA, followed by butyric acid (11.3% - 25.7%), and finally propionic acid (6.6% - 19.8%). The set of organic acids of high molecular weight (valeric acid, isovaleric acid) recorded very low and stable concentrations (4.8% - 6.2%). It is worth noting that the only results where propionic acid was recorded as the second most abundant organic acid was when the highest operating temperature (23°C) was reached.

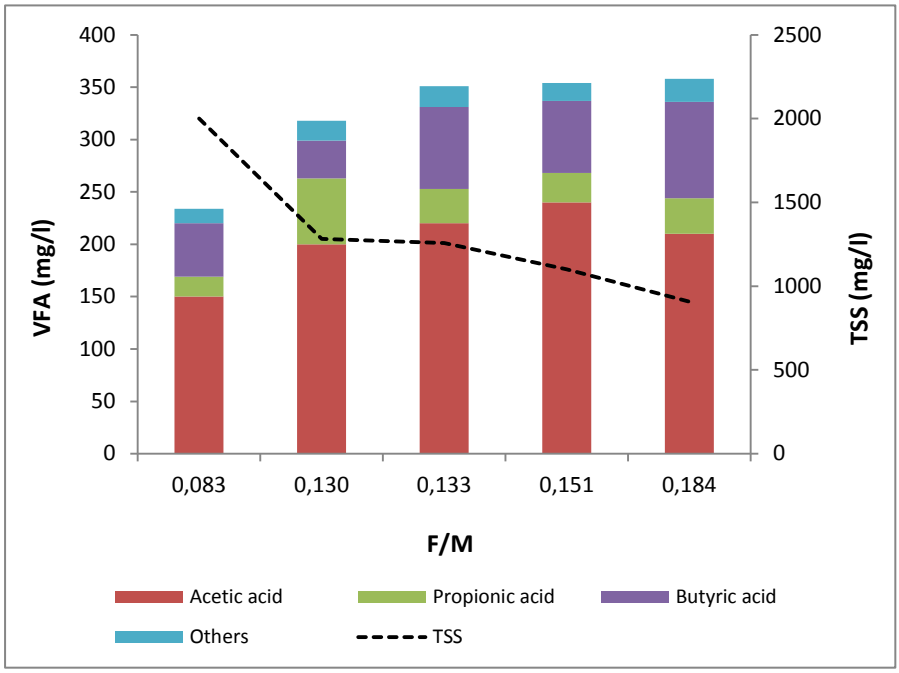

Figure 5.6: VFA composition at the end of the anaerobic phase against the F/M ratio and the TSS concentration in mixed liquor.

In turn, pH values between 4.5 and 4.8 were recorded when butyric acid was the second most abundant VFA produced, and a pH of 5.5 was recorded when butyric acid concentration decreased and the second most abundant VFA produced was in fact propionic acid (Figure 5.7).

Likewise, in research by Silva et al. (2013), using sugarcane molasses as substrate, the most abundant VFAs obtained were acetic, butyric and propionic acid.This research confirmed that the synthesis of long chain VFAs occurs at low pHs. Additionally, Albuquerque et al. (2007), investigated the fermentation of molasses, observing that when pH decreased from 7 to 5, the concentration of acetic and propionic acids decreased, and the concentration of butyric and valeric acid increased.

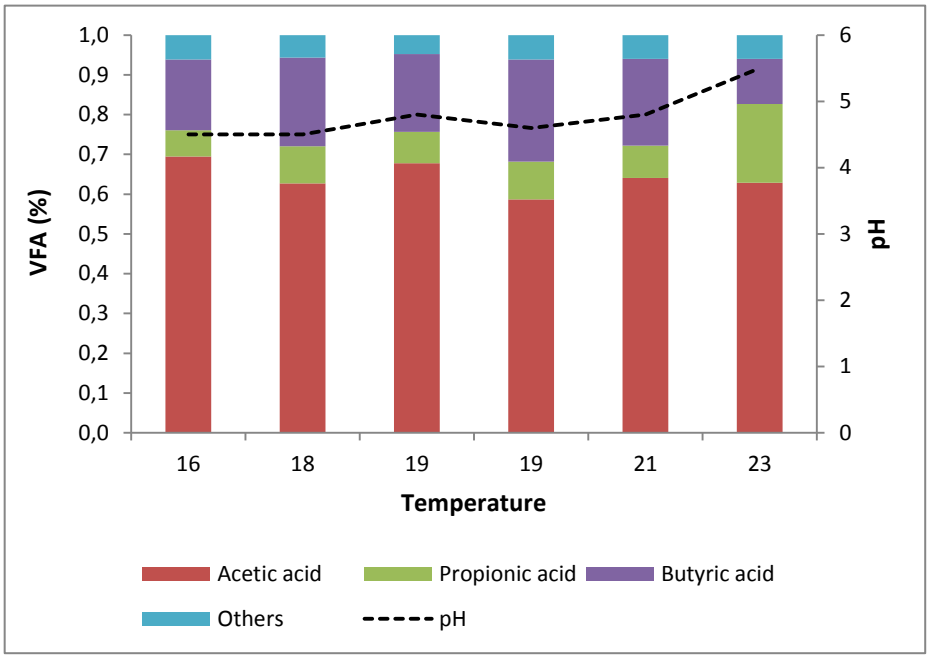

Figure 5.7: VFA composition at the end of the anaerobic phase against temperature and pH in mixed liquor.

Dinopoulou et al. (1987) investigated the influence of certain operational parameters on the VFA composition using meat extract as fermentation substrate. The most abundant VFAs obtained were acetic acid (45%) and propionic acid (40%), while butyric and valeric acid were present in concentrations between 4% and 17%. These proportions showed no variations as pH and temperature varied. Additionally, the authors noted that an increase of the influent COD influenced the concentration of propionic acid (R = 0.96) and an increase of the HRT and influent COD influenced the concentration of acetic acid (R = 0.88).

Apparently, none of the operational parameters mentioned so far have had a clearly observable influence on the VFA composition of acidogenic processes. However, several investigations mentioned that the VFA composition might be determined by the type of fermentation substrate used. Research by Cohen et al. (1979), Breure and van Andel, (1984), and Dohányos et al. (1985) evaluated the acidogenic process using glucose, sucrose, starch, pectin and gelatin as fermentation substrates, concluding that VFA composition is dependent on the substrate used.

Regarding glucose as a fermentation substrate, Alkaya and Demirer (2010) mentioned that the VFA composition obtained from carbohydrate fermentation included acetic, propionic and butyric acids. These correspond to the principal VFAs obtained in the present research. However, the high rates of acidogenic potential and high degrees of acidificationrecorded here were achieved using the most suitable HRT, TSS and F/M ratios.

5.4 Conclusions

1. With a reaction time of six hours during the anaerobic phase, the highest VFA production was recorded at the end of the fourth reaction hour. With operational conditions as TSS of 1 mg/l, F/M ratio of 0.381 gCOD/gTSS and HRT of 12 hours, and using glucose as fermentation substrate, it may be concluded that the anaerobic phase length may be reduced to four hours.

2. A total suspended solids concentration (TSS) in mixed liquor of about 1 g/l and F/M ratio of approximately 0.4 gCOD/gTSS were the most appropriate operational parameters to achieve the highest production of VFA. Further, increasing the TSS concentration in mixed liquor was detrimental to the VFA production.

It may therefore be concluded that to obtain good VFA production in fermentation processes, the substrate requirements of the biomass should be considered in order to avoid the consumption of the VFA by the same biomass.

3. Variations in the operational parameters TSS (g/l) and F/M ratio ($gBOD_5$/gTSS) showed no influence on the VFA composition at the end of the reaction time.

4. It is possible to promote the development of the acidogenic bacterial community when the HRT is kept constant within limits that do not permit the completion of the fermentation process.

The VFA production of the fermentation reactor was used as a carbon source in a subsequent study (Chapter 7) which aimed to develop different EBPR bacterial communities using different composition in the influent.

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CHAPTER 6: Assessment of the accumulation of PHA and phosphorus in EBPR bacterial communities using staining techniques and Scanning Electron Microscopy (SEM) coupled with Energy-Dispersive X-ray spectroscopy (EDX)

6.1 Introduction

In the present research, the changes observed in the storing of intracellular polymers in different Bio-P bacterial communities were used to assess the stability of the EBPR process.

Among the methodologies used to identify intracellular polymers in EBPR communities, light microscopy may serve to visualize intracellular polyphosphate granules (volutine granules) using specific staining techniques such as the Neisser staining technique, or to identify intracellular polyhydroxyalkanoates (PHA) granules using the PHA technique (sudan black).

Because of the incomplete information of the identity and abundance of microorganisms in EBPR processes, the possibility of linking specific morphotypes with their storage capacity of intracellular substances is still useful. With respect to the morphotypes commonly found in activated sludge samples, EBPR bacterial communities are composed of a mixed community with equitable composition of coccus-, rod- and bacillus-shaped cells (Jeon et al., 2000). Besides identifying the basic features of the morphotypes in EBPR systems, in this research the identification of the Tetrad Forming Organisms (TFO) and coccus-shaped bacteria with high phosphorus accumulation capacity was emphasized since these morphotypes were identified as possibly related to either the socalled G-Bacteria (Sudiana et al., 1998) or, respectively, Polyphosphate Accumulating Organisms (PAO) (Jeon et al., 2000).

According to the metabolic pathway proposed by Mino et al. (1987), the PAO bacteria can uptake carbon sources as VFA and to store it as polyhydroxyalkanoates (PHA) during the anaerobic phase. The energy required for this process can be met by glicolysis of a portion of the stored glycogen and a portion of the stored polyphosphate chains. Because of the degradation of the polyphosphate molecules, a portion of the phosphate ions will be released to the aqueous phase. Thus, at the end of the anaerobic phase it should be possible to visualize intracellular PHA granules and, at the same time, a small number of polyphosphate granules. Subsequently, during the aerobic phase, the PAO cells that are in the presence of oxygen could degrade the stored PHA molecules to use it in growth and reproduction processes. The catabolism

of the PHA may provide the proton motive forces (PMF) that would be used for growth and replenishment of polyphosphate stores (Seviour and Nielsen, 2010). Finally, at the end of the aerobic phase, the intracellular polyphsosphate granules can be observed, and also either the presence of only a small amount or the absence of PHA.

It is important to identify intracellular polymers in the TFO microorganisms since a possible relation between this morphotype and PAO competitors has been proposed. These microorganisms may compete with PAO for the carbon source during the anaerobic phase, but during the aerobic phase they will be unable to store polyphosphate, impairing the EBPR process. Cech and Hartmann (1993) identified a morphotype described as tetrad-arranged cocci, called G-bacteria. Sudiana et al. (1998) reported that the tetrads had the capacity to store lipophilic inclusions (possible PHA), which they determined using the staining techniques sudan black B and nile blue A. Additionally, the absence of intracellular polyphosphate granules in the tetrads was reported in several investigations (Cech and Hartman, 1990; Blackall et al., 1997; Sudiana et al., 1998). In order to confirm that the G-bacteria with the TFO morphotype were PAO competitors, Cech and Hartman (1993) isolated one of the tetrad-arranged cocci microorganisms, which was identified as *Amaricoccus kaplicensis* (Maszenan et al., 1997). However, Falvo et al. (2001) showed that this microorganism was not a PAO competitor since it was not able to uptake acetate or glucose as substrate.

Further molecular studies (Nielsen et al., 1999a) on a bacterial community which recorded a deficient EBPR process revealed the presence of a bacterial group (35% of the total bacterial community) with no polyphosphate accumulation capacity. These microorganisms were related to a phylogenetic group called a cluster novel of *Gammaproteobacteria* and described as a big coccus of about 3 to 4 micrometers in diameter. Subsequent investigations showed the impossibility of relating the TFO morphotype to the behavior of a PAO competitor (Seviour et al., 2000; Blackall et al., 2002). GAO (glycogen accumulating organism), so-called by Mino et al. (1995), are identified on the basis of a biochemical behavior of a microorganism group that competes with PAO for the carbon source during the anaerobic phase but without phosphorus assimilation during the aerobic phase. Thus, GAO is a classification based on a biochemical behavior while the TFO classification is based on a morphological description of a bacterial group which may or may not be related to the competitor group of PAO. Moreover, a GAO bacterium, called *Candidatus Competibacter phosphatis* (Gammaproteobacteria), has been identified that does not have a TFO morphotype (Crocetti et al., 2002).

However, it is not possible to determine the metabolic role of the TFO morphotype in the EBPR processes since this morphotype may include microorganisms from different phylogenetic groups with different metabolic strategies.

In fact, these staining techniques are still usable for monitoring the performance of the EBPR processes, although only visual. Techniques to visualize the intracellular PHA using sudan black and the intracellular polyphosphate granules using methylene blue may confirm that the EBPR process is functioning well, but it should be also considered that the phosphorus removed may not all correspond to the EBPR metabolic process.

Investigations have shown that, in EBPR processes, the phosphorus accumulation on the bacterial biomass is not restricted to the intracellular environment of the PAO bacterial group but that other locations in the cell or in the floc should be considered as well. In these locations, the phosphorus can be deposited through metabolic or adsorption processes. Previous studies reported that, although the TFO microorganisms were not able to store intracellular polyphosphate, their cell envelope recorded a positive staining reaction to polyphosphate in some cases (Cech and Hartman, 1990; Blackall et al., 1997; Sudiana et al., 1998). Additionally, He et al. (1996), using transmission electron microscopy (TEM) coupled with energy dispersive x-ray spectroscopy (EDX), suggested the possibility of orthophosphate adsorption as iron hydroxide on the extracellular polymeric substances (EPS) of the activated sludge floc. Finally, Oosthuizen and Cloete (2001), using scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX), recorded that a considerable amount of phosphorus was removed through adsorption processes on the EPS of the activated sludge flocs, so much so that 30% of the phosphorus content in the sludge biomass corresponded to the EPS.

In the present research, the main objective of the SEM/EDS analysis is to identify the principal morphotypes in the sludge samples and to determine which morphotype is associated with the highest phosphorus accumulation capacity.

6.2 Material and methods

The sludge samples were taken from the S and B reactors at the end of the anaerobic and aerobic phases. Additional sludge samples were taken from the aeration tank of the Lüneburg WWTP (Lüneburg-BS sludge sample). The staining techniques used to identify the intracellular storage polymers were the Neisser staining technique with chrysoidine Y as the contrast solution (Merck 9240) for intracellular polyphosphate granules, and the PHA staining technique with sudan black B (IV) 0.3% in ethanol (60%) to identify intracellular granules of PHA. Detailed descriptions of the staining techniques are presented in Chapter 4. The prepared slides were observed with a Zeiss light microscope and the images were captured using a USB camera from the brand BMS Systems with the Scopephoto software. The samples were observed using the oil-immersion objective (1000 X).

Additionally, sludge samples from the S reactors were taken at the end of the aerobic phase to perform SEM/EDX analysis in order to determine the phosphorus accumulation capacity of the principal morphotypes.

Each sludge sample was pretreated before performing the SEM-EDX analyses. The flowchart in Figure 6.1 shows the pretreatment procedure. This pretreatment was performed with the aim reduce the potential distortion of the EPS in the floc in order to maintain the phosphorus composition at exocellular level. The sludge samples were pretreated following a centrifugation procedure (10000 rpm for five minutes) in which they were rinsed three times using a solution of sterile double-deionized water and centrifuged each time. Once the sludge samples dried, these were transported to the Electron Microscopy Laboratory of the Osnabruck Hochschule (Niedersachsen-Germany). In the laboratory, the dried sludge samples were cut into small pieces and set on high purity carbon stubs before being coated under vacuum with a layer of high purity carbon.

For the electron microscopy analysis, a Zeiss- EVO MA10 Scanning Electron Microscope was used, coupled to an Oxford Inca Energy 250 EDX system.

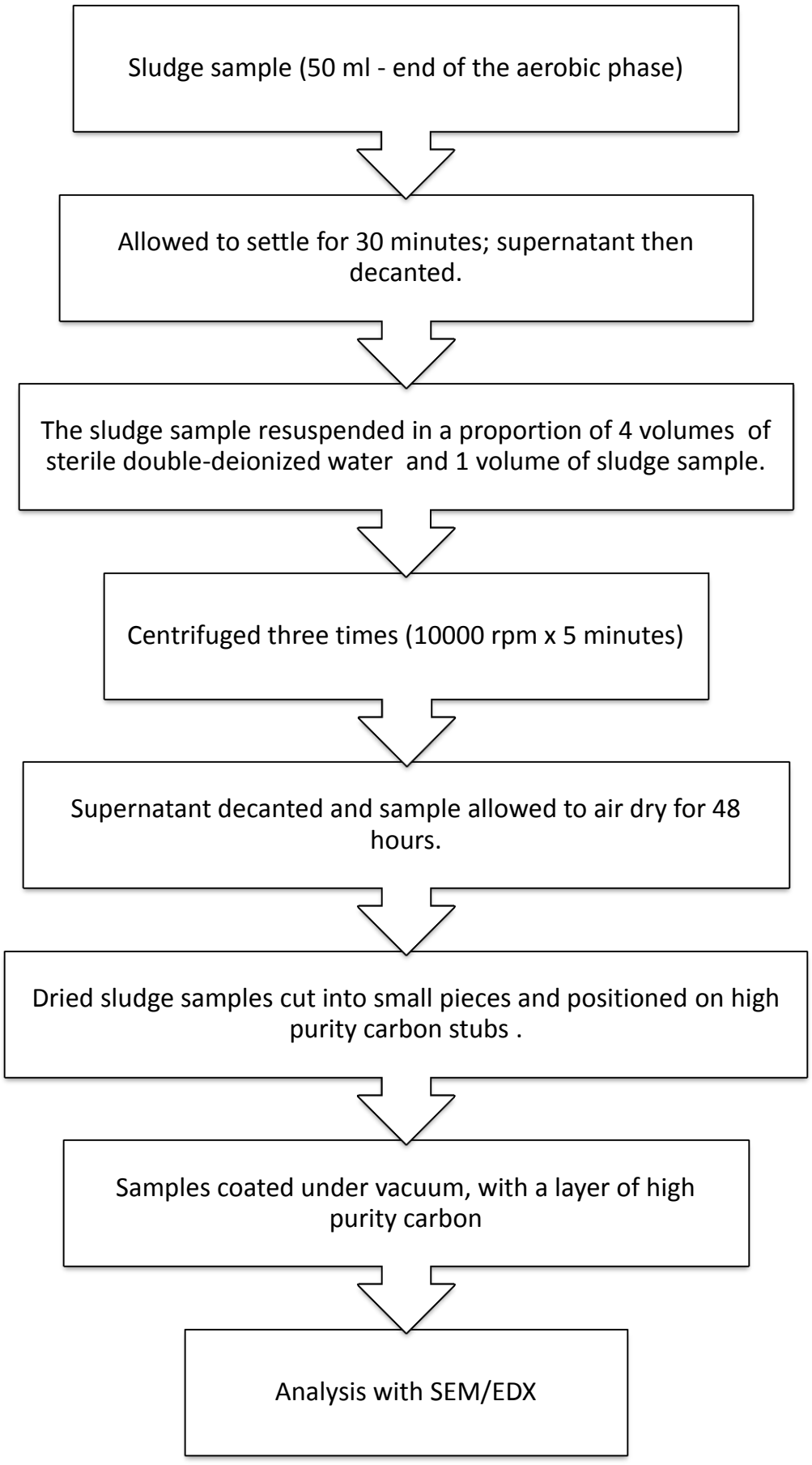


Figure 6.1: Flowchart of the pre-procedure for the SEM/EDX analyses.

6.3 Results and discussion

6.3.1. Identification of the principal bacterial morphotypes in the sludge samples using light microscopy

Figure 6.2 shows the basic appearance of each bacterial community in the sludge samples. For the morphological characterization, the slides from the PHA staining technique were used because of the better contrast and resolution of these images.

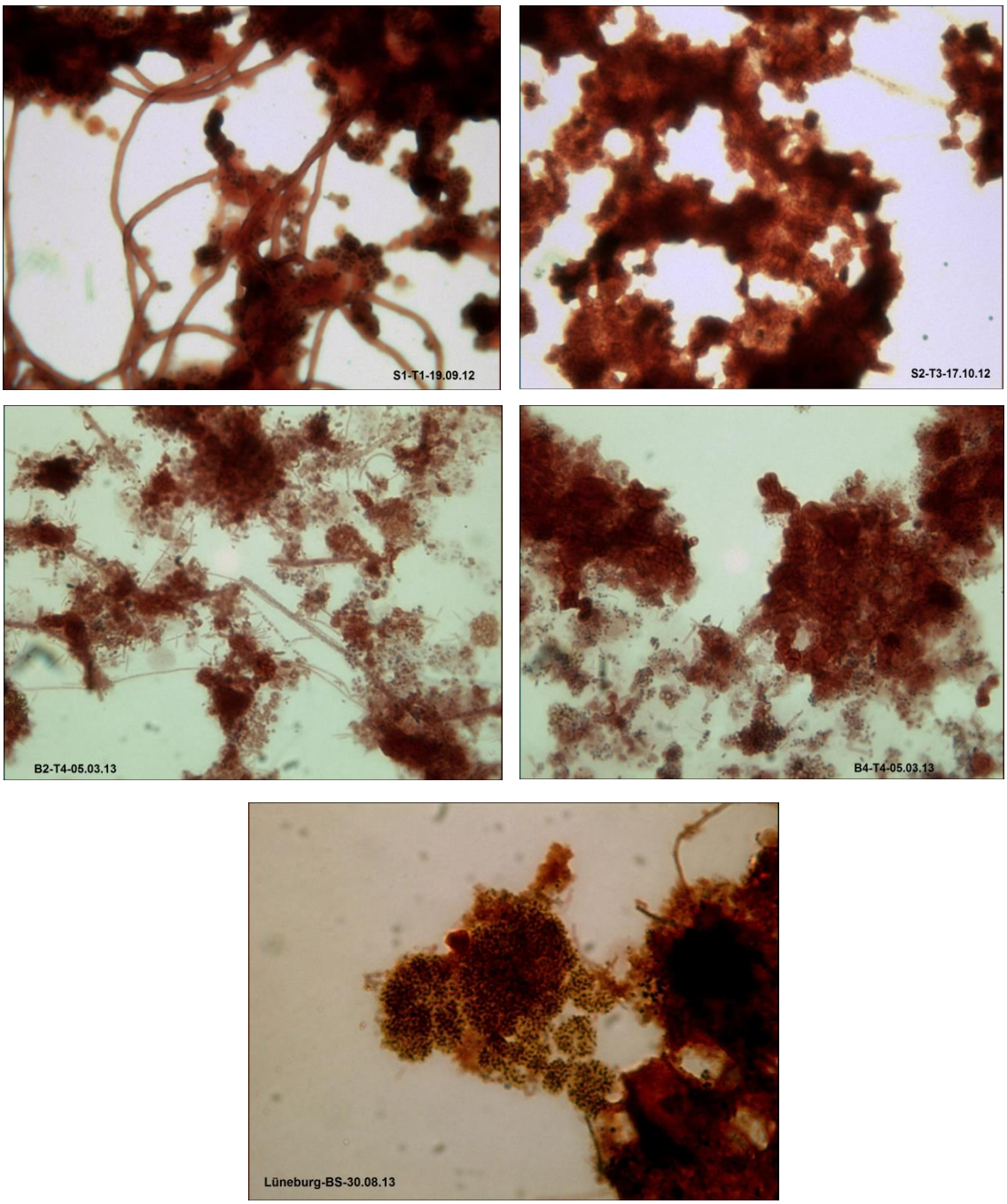


Figure 6.2: Basic appearance of the bacterial communities in the activated sludge samples using the sudan black staining technique (1000 X).

Figure 6.2 shows that the S1 bacterial community was dominated by the TFO morphotype but other morphotypes, such as filamentous and coccus bacillus, were also identified. The dominant filamentous bacteria corresponded to the genus *Nostocoida* (Kunst et al., 2000) but the concentration of these microorganisms did not adversely affect the settling process. The S2 bacterial community showed a similar composition to that observed in the S1 sludge sample, but the concentration of filamentous bacteria was significantly lower. Because of the dominance of the TFO morphotype in the S reactors, these sludge samples are refered to as the TFO or glucose bacterial community. The dominance of the TFO morphotype has been reported in: reactors fed with glucose as carbon source (Jeon et al., 2000), in reactors fed with a mix of glucose and acetate (Cech and Hartmann, 1993), and in reactors fed with wastewater containing high concentration of carbohydrates (Tsai and Liu, 2002). Other research relates the use of glucose as a carbon source to the reduction of the EBPR performance (Cech and Hartmann, 1990, 1993; Rustrian et al., 1996). Meanwhile, however, other research has recorded good EBPR performance using glucose as the sole carbon source (Tracy and Flaminno, 1987; Carucci et al., 1994). According to Jeon and Park (2000), two groups of microorganisms are involved in achieving good EBPR performance in reactors fed with glucose. One of these groups is the LPO morphotype (lactic acid producing organism) which uptakes glucose and simultaneously stores glycogen. To cover the energy demand of this process, the cell metabolizes part of the glucose through glycolysis, obtaining in turn not only energy but also lactate. Subsequently, the glycogen is converted into PHA and storage polymers. The second group of microorganisms is the polyphosphate-accumulating organism (PAO), which uptakes the lactate, converting it into PHA and simultaneously releasing orthophosphate during the anaerobic phase. Finally, PAO microorganisms accumulate polyphosphate intracellularly during the aerobic phase.

Unfortunately Jeon and Park (2000) did not have enough microbiological evidence to support the metabolic pathway they proposed. Recently, however, Wong and Liu (2007) have reported that *Defluviicoccus vanus* related GAO microorganism was able to uptake glucose directly and according to Seviour and Nielsen (2010) *Defluviicoccus* shows the TFO morphotype in FISH probes. This offers some support for the model of Jeon and Park (2000). In the present research, even with the dominance of the TFO morphotype, good EBPR performances were achieved, but clarifying the metabolic pathways behind this process is difficult since the microorganisms involved are still unknown.

Figure 6.2 also shows the microscopic appearance of the B2, B4 and Lüneburg-BS sludge samples. These sludge samples corresponded to EBPR bacterial communities fed with a mix of VFA, propionic acid, and domestic wastewater, respectively. These bacterial communities recorded typical EBPR performance, releasing and accumulating polymers between the anaerobic and the aerobic phases. The microscopic structure of these sludge samples corresponds to a mixed community consisting of coccus, rod, bacillus, cuboidals (Jeon et al., 2000) and filamentous bacteria. Additionally, none of the mentioned morphotypes was observed to be dominant in any case. These mixed bacterial communities are called EBPR communities in this research.

6.3.2 Storage of intracellular polymers during the anaerobic and the aerobic phases.

The confirmation of the release and storage of intracellular polymers, as part of the EBPR process, is described below. In each reactor, a sludge sample was taken at the end of the anaerobic and aerobic phases. These samples were subjected to staining processes to identify intracellular PHA granules using the PHA staining technique, and to identify intracellular polyphosphate granules using the Neisser staining technique. A positive reaction for the Neisser staining reaction is the presence of intracellular black-purple granules that are distinguishable from each other, and this indicates that the intracellular phosphorus concentration is not so high. In the case that the concentration of intracellular phosphorus is high, for example at the end of the aerobic phase, the whole cell may be stained black-purple (Lindrea et al., 1999). In turn, a positive reaction for the PHA staining technique is described as intracellular blue-black granules on a clear (sometimes slightly colored) background. It should be noted that Sudan black is a lipophylic dye and can dye all intracellular lipid material, among which is usually PHA (Bartholomew, 1981; Murray et al., 1994; Seviour and Nielsen, 2010). For the TFO morphotype, the staining reaction observed at the end of the anaerobic phase, using the PHA staining technique, was the presence of intracellular black-blue granules and, additionally, the whole intracellular space was stained with a blackish color. This coloration was not easily observable at the end of the aerobic phase and, therefore, a relation was established between this staining reaction and the typical PHA staining reactions.

Figure 6.3 shows the results of the PHA and Neisser staining techniques for the S1, S2, B2 and B4 sludge samples at the end of the anaerobic phase.

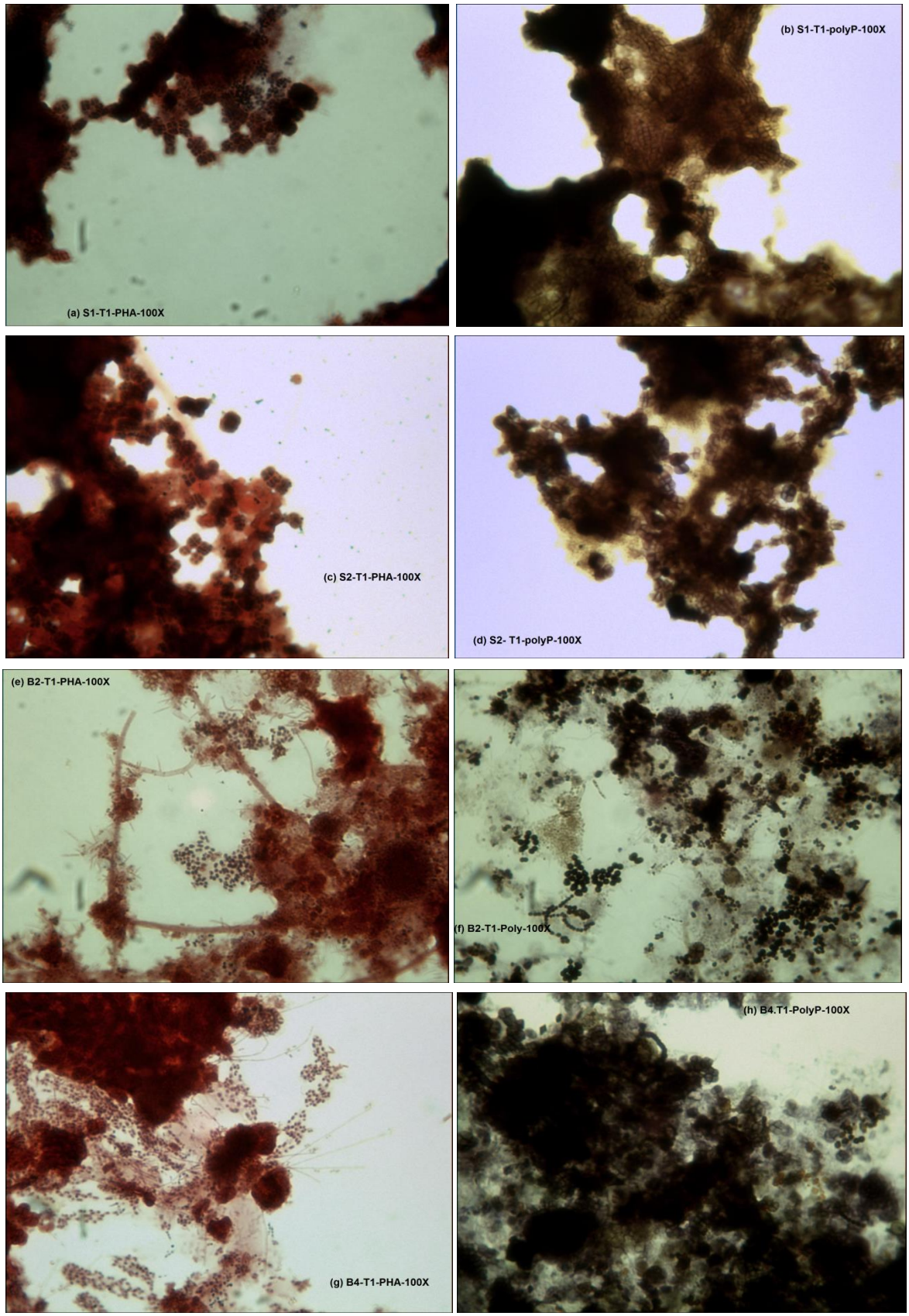


Figure 6.3: Results of the PHA (6.3.a, 6.3.c, 6.3.e, 6.3.g) and Neisser (6.3.b, 6.3.d, 6.3.f, 6.3.h) staining techniques for the S1, S2, B2 and B4 sludge samples at the end of the anaerobic phase.

Figures 6.3.a, 6.3.c, 6.3.e, and 6.3.g show the results of the PHA staining technique at the end of the anaerobic phase for the S1, S2, B2 and B4 sludge samples, respectively. Figures 6.3.a (S1) and 6.3.c (S2) show the dominance of the TFO morphotype as well as the positive reaction for the PHA staining technique. In the TFO morphotype, the whole intracellular space was stained blue-black and areas with blue-black granules on a clear background were also observed. In Figures 6.3.e (B2) and 6.3.g (B4), a positive reaction for the PHA staining technique was also observed but with the form of intracellular granules on the coccus bacillus morphotype. In these sludge samples, the absence of a positive reaction with the PHA technique in the TFO morphotype should be noted, since this reaction confirms the difficulty this bacterial group has uptaking VFA as a carbon source during the anaerobic phase.

Figures 6.3.b, 6.3.d, 6.3.f, and 6.3.h show the results of the Neisser staining technique for the S1, S2, B2 and B4 sludge samples, respectively, at the end of the anaerobic phase. In Figures 6.3.b (S1) and 6.3.d (S2) it can be seen that the TFO morphotype does not show intracellular polyphosphate granules but an apparently positive Neisser reaction in the cell surface can be observed, as other research has also reported (Cech and Hartman, 1990; Blackall et al., 1997; Sudiana et al., 1998). In Figures 6.3.f (B2) and 6.3.h (B4) the black-purple reaction was observed only in the cell surface of the TFO morphotype and in some coccus bacillus, which were stained blackish.

Figure 6.4 shows the results of the staining tests using the Neisser and PHA staining techniques for the S1, S2, B2, and B4 sludge samples at the end of the aerobic phase. Figures 6.4.a (S1), 6.4.c (S2), 6.4.e (B2), and 6.4.g (B4) show the results using the PHA staining technique. The S1 and S2 sludge samples showed positive reaction for the PHA staining technique in the coccus bacillus morphotype, while the TFO morphotype did not show intracellular accumulation of PHA granules. Additionally, the B2 sludge sample showed no positive PHA reaction, but in the B4 sludge sample some coccus bacillus showed intracellular granules of PHA.

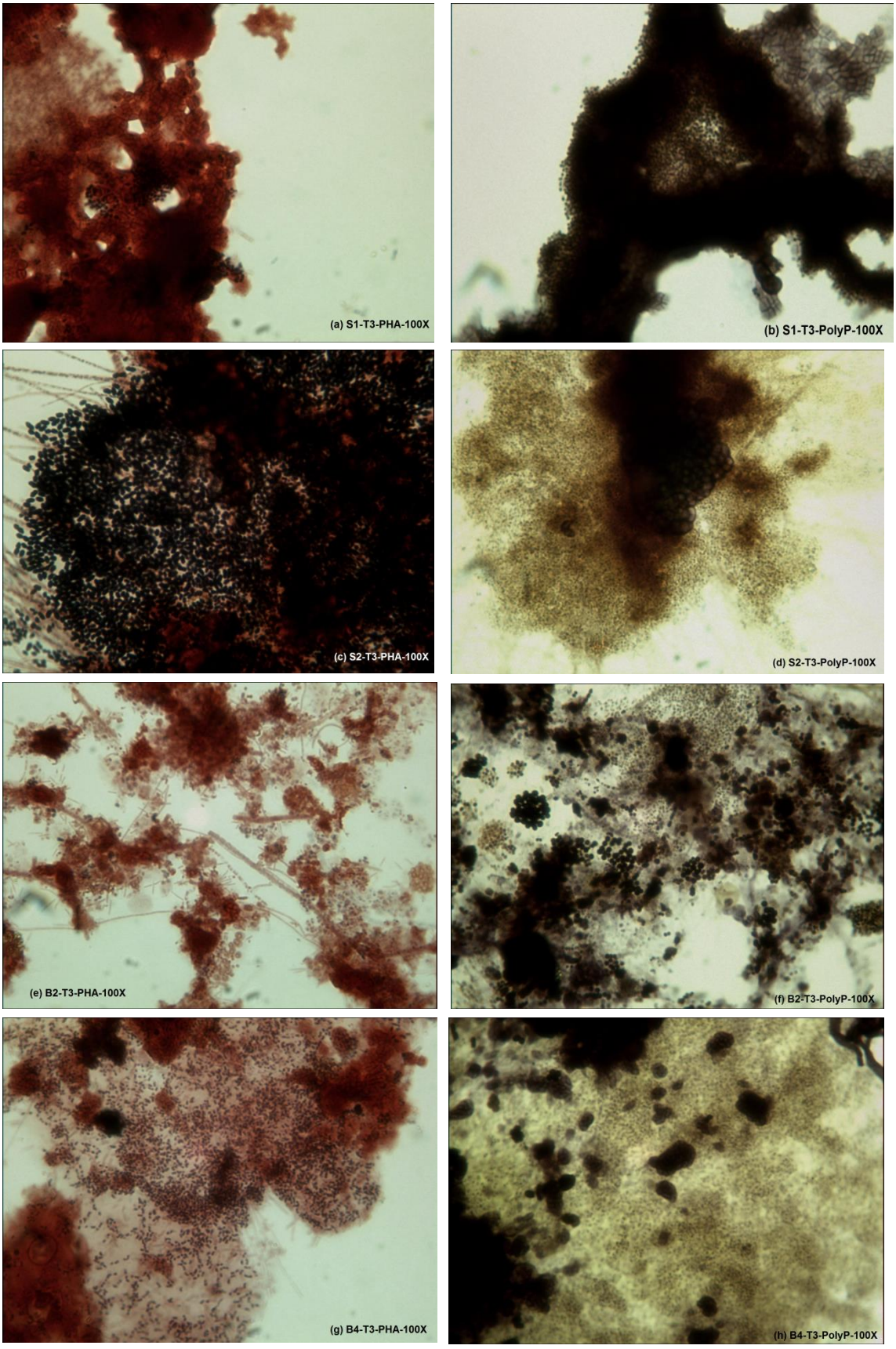


Figure 6.4: Results of the PHA (6.4.a, 6.4.c, 6.4.e, 6.4.g) and Neisser staining techniques (6.4.b, 6.4.d, 6.4.f, 6.4.h) for the S1, S2, B2 and B4 sludge samples at the end of the aerobic phase.

The S1 sludge sample (Figure 6.4.a) showed a positive PHA staining reaction of blue-black granules on a clear background, but specific morphotypes did not show any reaction to the sudan black stain. Additionally, for the same bacterial community, a positive Neisser staining reaction was also observed (Figure 6.4.b) as intracellular black-purple granules in the coccus bacillus morphotype.

The S2 sludge sample showed a positive PHA staining reaction (Figure 6.4.c) in the coccus bacillus morphotype, although this sample was taken at the end of the aerobic phase. In reviewing the phosphorus removal performance in the S2 sludge sample, an inefficient EBPR process was reported for this sampling date, with phosphorus content in the effluent up to 2.3 mg/l recorded. Comparing the results of the S2 and S1 sludge samples, a positive PHA staining reaction was also reported in the latter but with less intensity, and the S1 reactor also recorded a phosphorus concentration in the effluent up to 0.4 mg/l. The reason for the failure of the EBPR process in the S2 sludge sample lies in an operational problem reported for this reactor, which had been in an anaerobic phase for more than the previous 48 hours. Meanwhile, the consumption of the PHA previously stored during the anaerobic phase was evident in the B2 and B4 sludge samples, as either no or very little PHA staining reaction was observed, as shown in Figures 6.4.e and 6.4.g, respectivelly.

Figure 6.5 shows the results corresponding to the PHA and Neisser staining techniques for the Lüneburg-BS sludge sample at the end of the aerobic phase. In Figure 6.5.a, a positive reaction for the PHA staining technique is observable, but only to a small extent and only in the coccus bacillus morphotype.

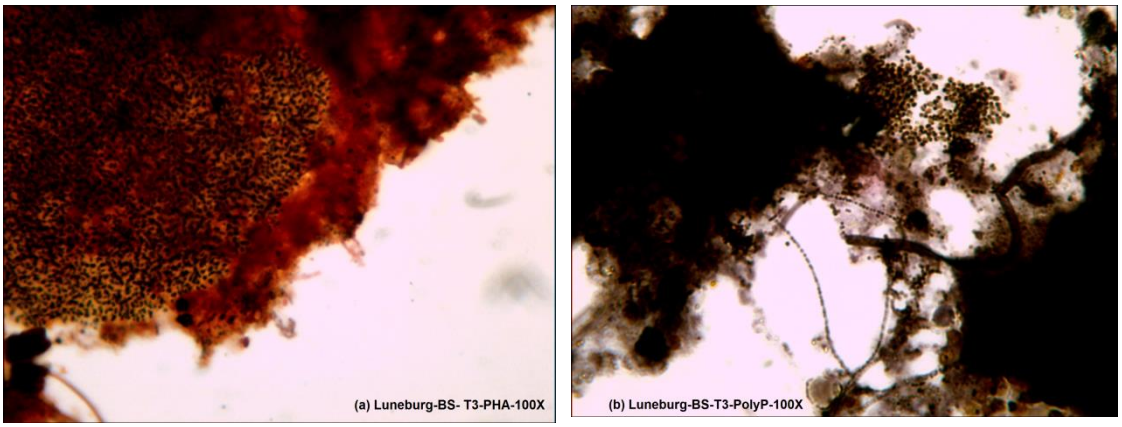


Figure 6.5: Results of the PHA (6.5.a) and Neisser (6.5.b) staining techniques for the Lüneburg-BS sludge sample at the end of the aerobic phase.

Finally, Figures 6.4.b, 6.4.d, 6.4.f, and 6.4.h show the results corresponding to the Neisser staining technique for the S1, S2, B2 and B4 sludge samples, respectively, at the end of the aerobic phase. A positive reaction for the Neisser staining technique was

recorded in all sludge samples for the coccus bacillus morphotype and also in the cell surface of the TFO morphotype for the S1 and S2 sludge samples.

A high concentration of polyphosphate at intracellular level was observed in the S1, B2 and B4 sludge samples, where all the coccus bacillus cells were stained black-purple, sometimes so much so that it was difficult to recognize the cell shape. Meanwhile, for the S2 sludge sample, only black-purple granules were observed. Similar results were obtained in the Lüneburg-BS sludge sample, where intracellular black-purple granules in the coccus bacillus morphotype were observed, as well as complete masses of stained cells. These staining results were consistent with the results of the EBPR performance reported for each sludge sample, with total phosphorus concentrations in effluent of up to 0.4 mg/l (S1), 0.7 mg/l (B2), 0.2 mg/l (B4), and 0.3 mg/l (Lüneburg-BS). It should also be mentioned that intracellular granules of polyphosphate were not observed in the TFO morphotypes in any sludge sample.

In summary, the typical fluctuations of the intracellular polymer storage between the anaerobic and aerobic phases in EBPR systems were observed in all sludge samples, except in the S2 sludge sample, where a previous operational problem has been reported. Storage of PHA was observed during the anaerobic phase and intracellular polyphosphate accumulation was also observed during the aerobic phase. The staining results complement the results of the EBPR process performance, both confirming the occurrence of the EBPR processes in the sludge samples. Additionally, the positive PHA staining reaction on the TFO and coccus bacillus morphotype was observed, as was the intracellular polyphosphate accumulation capacity of the coccus bacillus morphotype. The capacity of the TFO morphotype to accumulate polyphosphate should be noted, but it only occurred at exocellular level.

6.3.3 Assessment of phosphorus accumulation in activated sludge samples using Scanning Electron Microscopy (SEM) coupled with Energy-dispersive X-ray spectroscopy (EDX)

To estimate the amount of phosphorus deposited on each bacterial morphotype in the activated sludge samples, a SEM/EDX analysis was performed.

In this technique, a solid sample is bombarded with a focused electron beam. This bombardment causes a sample to emit X-ray spectra, where each X-ray spectrum is specific to a chemical element. To quantify the chemical element concentration in the sample, the energy of the released X-ray photons is measured. This measurement may be performed by a detector that emits output pulses which are proportional in height to

the energy of the emitted photon. A pulse height analyzer (multichannel type) is used to measure the height of the pulses.

The best SEM images were obtained with the S sludge samples. The SEM/EDX results of the TFO, coccoid cells and filamentous morphotypes are shown in Figures 6.6, 6.7 and 6.8, respectively. The EDX spectra in Figures 6.6.b, 6.7.b, and 6.8.b show the X-ray energy in kiloelectron volt (keV) on the X axis, and on the Y axis shows the number of counts obtained for each chemical element. The image of the TFO morphotype was capturered (Figure 6.6.a) before it lost its characteristic shape because of the intensity of the bombardment of the electron beams. The EDX spectra for the TFO morphotype (Figure 6.6.b) recorded up to approximately 2400 counts per channel for the element phosphorus.

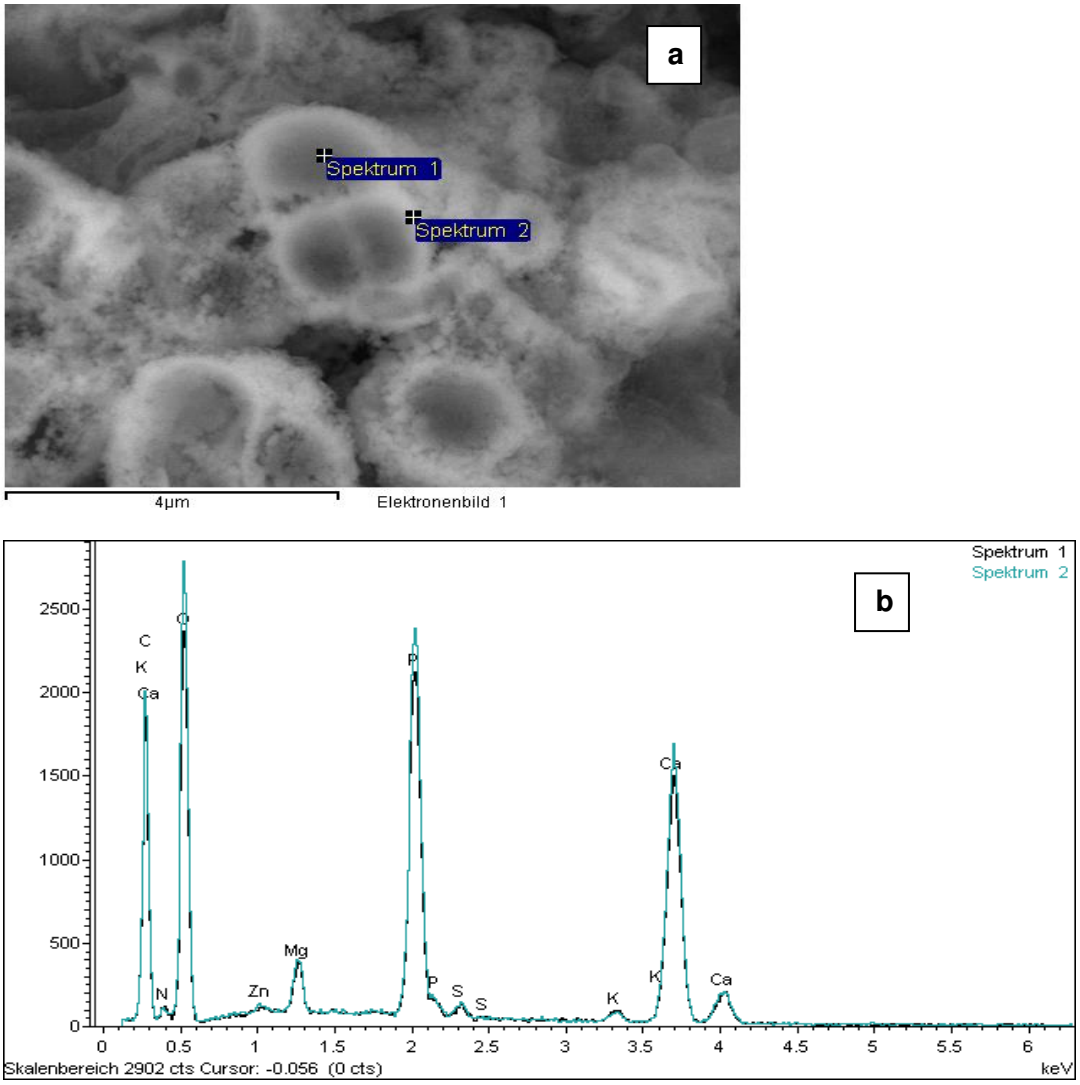
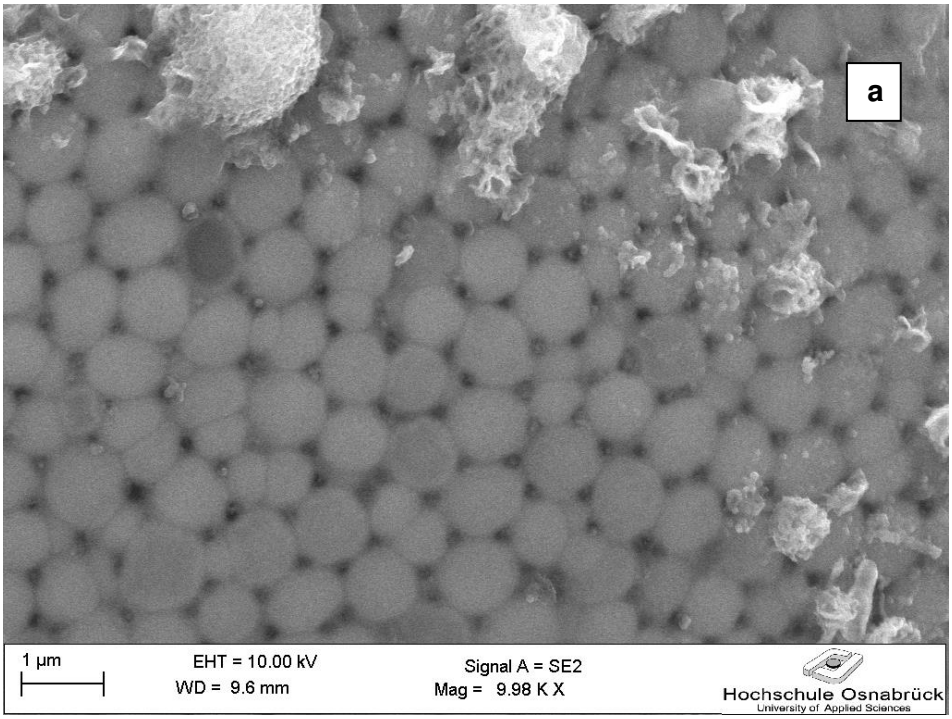


Figure 6.6: SEM image of the TFO morphotype (6.6.a) and EDX spectra corresponding to the marked spectrum- points (6.6.b)

The other morphotype identified was a coccoid cells of less than 1 µm diameter (Figure 6.7.a). The EDX spectra of this morphotype recorded up to approximately 450 keV for the element phosphorus.



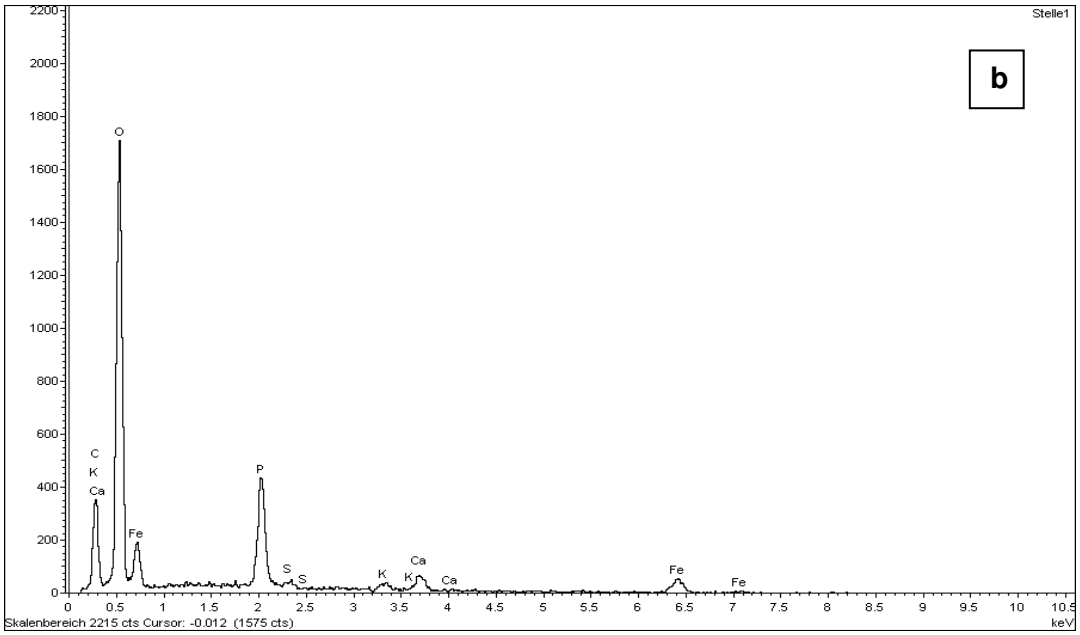


Figure 6.7: SEM image of a cluster of the coccoid cell morphotype (6.7.a) and its respective EDX spectra (6.7.b)

Finally, a filamentous morphotype was identified (Figure 6.8) and its EDX spectra recorded to up 200 keV for the element phosphorus. The identification of additional morphotypes was not possible because of the intensity of the bombardment of the electron beams on the sludge sample, which led to the morphotypes losing their shapes.

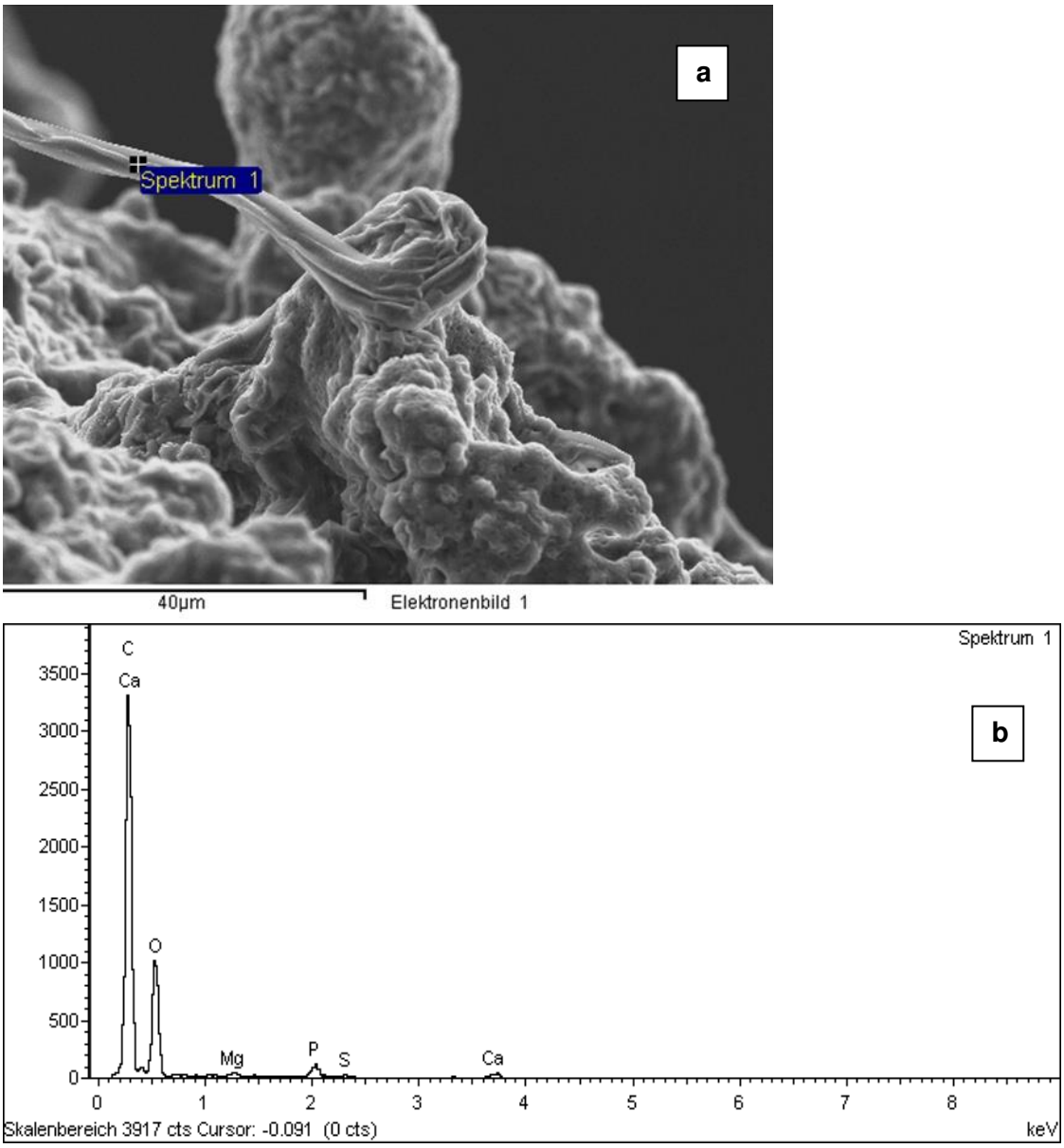


Figure 6.8: SEM image of the filamentous morphotype (6.8.a) and the EDX spectra corresponding to the marked spectrum point (6.8.b)

Among all the morphotypes assessed, the TFO recorded the highest amount of phosphorus. However, the coccus bacillus morphotype, which recorded a positive reaction for the Neisser staining technique at the end of the aerobic phase, was not identified; therefore, it is not possible to know which of the two morphotypes would have the highest phosphorus accumulation capacity.

6.4 Conclusions

1. The bacterial communities in the activated sludge samples were identified according to their basic microscopic appearance using light microscopy and the PHA staining technique. The bacterial communities identified were TFO, obtained in reactors fed with glucose as carbon source (S1 and S2 reactors), and EBPR, obtained in reactors fed with VFA and propionic acid separately (B2, B4 and Lüneburg-BS reactors). The TFO bacterial community was dominated by cuboidal bacteria disposed in groups of four cells, which were correspondigly called Tetrad Forming Organisms. The appearance of the EBPR bacterial community may be described as a mixed community, where no dominance by a specific morphotype was observed, but rather the equal presence of coccoids, coccus bacillus, rod shape, cuboidals and filamentous morphotypes.

2. The TFO and EBPR bacterial communities showed characteristic staining reactions using the Neisser and PHA staining techniques during the anaerobic and aerobic phases in the EBPR processes. Apparently, the TFO bacterial community, in reactors fed with glucose, uptakes most of the carbon source at the end of the anaerobic phase, as the positive PHA staining reaction was abundant in this morphotype.

3. The bacterial communities obtained using glucose as a carbon source show the dominance of the TFO morphotype but a coccus bacillus morphotype was also identified. The TFO morphotype showed positive reaction to the Neisser staining technique at exocellular level and the coccus bacillus morphotype showed a strong positive reaction with the Neisser staining technique at the end of the aerobic phase. These two bacterial groups showed typical accumulation of intracellular polymers observed in efficient EBPR processes.

   Bacterial communities fed with glucose as the sole carbon source are able to show typical staining reactions observed in efficient EBPR processes.

4. Three different morphotypes were identified using the SEM/EDX technology: TFO, coccoid and filamentous morphotypes. Of these morphotypes, the highest counts per channel for phosphorus on the EDX spectra were recorded for the TFO morphotype.

Although the results of the staining techniques showed that the TFO morphotype had no capacity to store phosphorus intracellularly, the results obtained using the SEM/EDX technology lead to the conclusion that this morphotype was able to store phosphorus, it is assumed, at exocellular level.

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CHAPTER 7: Assessment of the performance of the EBPR process using different carbon sources and different C:N:P ratios in synthetic wastewater

7.1 Introduction

Activated sludge systems able to perform EBPR processes are among the most efficient technologies for phosphorus removal from wastewater, achieving phosphorus concentrations in effluent below 0.1 mg/l (Strom, 2006). However, wastewater treatment plants (WWTP) that apply this process generally also have additional phosphorus precipitation technology, using for this purpose compounds of calcium, aluminium, and iron (Tchobanoglous et al., 2003). The need for an additional method for phosphorus removal suggests that the EBPR process is not stable (Hartley and Sickerdick, 1994; van Loosdrecht et al., 1997; Blackall et al., 2002; Seviour and Nielsen, 2011). The metabolic mechanisms causing this instability have not yet been explained and do not fit in the metabolic models proposed for the EBPR process (Comeau et al., 1986; Wentzel et al., 1986; Mino et al., 1987).

So far, investigations into the stability of the EBPR process have focused on the applied operational parameters, focussing on identifying the most suitable substrate for the development of the polyphosphate accumulating organisms (PAO). It is often proposed in the literature, that volatile fatty acids (VFA), mainly in the form of acetic acid, are assimilated by PAO more efficiently, benefiting the polyphosphate uptake during the aerobic phase (Comeau et al., 1986; Wentzel et al., 1986; Mino et al., 1987; Satoh et al., 1996; Pitman, 1999; Mulkerrins et al., 2004).

Aditionally, research on the availability of substrate and the main nutrients required for the development of an efficient EBPR process has also been performed. This research focused on the proportions of carbon source (BOD_5) in relation to nitrogen source (Total Nitrogen) and phosphorus source (Total Phosphorus) in influent. The most appropriate ratios for the EBPR process have been determined as a C:N:P ratio of 100:10:1 (Yu et al., 2007); the ratio C/N of 4 COD/N-TKN - 30 COD/Ntot (Puig Broch, 2007; Jenkins, 2003), and the ratio C/P of 40 - 140 COD/Ptot (Randall et al.,1992; Jenkins, 2003). However, numerous experiments have recorded occasional low yields of the EBPR process even under optimum operational conditions and even with adequate nutritional requirements (Cech and Hartman, 1990; Seviour and Nielsen, 2010).

Generally, in real conditions, municipal wastewater will not have C:N:P proportions that are optimal for the development of the EBPR process. The C:N:P proportion in municipal wastewater will vary due to the different industrial activities performed in the region, due to different eating habits of the community, because of the nature of the soil and drinking water, and because of climatic variations such as the onset of the rainy season. Because of these reasons, municipal wastewater mainly records a C:N:P ratio of about 20:4:1 (Winkler, 2012).

In this investigation, the influence of the carbon source and the C:N:P ratios on the EBPR performance used in influent were evaluated simultaneously. The carbon sources used were a mixture of VFA, propionic acid, and glucose, and the C:N:P ratios were 18.1:1.9:1; 37:2.5:1 and 45.2:4:1, respectively. The principal objective of this investigation was to promote the development of different bacterial communities able to perform EBPR processes. Additionally, a secondary objective was to clarify which factors regarding influent composition are the most influential on the well-functioning of the EBPR systems.

Subsequently, these bacterial communities were used in further research (Chapter 8), aiming to evaluate their phosphorus sorption capacities.

7.2 Material and Methods

7.2.1 Composition of the synthetic wastewater

Different carbon sources (glucose, mix of VFA, and propionic acid) were employed to produce three different types of wastewater. The compositions of the synthetic wastewaters are shown in Table 7.1.

Table 7.1: Initial basic composition of the synthetic wastewaters (Modified from Liu et al., 2006).

	Carbon source		
Compound (mg/l)	Glucose (S)	Volatile fatty acids (VFA)	Propionic acid (Propionic)
Carbon source	589.5**	48.1	54.6
P (KH_2PO_4)	7.5	3.21	2.39
NH_3-N (NH_4Cl)	21	5	5
COD	600	67.2	71.9
$MgCl_2$	10	10	10
$CuSO_4$	0.1	0.1	0.1
$CaCl_2$	5	5	5
$MnSO_4$	0.1	0.1	0.1
$ZnCl_2$	0.1	0.1	0.1
Peptone	10	10	10
C:N:P	33.3:2.8:1	9:1.6:1	12.5:2.1:1

** Initially, the synthetic wastewater was prepared using glucose as carbon source. The so-called glucose community in S reactor was obtained using this carbon source.

In this research, the term VFA is used to describe the volatile fatty acids obtained in the laboratory during fermentation processes using glucose as substrate. In summary, the VFA had the following composition: 64.3% acetic acid, 19.3% butyric acid and 10.6% propionic acid. Other organic acids, such as isobutyric acid, valeric acid and isovaleric acid, accounted for 5.8% of the organic acid concentration. More details about the VFA composition are discussed in Chapter 5.3.4

As shown in Table 7.2, the C:N:P ratio in each influent wastewater (Table 7.1) was gradually changed in order to assess the effects of such modifications on the phosphorus removal percentage (Prem %) and on the mass ratio of phosphorus uptake per biomass unit as volatile suspended solids (MR mgPtot/gVSS).

Table 7.2: Carbon sources and C:N:P ratios used in synthetic wastewater in each reactor during the assessment of the performance of the EBPR process.

		C:N:P ratio			
Reactor	Carbon source	Month 1 - 6	Month 7 - 12	Month 13 - 18	Month 19 - 24
S	Glucose	10-30:3:1	35:3:1	40:4:1	45:4:1
B1	Volatile fatty acids	-------	10-20:2:1	35:3:1	45:4:1
B2					
B3	Propionic acid				
B4					

7.2.2 Inoculum

The bacterial community used in this experiment was taken from the activated sludge system of the Uelzen WWTP. Table 7.3 shows some characteristics of the inoculum at the time it was collected. This bacterial community was moved to the laboratories of the Faculty of Civil and Environmental Engineering of Ostfalia University and added to reactors S, B2 and B4. After six months of operation, an inoculum of the S reactor was used as inoculum for reactors B1 and B3.

Table 7.3: Characteristics of the inoculum used in the research of EBPR performance.

Parameter	Value
Total suspended solids (TSS)	4.0 g/l
PO_4-P in supernatant in aeration tank	0.35 mg/l
NH_4-N in supernatant in aeration tank	0.49 mg/l
Excess sludge	483 m^3/d

It should be noted that the Uelzen WWTP regularly uses iron chloride ($FeCl_2$) as a complementary technology for biological phosphorus removal from wastewater.

7.2.3 Location

The present research was performed in the laboratories of the Civil and Environmental Engineering Faculty of Ostfalia University - Campus Suderburg (Niedersachsen, Germany) from March 2011 to October 2013.

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7.2.4 Reactors

Six sequential batch reactors (SBR) were used and their characteristics are presented in Table 7.4.

Table 7.4: Basic characteristics of the SBR reactors used in the EBPR process assessment.

Reactor	Material	Total Volume (l)	Devices adapted*
S1, S2	Plexiglass	35	Yes
B1, B2, B3, B4	Glass	2	No

* Devices adapted refer to the systems already in reactors for loading, unloading, mixing, and aeration.

The charge of the influent and discharge of the effluent were carried out using peristaltic pumps. The charge and discharge levels were controlled considering the length of each process and the pumping rates. Air injection was carried out using membrane pumps. The agitation systems of the S reactors consisted of two blades disposed about a central axis. For the B1, B2, B3 and B4 reactors, magnetic stirrers were used. The daily sludge discharge was carried out manually.

All equipment was operated automatically using programmable timers. The basic structure of a reactor is illustrated below in Figure 7.1.

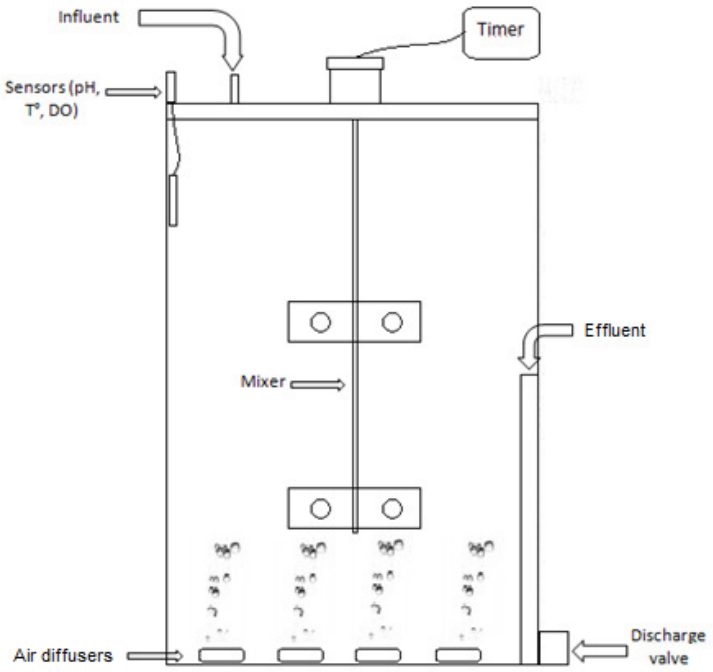


Figure 7.1: Design of the SBR reactor used in the assessment of the EBPR process.

7.2.5 System Operation

The reactors were batchwise operated with three cycles per day, each of 8 hours. The length and sequence of each phase in a cycle is shown below, in Figure 7.2.

Figure 7.2: Length and sequence of phases within each cycle of operation.

Except for the organic loading rate (OLR), the other operational parameters were maintained under stable conditions throughout the experimental process (Table 7.5). Initially, for B1, B2, B3 and B4 reactors, low OLRs were used because of the need to adapt the biomass to organic acids. Subsequently, the OLRs were gradually increased.

Table 7.5: Operational parameters used in the SBR reactors during the assessment of the EBPR process.

		Reactors	
Parameter	Units	S1, S2	B1, B2, B3, B4
Organic loading rate (OLR)	mg CODt/l.cycle	100 - 200	22.4 - 217.6
Total volume (V_t)	l	35	2
Filling volume (V_f)	l	30	1.5
Total Suspended Solids (TSS)	g/l	4	1 - 2
Sludge Volume Index (SVI)	ml/g	87.4	93.5
Hydraulic Retention Time (HRT)	h	24	24
Mean Cell Residence Time (MCRT)	days	15	15
Reaction time (RT)	min	383	383

7.2.6 Sampling

At the beginning of the filling phase, samples of influent synthetic wastewater were taken to determine its C:N:P ratio and the concentration of VFA. Within operating cycles, mixed liquor samples were taken during and at the end of each anaerobic and aerobic phase (Figure 7.3). The samples were immediately filtered using folded filters and the filtrate was deposited in polyethylene bottles and stored at -20°C until analysis. In the case of samples taken for measurement of total phosphorus content, a solution of H_2SO_4 (1 Molar) was added to the samples until pH 2 was reached, at which point they were frozen.

The samples for measurement of COD and BOD_5 concentration were either analyzed immediately or were refrigerated under temperatures between 1 and 4°C for up to 24 hours and then analyzed. Samples taken to measure the concentration of total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed immediately.

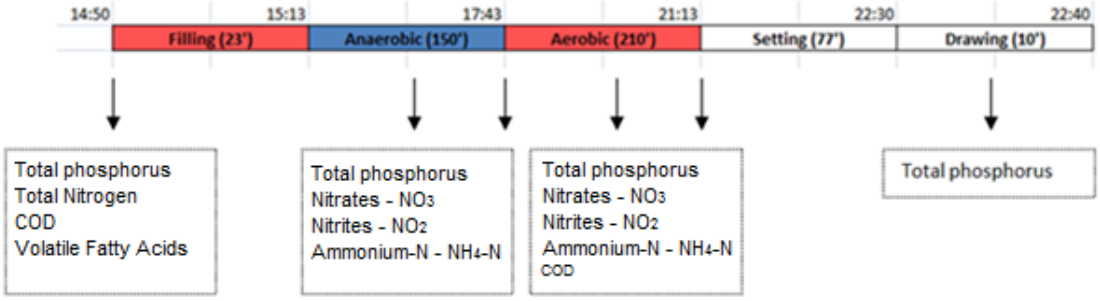


Figure 7.3: Sampling cycle during the assessment of the EBPR process.

7.2.7 Sample Analysis

The samples were collected, preserved, stored, and analyzed following the guidelines described in the relevant DIN Norms of the German Institute for Standardization. The DIN Norms used are shown in Table 7.6.

Table 7.6: DIN Norms used for collection, preservation, storage, and analysis of the samples of the EBPR process.

Parameter	Sampling and analysis	Preservation and storage
Total phosphorus	DIN EN ISO 6878	DIN EN ISO 5667 – 3 (A21) May 2004
COD	DEV - H41 - 1	
BOD_5	DIN 38409 - H51 & DIN 38409 - H52	
NH_4-N	DIN 38406 T5	
TSS	DIN 38414 - S2	
VSS	DIN 38409 - 1	
Organic acids	DIN 38414 - S19	
Ortophosphate	DIN 38405 - D - 11	

Other analysis techniques were used to assess the composition of the influent wastewater and the presence of nitrite and nitrate in mixed liquor during the anaerobic and aerobic phases. These tests are listed in Table 7.7.

Table 7.7: Other parameters evaluated and the techniques used during the assessment of the EBPR process

Parameter	Techniques
Total nitrogen	Dr. Lange LCK 238
Organic acids	Dr. Lange LCK 365
Nitrite (NO_3-N)	Quantofix 913 13
Nitrate (NO_2-N)	Quantofix 913 13

7.3 Results and discussions

7.3.1 Stability assessment of phosphorus removal in EBPR processes considering the use of different carbon sources in the influent.

a. Effects on the phosphorus removal percentage

The analyses using volatile fatty acids (VFA) and propionic acid as carbon sources in wastewater (Table 7.2) started after a month acclimatization period of the bacterial communities.

To compare the phosphorus removal performance between reactors, either as phosphorus removal percentage (Prem %) or phosphorus uptake (MR mgPtot/gVSS), three different C:N:P ratios in the influent were considered. These C:N:P ratios were: low - adverse (18.1:1.9:1), medium (37:2.5:1) and high (45.2:4:1).

It should be taken into account that the initial concentration of wastewater (Table 7.1) was varied to reach each of the C:N:P proportions. The phosphorus removal percentage was calculated considering the initial total phosphorus concentration in the influent and the total phosphorus concentration in the effluent. This initial total phosphorus concentration in the influent (Table 7.1) was almost constant for all three different C:N:P ratios during the experimental process. The variations of the C:N:P ratios were controlled by the COD/BOD concentration in the influent.

Initially, an adverse C:N:P ratio was used in all influent synthetic wastewaters. This ratio was gradually varied as shown in Table 7.2. The results of phosphorus removal (Prem) corresponding to the adverse C:N:P ratio in the influent are shown in Figure 7.4. With a C/P ratio of 18.1 in the influent, the reactors showed very different percentages of phosphorus removal. The highest Prem (%) was obtained in the B2 reactor (EBPR community - VFA) followed by the B4 reactor (EBPR community - propionic acid). In both cases, the removal percentages were much higher than those recorded for the other reactors, and so high that a maximum difference of 58% was observed between B2 (EBPR community - VFA) and B3 (Glucose community - propionic acid) reactors.

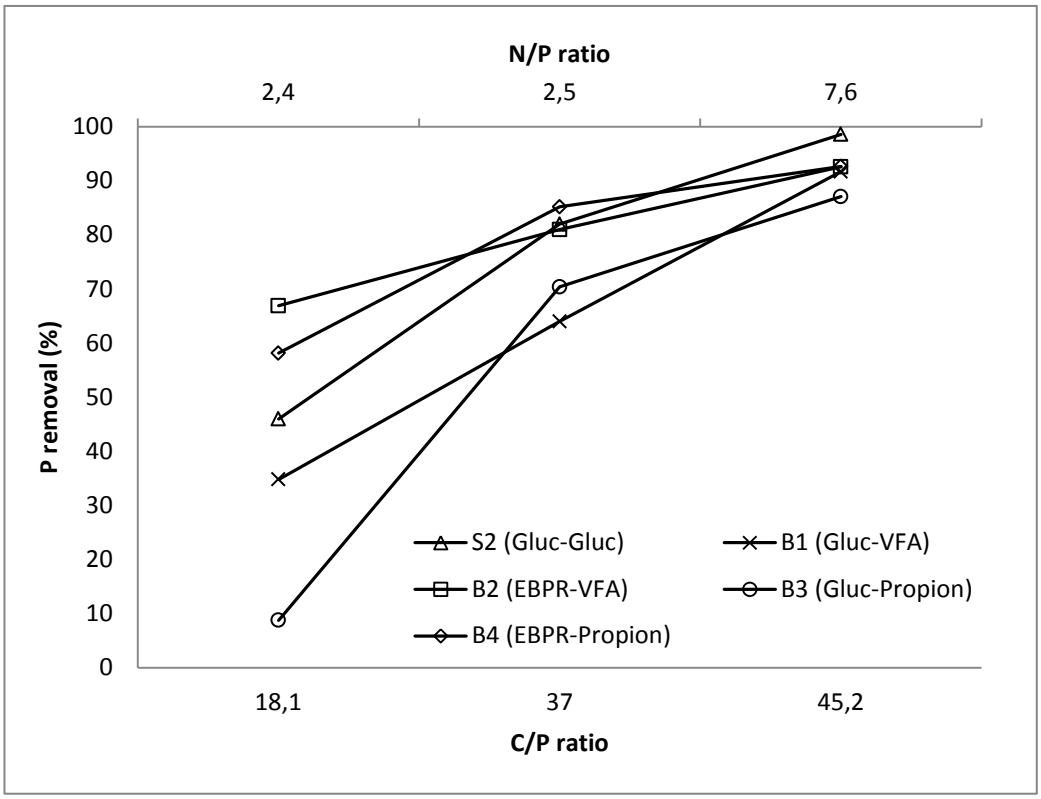


Figure 7.4: Phosphorus removal percentages (Prem %) according to the C/P ratio (BOD_5/Ptot) and the N/P ratio (Ntot/Ptot) in the influent synthetic wastewater.

These results agree with investigations by Randall and Liu (2002), who observed that, using acetic acid as carbon source in the influent wastewater, 3-hydroxy butyrate (3HB) was obtained as reserve material, increasing the phosphorus uptake during the aerobic phase. In comparison, using propionic acid as carbon source, such good results of phosphorus uptake were not obtained. Additionally, Abu-Ghararah and Randall (1991) showed that propionic acid was less effective than acetic acid for the EBPR system. Both experiments were conducted in short-term batch tests.

For the B4 reactor, a similar yield but with a lesser removal percentage (58.2%) was observed using propionic acid as carbon source. It should be mentioned that it was assumed that the EBPR bacterial community (PAO included) was present in the inoculum obtained from the Uelzen WWTP, which is supported by its performance of phosphorus removal and by staining tests (Chapter 5.3). Given the presence of the EBPR bacterial community, the results of the B4 reactor agree with the results obtained by Hood and Randall (2001) who have suggested that propionic acid can be rapidly assimilated by the PAO (Polyphosphate Accumulating Organisms) while not being easily used by other microorganisms present in the activated sludge community. Additionally, Lopez-Vasquez et al. (2009) observed a high enrichment of *Accumulibacter* (PAO) when propionic acid was used as carbon source in the influent. However, the abundance of PAO with respect to all of the microbial community should

be considered. For example, Pijuan (2004) recorded an increase in the amount of PAOMIX-binding cells (*Accumulibacter*) from 7% to 54% after 15 days of operation using propionic acid as carbon source.

The low phosphorus removal percentage of 46% of S reactor is attributable to the C:N:P ratio in influent which was not suitable for the EBPR process (Yu et al., 2007).

Before analyzing the phosphorus removal performance of the B1 and B3 reactors, the relationship between the bacterial communities GAO (Glycogen Accumulating Organism) and TFO (Tetrad Forming Organism) should be mentioned. It has been shown that GAO and LPO (Lactate Producing Organisms) are the dominant bacterial communities in reactors fed with glucose as carbon source (Jeon et al., 2000). The morphotype of the LPO microorganisms is described as eight coccus-shaped cells, when this observation was performed using SEM technology. Under light microscopy, the LPO cell group is described as Tetrad Forming Organisms (TFO). Unlike GAO, TFO bacteria are classified based only on their morphological characteristics (cuboidal cells disposed in groups of four bacteria), and GAO bacteria are classified based on their metabolic behavior (carbon source consumption during the anaerobic phase and phosphorus uptake absence during the aerobic phase). It should also be noted that some investigations relate some TFO organisms to GAO bacteria, but this does not mean that all TFO should be classified as GAO (Seviour and Nielsen, 2011). In the present research, the bacterial community in the S1 and S2 reactors (Glucose community) was dominated by the TFO morphotype.

The lowest phosphorus removal percentages were recorded in the B1 (Glucose community - VFA) and B3 (Glucose community - propionic acid) reactors with 34.8% and 8.8% respectively. Regarding these results, research by Lopez-Vasquez et al. (2009) indicates that using acetic acid and propionic acid as carbon source does not promote the development of the GAO bacterial community which is dominant in reactors fed with glucose (B1 and B3 reactors). Similarly, Lu et al. (2006) suggested that the carbon source in influent should change from acetate to propionate in order to eliminate the GAO community in lab-scale reactors. This latter study provides a relationship that explains the detriment of the Glucose-TFO community (Figure 7.5) in the B3 reactor and the loss of its phosphorus removal performance when receiving a new carbon source. This relationship also raises the possibility that some TFO might participate actively in the good performance of the EBPR process.

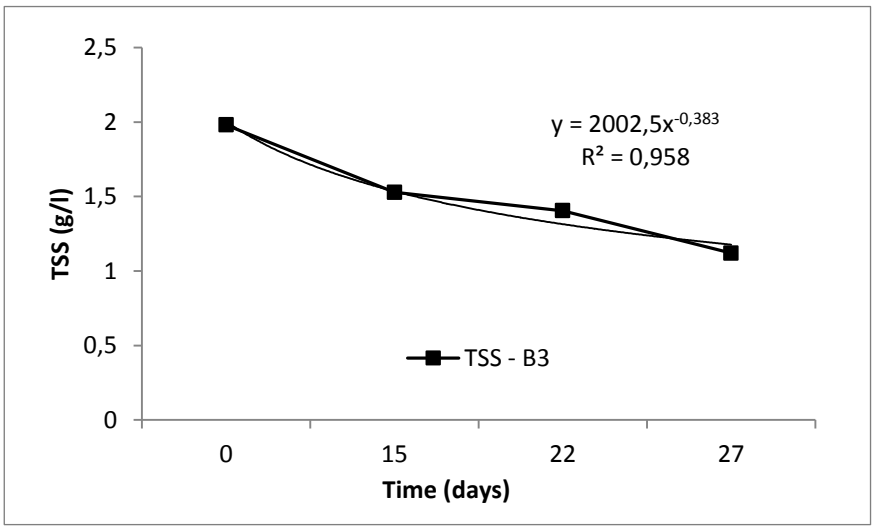


Figure 7.5: Decrease of the bacterial concentration as TSS in the B3 reactor during the first month of the experimental process.

After the experiment at the C:N:P ratio of 18.1:1.9:1, the carbon concentration (BOD_5) in the influent wastewater was gradually increased up to a C:N:P ratio of 37:2.5:1. The results, summarized in Figure 7.4, show that the differences in phosphorus removal percentage between reactors were not as large as those recorded when a C:N:P ratio of 18.1:1.9:1 was used. For a C/P ratio of 37, the highest removal percentages were obtained in B4 (EBPR community - propionic acid), S (Glucose community - glucose), and B2 (EBPR community - VFA) reactors. In these reactors, the phosphorus removal percentages recorded were up to 21.2% higher than those obtained in the other reactors.

According to results of the microscopic tests (Chapter 5.3), the dominant bacterial community in the S reactor was the so-called TFO community. Using staining techniques, such as Neisser and Sudan Black, coccobacillus-shaped cells were also observed and were in fact most abundant. This TFO bacterial community has been consistently observed, to be most abundant in reactors fed with glucose as carbon source. Research has repeatedly shown the ability of this community to synthesize and store glycogen (Pijuan, 2004). Additionally, the GAO community has been identified as unable to perform efficient EBPR processes. It should be noted that the malfunction of the EBPR process, in reactors dominated by this community, was identified as a response to the presence of microorganisms described as “coccoid cells in the form of tetrads” (Cech and Hartman, 1990, 1993), i.e. the malfunction of the EBPR process was related to the presence of a specific morphotype. However, further research recorded that it was possible to obtain good and stable yields for EBPR processes using glucose as carbon source in laboratory scale reactors (Carucci et al., 1994; Wang et al., 2002).

Additionally, it should be considered that glucose as carbon source has been identified as a substrate that allows a significant accumulation of intracellular glycogen (Pijuan, 2004) and that glycogen could play an important role in EBPR processes (Mino, 1998). Furthermore, Jeon and Park (2000) have proposed that at least two bacterial groups might be involved in EBPR processes in reactors fed with glucose. They suggest that one of these groups is the LPO microorganisms (Lactic Accumulating Organisms) and the other group is the PAO community. The first group would be able to take up glucose directly and to transform it into glycogen, covering the energy demand of this process with a glycolisis process that, in turn, would produce lactate. Subsequently, the lactate produced by the LPO microorganisms would be used as carbon source by the PAO community, which would release phosphorus during the anaerobic phase and would take up phosphorus during the aerobic phase. This theory could explain the phosphorus removal performance observed in the S reactors in the present research. The good EBPR performance recorded for the S reactors was also confirmed by the results of the Neisser staining technique (Chapter 6.3.1) which showed a considerable amount of intracellular polyphosphate accumulation at the end of the aerobic phase for these sludge samples.

In turn, the microscopic examination of the B2 and B4 reactors showed the same structure of EBPR bacterial communities as previously seen at the Uelzen WWTP, from where the sludge sample came, when the seasonally-recorded EBPR processes were efficient. Thus, good yields of phosphorus removal were also observed in the B2 (EBPR community - VFA) and B4 (EBPR community - propionic acid) reactors, recording phosphorus removal percentages of more than 80%. These results also confirm the ability of the EBPR bacterial communities to improve the phosphorus uptake when receiving VFA as carbon source.

Finally, the B3 reactor (Glucose community – propionic acid) continued to record the lowest phosphorus removal percentages, confirming its low adaptability to using propionic acid as carbon source.

The results obtained in the B1 reactor were not considered in this second group of samples because of the presence of nitrate (NO_3^-) during the anaerobic phase, resulting in the drastic deterioration of the EBPR process (Figure 7.6).

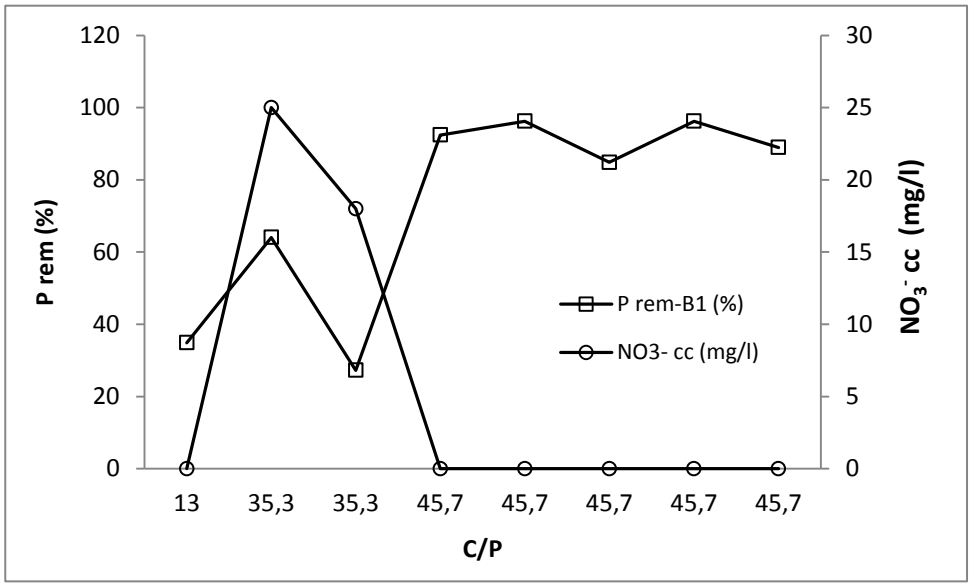


Figure 7.6: Deterioration of the EBPR process in the B1 reactor because of the presence of nitrate (NO_3^-) during the anaerobic phase.

Finally, the carbon concentration in the influent wastewater was gradually increased up to a C:N:P ratio of 45.2:4:1. Using this C:N:P ratio, all reactors reached optimum percentages of phosphorus removal, i.e. phosphorus removal percentages of around 90%. The results of phosphorus removal were similar in each reactor, with the largest difference recorded between the S (98.6%) and B3 (87%) reactors. The highest percentages of phosphorus removal were recorded again in the S reactor, followed by the B2, B4 and B1 reactors. Again the B3 reactor recorded the lowest removal percentage. In all cases, the reactors reached a total phosphorus concentration in effluent of less than 0.4 mg/l.

b. Effects on the amount of phosphorus removed per mass unit of volatile suspended solids (mgPtot/gVSS):

The results obtained in section 7.3.1.a showed the S reactor to be the most efficient in terms of phosphorus removal percentage. However, these results do not consider the difference between the biomass concentrations in each reactor. Based on the different growth rates of each community, the phosphorus uptake capacity per gram of Volatile Suspended Solids (mgPtot/gVSS) in each reactor was calculated.

Figure 7.7 shows the results obtained using an influent with a C:N:P ratio of 18.1:1.9:1. With a C/P ratio of 18.1, the highest rates (mgPtot/gVSS) were recorded in the B2 and B4 reactors, followed by the S, B1 and B3 reactors. These results agree with those

obtained for the percentages of phosphorus removal described in the previous section. The limited capacity of phosphorus uptake showed by the B1 and B3 reactors should be noted. Both bacterial communities recorded good yields of phosphorus removal when receiving glucose as carbon source, but when the carbon source changed to VFA for the B1 reactor and to propionic acid for the B3 reactor, the EBPR processes were severely impaired.

Using a C:N:P ratio of 37:6.5:1 in the influent, the best ratio of phosphorus uptake was obtained in the S reactor (1.47 mgPtot/gVSS) followed by the B4, B2, and B3 reactors.

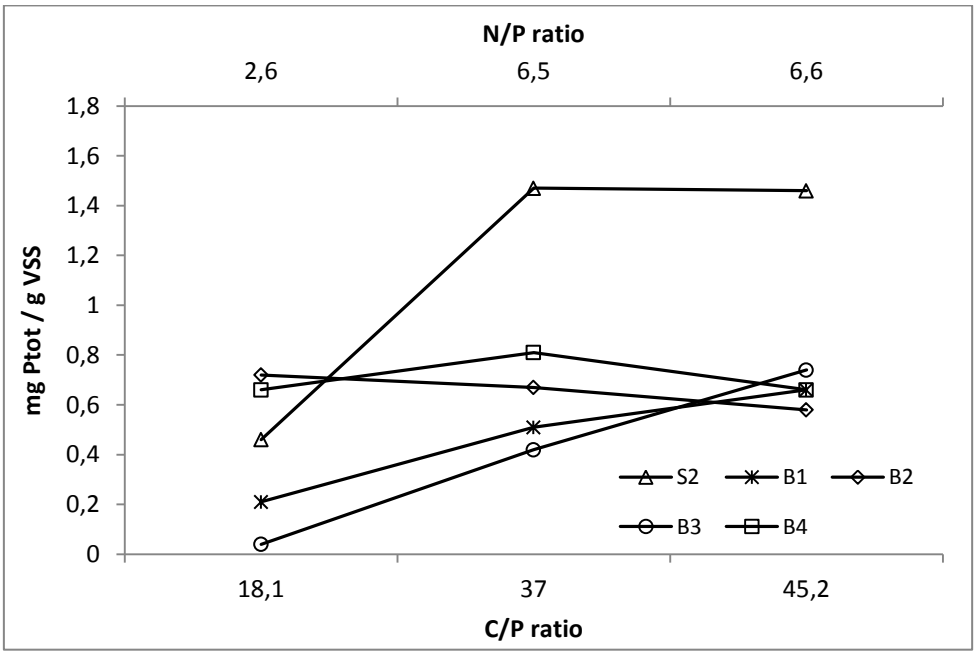


Figure 7.7: Phosphorus uptake per mass unit of VSS vs the C/P (BOD_5/Ptot) and N/P (Ntot/Ptot) ratios in the influent.

Finally, using a C:N:P ratio of 45:6.6:1 in the influent, the best rates of phosphorus uptake were recorded in the S reactor with 1.46 mgPtot/gVSS. The B1, B2, B3 and B4 reactors showed very similar results, each approximately 0.66 mg Ptot/gVSS.

With this later C:N:P ratio, a slight increase in the phosphorus uptake rate was observed in the B3 reactor. This result may be because of the adaptation of this bacterial community to using propionic acid as carbon source after ten months of operation. The phosphorus uptake results for the B2 and B4 reactors decreased considerably, a fact that contradicts the results obtained for the same reactors in terms of removal percentage, which continued increasing during the experimental process. The drop of the mgPtot/gVSS rates can be explained by the type of bacterial community present in both reactors (EBPR community) and the C/P ratio in the influent

wastewater. The phosphorus uptake decrease in the B2 and B4 reactors is explained in more detail in section 7.3.2.c.

c. Relationship between the VFA concentration in the influent and the phosphorus uptake capacity.

For reactors that used VFA and nutrient broth as carbon sources, the VFA concentrations were gradually increased from approximately 36.7 mg/l to 90.9 mg/l. During the first four months of operation for the B1 and B3 reactors (Figure 7.8), it was observed that the mgPtot/gVSS rate generally decreased as the VFA concentration in the influent increased, demonstrating once again the poor adaptability of the glucose community to VFA as carbon source. The results of the biomass concentration depletion over time in the B1 and B3 reactors (Figure 7.5) explain why the lowest results of phosphorus removal percentages and phosphorus uptake were recorded in these reactors.

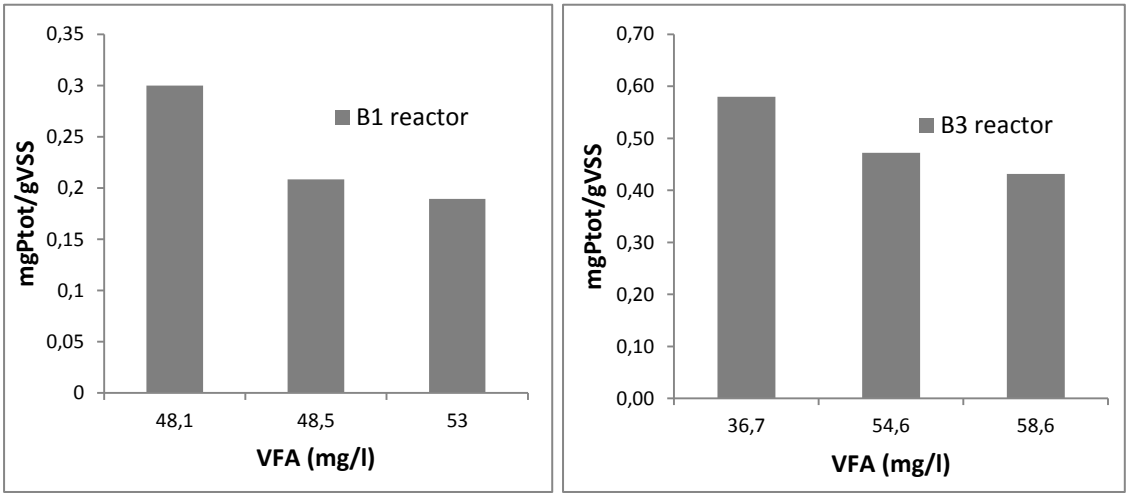


Figure 7.8: Effect of the VFA concentration in the influent on the phosphorus uptake capacity of the B1 and B3 reactors (Glucose bacterial communities) during the first months of operation.

On the other hand, the phophorus uptake ratio for the B2 and B4 reactors (Figure 7.9) increased as the VFA concentration in the influent increased. The highest mgPtot/gVSS rate was reached using a VFA concentration of approximately 54 mg/l in the influent, to subsequently decrease as the VFA concentration in the influent increased.

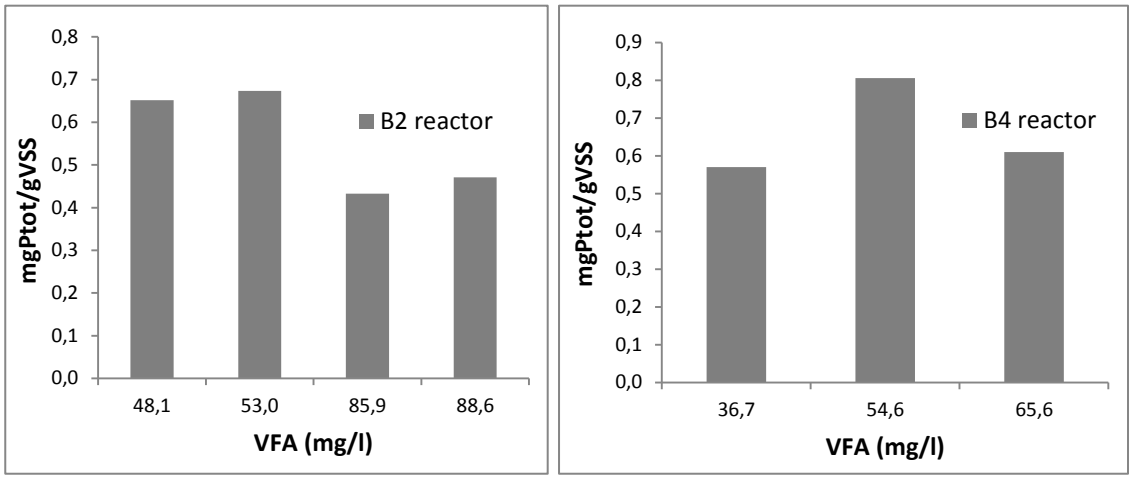


Figure 7.9: Effect of the VFA concentration in the influent on the phosphorus uptake capacity of the B2 and B4 reactors (EBPR bacterial communities).

Similar research into the gradual increase of VFA concentration in the influent were performed by Pijuan (2004), who recorded that increasing the propionate concentration in the influent from approximately 1.35 mmolC/gVSS to 4.9 mmolC/gVSS did not result in an increase of the phosphorus uptake rate, which remained at 0.012 mmolP/gVSS.min. Subsequently, when the concentration of acetic acid increased from 1.42 mmolC/gVSS to 5.3 mmolC/gVSS, the phosphorus uptake remained at 0.016 mmolP/gVSS.min to later decrease when the highest concentrations of acetate were achieved. Other research performed under similar operational conditions (Randall and Liu, 2002) used a VFA concentration in the influent (acetic acid and propionic acid) of about 50 mg/l. This latter study obtained a relatively efficient EBPR process, with a maximum phosphorus removal percentage of up to 77.2%, considering that the total phosphorus concentration in the influent was 22 mg/l.

Additionally, according to Randall and Chapin (1997), a high concentration of acetate in the influent may adversely affect the operation of EBPR processes, while Chuang et al. (1998) reported that sludge samples with low COD-SS loading rates may have a high potential for phosphorus uptake.

7.3.2 Stability assessment of phosphorus removal in EBPR processes using different C:N:P ratios in the influent.

a. Influence of the C/N (COD/Ntot) and C/P ratios (BOD_5/Ptot) in the influent on the phosphorus removal performance in EBPR processes

With regard to the necessary C/N ratio for denitrification processes in EBPR systems, research by Puig et al. (2007) stated that values lower than 4gCOD/gN-TKN cannot

meet the carbon requirements of the denitrification process and hence nitrate ions will accumulate in the reactor, causing the deterioration of the EBPR process. Additionally, Jenkins (2003) showed that if the influent contains a C/N ratio equal to or greater than 30, the effects of nitrogen deficiency can be observed. These effects are: increase of PHA production during the anaerobic phase, gradual decrease of COD and acetate uptake, and decrease of the phosphorus release per acetate uptake ratio.

In the present research, all reactors received influents with C/N ratios higher than 4, with values ranging between 5.7 and 109 gCOD/gN-TKN. These values are within the nitrogen ranges identified as excess, limitation, and deficiency by Jenkins (2003). Given the C/N ratio present in the influent, the reactors were not exposed to situations of carbon deficiency.

Turning from nitrogen limitation to carbon limitation, research has suggested that the EBPR process is carbon limited when the C/P ratio in the influent is low. According to Randall et al. (1992), for settled domestic sewage, when the COD/TP ratio is less than 40, or in the case of BOD_5/TP less than 20, the carbon source cannot cover the metabolic requirements needed for the EBPR process. In case of carbon limitation, the PHA reserves will not be enough to promote the subsequent storage of Poly-P and the replenishment of glycogen during the aerobic phase.

In the present research, the influent wastewater did not show any phosphorus deficiency, since the C/P (BOD_5/Ptot) ratio was always less than 140 (Jenkins, 2003).

b. The influence of the N/P ratio in the influent on the performance of phosphorus removal in EBPR processes

As mentioned above, the most suitable wastewater composition for the EBPR process consists of the well-known C:N:P ratio of 100:10:1 as BOD_5:Ntot:Ptot (Yu et al., 2007), the C/N ratio between 4 as COD/N-TKN and 30 as COD/Ntot (Puig et al., 2007; Jenkins, 2003), and the C/P ratio between 40 and 140 as COD/Ptot (Randall et al., 1992; Jenkins, 2003). In the present research, it has been observed in some cases that, although the composition of the influent was optimal for the EBPR process, low phosphorus removal percentages were nonetheless recorded (Table 7.8).

The results presented in chapter 7.3.1.a showed the influence of the C/P ratio on the percentage of phosphorus removal for N/P ratios between 1.9 and 4.3. Additional tests with N/P ratios between 1.3 and 4.4 were performed in order to show the influence of

the N/P ratio on the phosphorus removal performance. These tests were performed only in the S reactors.

Table 7.8: C/P, C/N and N/P ratios in the influent and the phosphorus removal percentages obtained in the S reactors.

C/P (BOD_5/P_{tot})	C/P (COD/P_{tot})	C/N (COD/N_{tot})	N/P (N_{tot}/P_{tot})	Prem (%)
55.5	133.2	28.6	4.6	92.9
21.4	51.4	28.6	1.8	59.8
22.1	53	28.2	1.9	46.0
22.1	53	27.9	1.9	45.1
36.9	88.6	9.2	4	83.6
42.4	101.8	25.1	4	98.4
43	103.2	25.6	4	98.6

The results (Figure 7.10) show a direct relationship between the N/P ratio and the percentage of phosphorous removal. Removal percentages higher than 80% were obtained when the N/P ratio was equal to or greater than 2. Even with optimal C/P and C/N ratios in the influent, for example 21.4 and 28.6 respectively, if the N/P ratio was less than 2 the EBPR process was heavily impaired.

The N/P ratio can be graphed against the C/P ratio and the phosphorus removal percentages to show the nitrogen requirement of EBPR processes. This nitrogen requirement can be explained using the metabolic model proposed by Smolders (1994) for PAO metabolism under aerobic conditions. According to this model, during the aerobic phase a portion of the PHB stored is consumed for biomass formation, where for each 1.27 mole of PHB degraded 0.2 mol of NH_3 were consumed to produce 1C-mol biomass. Additionally, 1.5 mol ATP would be required to produce 1C-mol biomass.

Figure 7.10 shows the minimum nitrogen requirement for biomass synthesis, a process prior to phosphate transport.

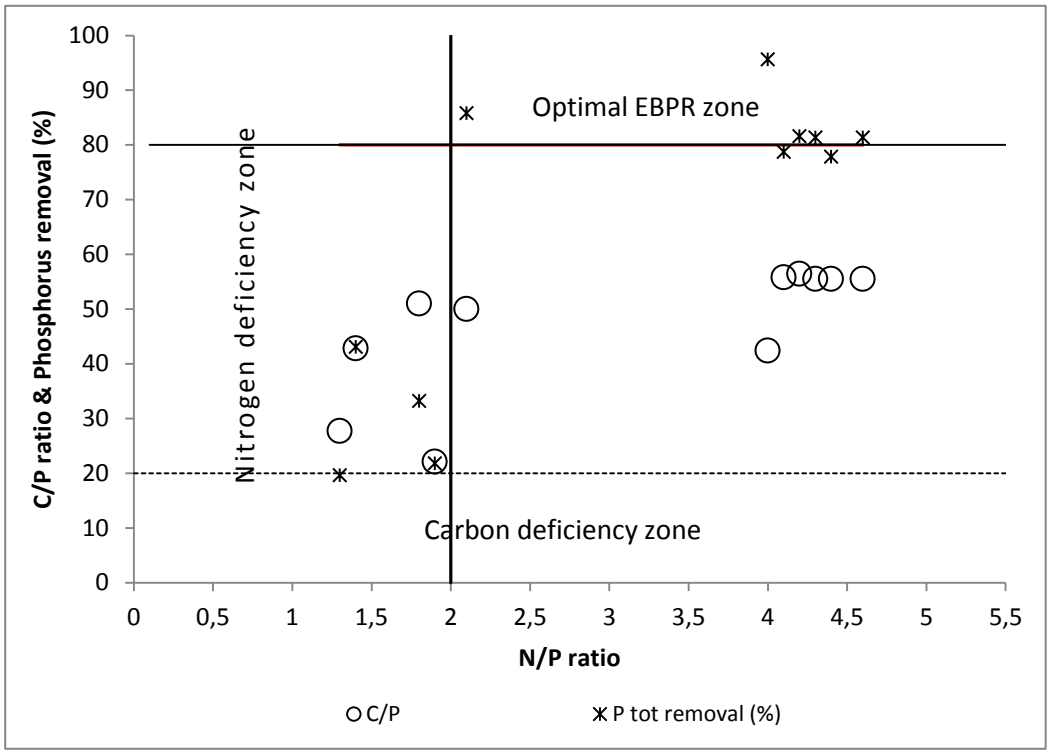


Figure 7.10: Phosphorus removal percentage against the N/P and C/P ratios in the influent for the S reactors.

c. Effects on the phosphorus removal percentage

The influence of the C:N:P ratio in the influent on the phosphorus removal performance was shown in Chapter 7.3.1. In this section, the influence of the C/P (BOD_5/Ptot) ratio on the phosphorus removal percentage and phosphorus uptake ratio (mgPtot/gVSS) is shown in more detail.

Figure 7.11 shows that as the C/P ratio in the influent was increased, the phosphorus removal percentage increased in all cases. Initially, C/P ratios between 13.1 and 22.1 in the influent were used, with both the C/P and N/P ratios then being gradually increased, as shown in Table 7.9.

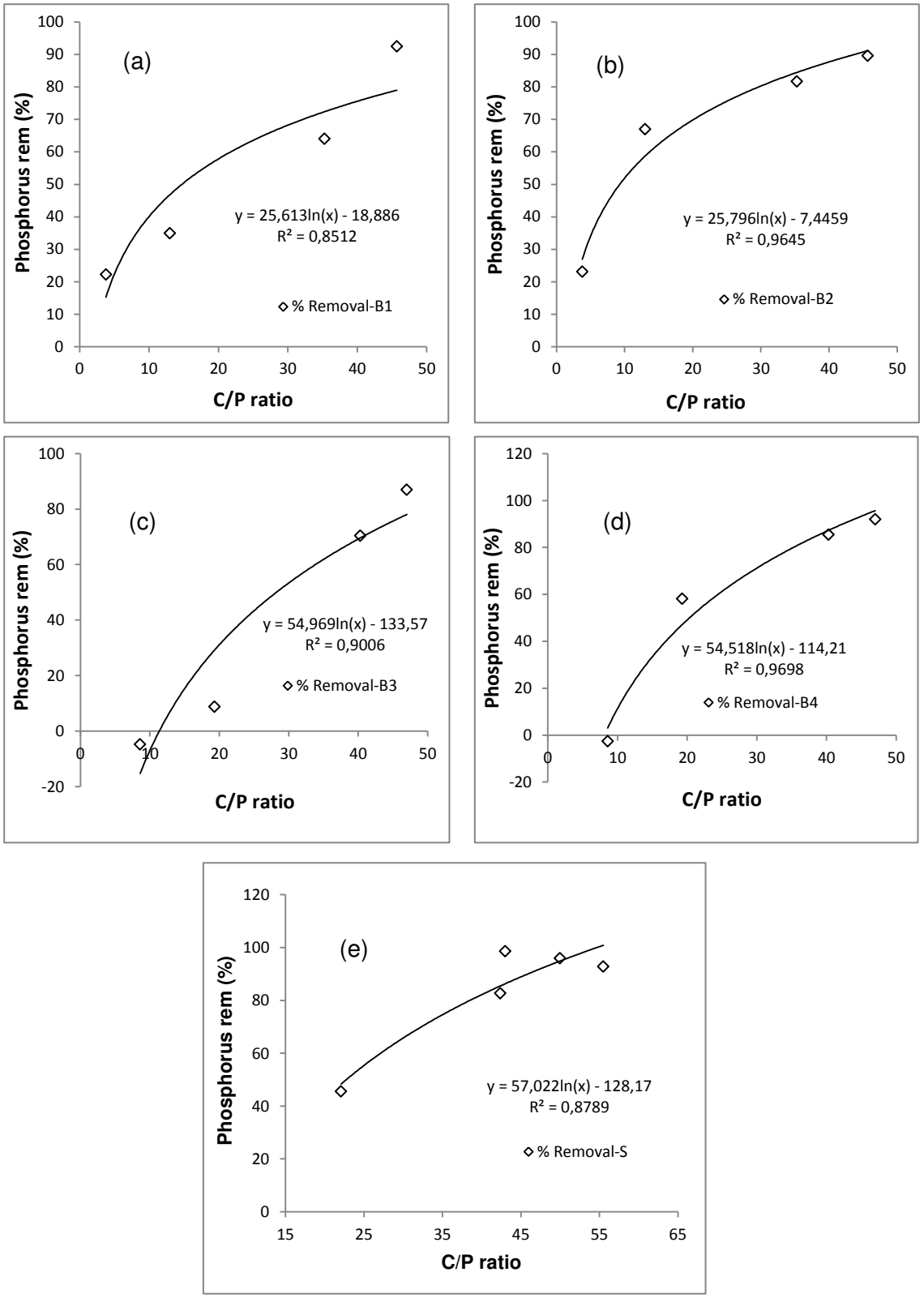


Figure 7.11: Trend lines determined by the C/P (BOD_5/Ptot) ratio in the influent against the phosphorus removal percentage.

Table 7.9: Gradual increase of the C/P (BOD_5/Ptot) and N/P (Ntot/Ptot) ratios in the influent during the experimental process.

Ratio	Month 1 - 6	Month 7 - 9	Month 10 - 12	Month 13 - 15	Month 16 - 18	Month 19 - 24	Month 25 - 27
C/P	13-22.1	22.1	35	38	40	45	56
N/P	1.9	1.9	3	4	4	4	4.1

As shown in Figure 7.11, the removal percentages between reactors become more similar as the C/P and N/P ratios in the influent increase. Finally, when a C:N:P ratio of 45:4:1 was achieved, very similar removal percentages were recorded, at approximately 90% in all reactors. At this point, the type of carbon source had almost no influence on the phosphorus removal percentage.

The trend lines in Figure 7.11 show positive slope regression coefficients for all reactors. From these coefficients, it can be seen that the increase of the removal percentage was continuous with the increase of the C/P ratio in the influent. These results contradict research by Liu et al., (1996) which suggested that when the loading rate COD-SS was high, the biomass could store a large amount of poly-hydroxyvalerate (PHV), the typical storage substance of GAO microorganisms, and consequently the EBPR system may decrease the efficiency of phosphorus removal. It may be significant, however, that the bacterial community in Liu et al. (1996), was described as an "anaerobic-aerobic activated sludge culture which used a P/TOC ratio in the influent appropriated for the development of PolyP accumulating bacteria (PAO)". Therefore, the decrease in the phosphorus removal efficiency observed by Liu et al. (1996) might be a response of the EBPR community facing a high COD-SS loading rate. In the present research, the increase of the C/P ratio in the influent up to 56/1 did not result in the detriment of the phosphorus removal percentage, not for EBPR communities (B2 and B4 reactors) nor in glucose communities (S, B1 and B3 reactors).

d. Effects on the phosphorus uptake per mass unit of volatile suspended solids (mgPtot/gVSS)

Initially, when a C/P ratio of less than 20 was used, the reactors recorded significant differences in their phosphorus uptake ratios. Subsequently, when a C/P ratio of more than 35 was used, the S reactors achieved the highest phosphorus uptake ratio, of approximately 1.47 mgPtot/gVSS. The difference of phosphorus uptake ratios between reactors was considerable when a C/P ratio of more than 40 was used. The S reactors

recorded phosphorus uptake ratios up to three times higher than those observed in the B reactors. Although remaining different from the S reactors, the phosphorus uptake ratios obtained in the B1, B2, B3 and B4 reactors became more similar to each other as the C/P ratio was increased. Thus, when a C/P ratio of more than 45 was reached, only non-significant differences between phosphorus uptake ratios in the B reactors were observed (0.44 - 0.53 mgPtot/gVSS).

In more detail, the gradual increase of the C/P and F/M ratios in the influent did not exert the same influence on the phosphorus uptake capacity in all reactors. Unlike the results of phosphorus removal percentage, where all reactors showed a constant increase of the phosphorus removal as the C/P ratio in the influent increased, the phosphorus uptake ratios did not show a constant increase with an increase of the C/P ratio in the influent. However, it was possible to differentiate two groups of results. The first group consisted of the B1 and B3 reactors which recorded a constant increase of the P uptake ratio according to the increase of the C/P ratio in the influent (Figure 7.12).

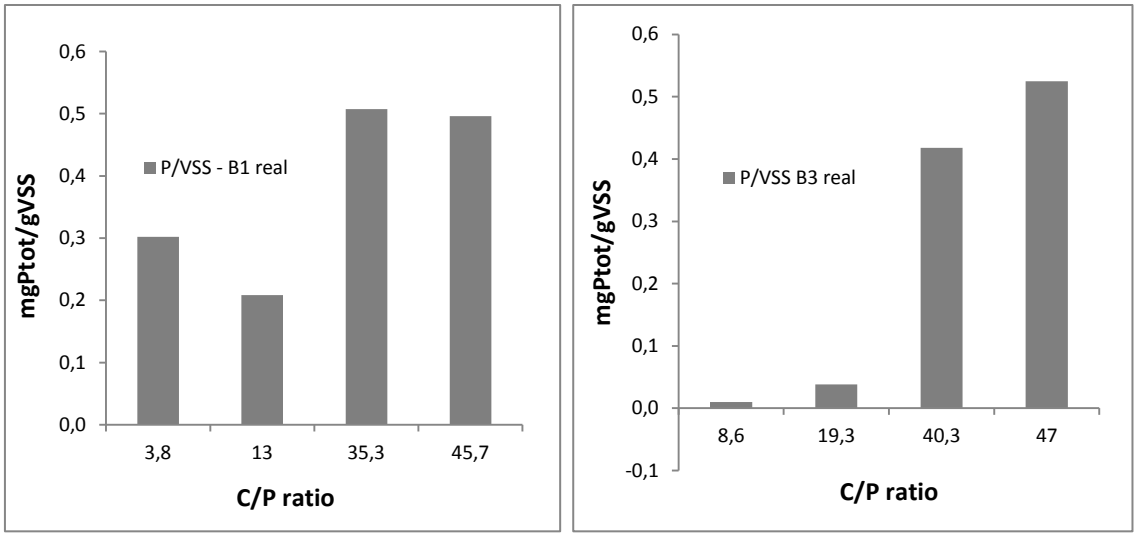


Figure 7.12: Phosphorus uptake per mass unit of Volatile Suspended Solids (mgPtot/gVSS) against the C/P ratio (BOD_5/Ptotal) in the influent for the B1 and B3 reactors.

The second group of results consisted of the S, B2 and B4 reactors (Figure 7.13), which recorded an increase in their P uptake ratios when C/P ratios (as BOD_5/P_{tot}) greater than 20 were achieved in the influent. Subsequently, as the C/P ratio continued increasing (35.3 for B2, 40 for B4 and 43 for S reactors) a constant decrease in the P uptake capacity was observed. The results obtained in the B2, B4 and S reactors were consistent with the results of research by Jenkins (2003). In Jenkins's research, it was observed that, during the anaerobic phase in an EBPR system, as the C/P ratio in the influent increased the acetate uptake ratio decreased, both of which could affect the phosphorus uptake during the incoming aerobic phase. Thus, Jenkins's work suggests

that the decrease of the P uptake capacity in the B2 and B4 reactors were to be expected since both biomasses corresponded to EBPR bacterial communities, which also include PAO bacteria as the most important bacterial group. For the S reactors, similar decrease in P uptake capacity was observed although this bacterial community corresponds to a glucose community which was dominated by the TFO morphotype. The decrease of the P uptake ratio for the B4 and S reactors was recorded when C/P ratios of 40.3 and 43 had been achieved, respectively.

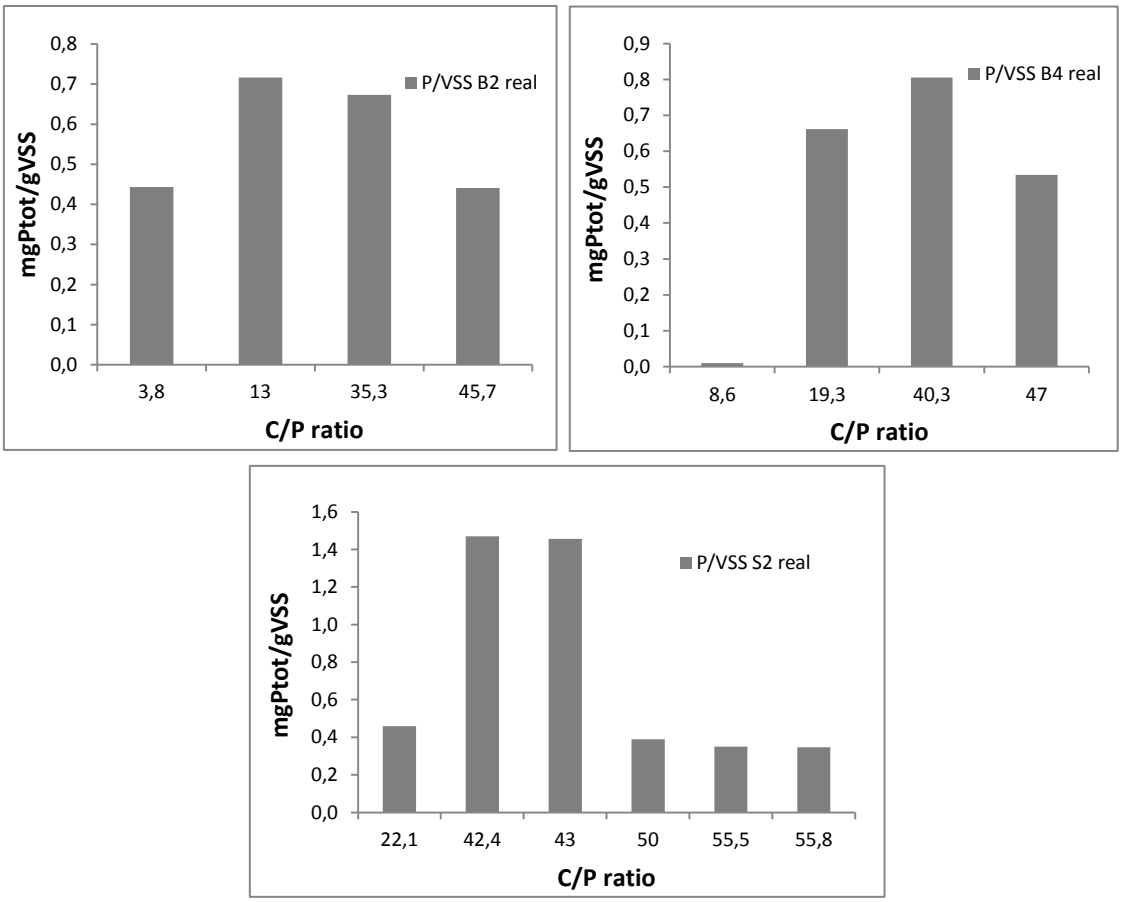


Figure 7.13: Phosphorus uptake ratio (mgPtot/gVSS) against the C/P ratio (BOD_5/Ptotal) in the influent for the B2, B4 and S2 reactors.

The relation between the P uptake ratio and the F/M ratio in mixed liquor showed similar results (Figure 7.14). For the B2 and B4 reactors, a slight and gradual decrease of the P uptake ratio was observed as the F/M ratio increased in mixed liquor, and for the S reactors, a slight decrease of the P uptake ratio was observed when an F/M ratio of 183 mgBOD_5/gVSS was achieved. As mentioned above, Liu et al. (1996) observed that as the COD-SS ratio increased in mixed liquor, the EBPR performance decreased.

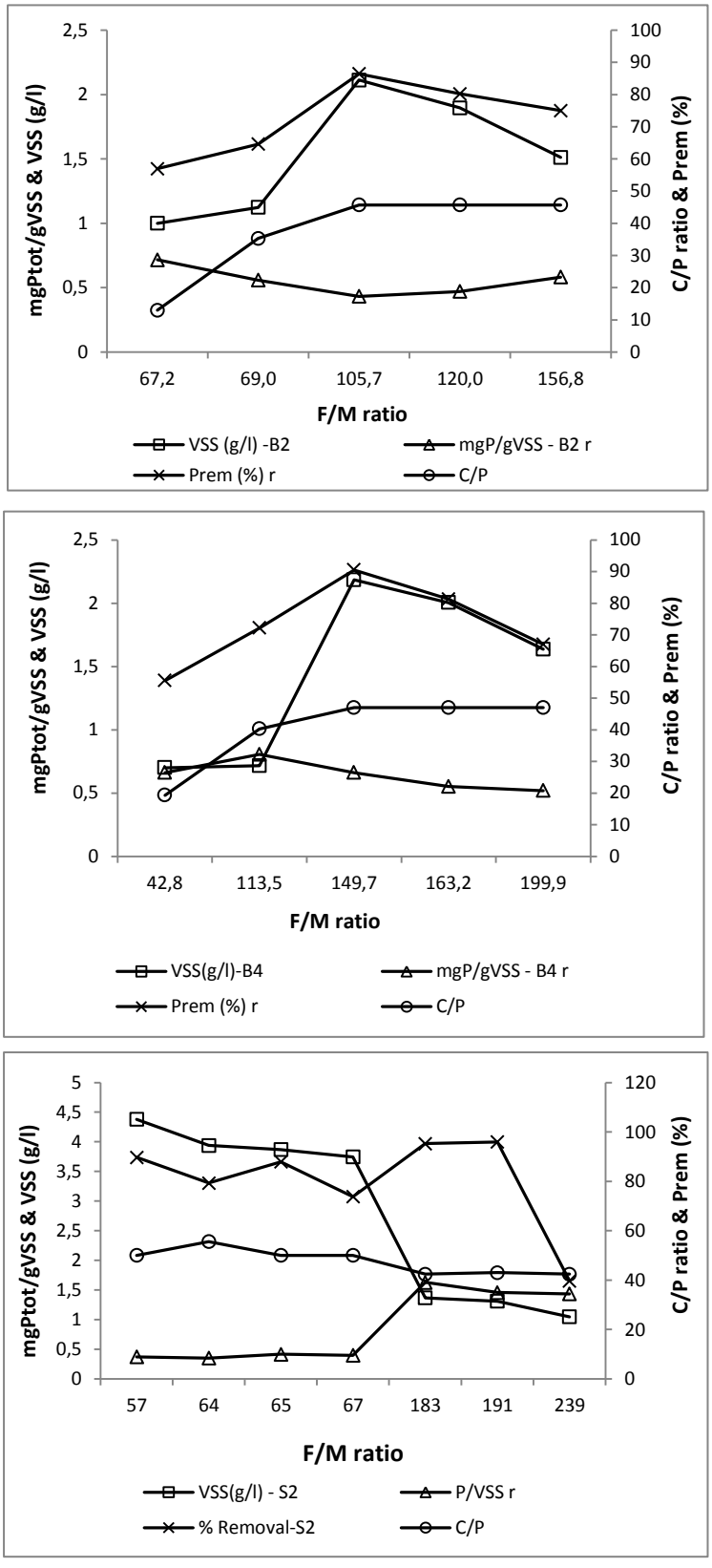
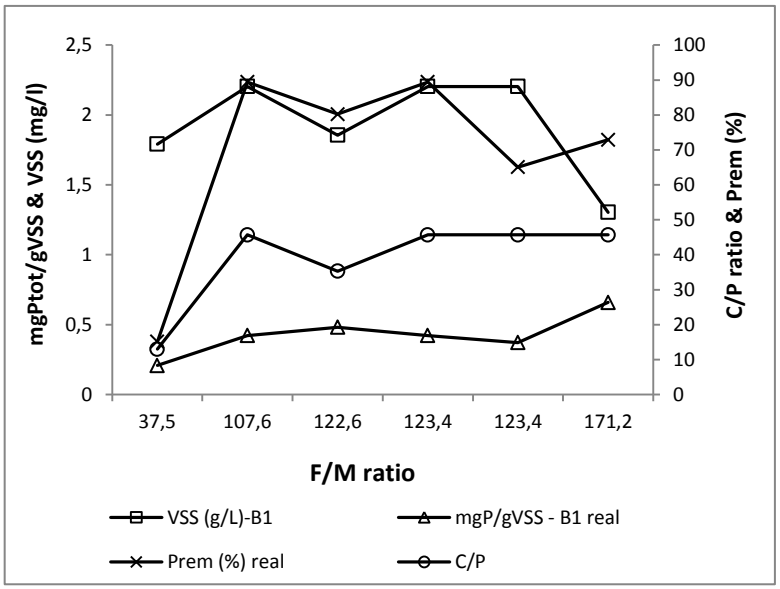


Figure 7.14: Phosphorus uptake ratio (mgPtot/gVSS) against the F/M ratio (mgBOD_5/gVSS) in mixed liquor for the S, B2 and B4 reactors.

Additionally, for the B1 and B3 reactors, a gradual increase of the P uptake ratio was observed as the F/M ratio in mixed liquor was increased. These results coincide with the results obtained for the phosphorus removal percentage in the same reactors.

It is notable that the B1 and B3 reactors recorded the lowest phosphorus removal yields, a fact that can be attributed to the adverse effect of the VFA in the influent on glucose bacterial communities. Later, a slow and steady increase of the P uptake ratio in both reactors (Figure 7.15) was observed. This can be attributed to a process of adaptation of these communities to new carbon sources.



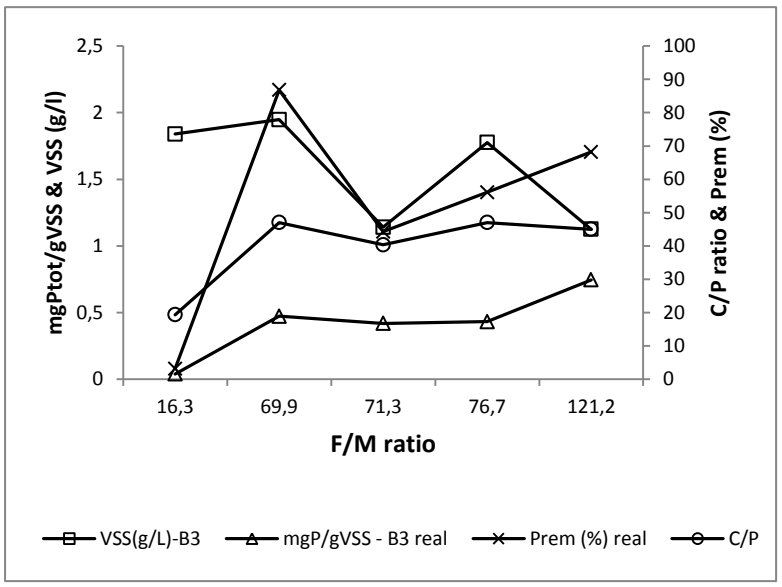


Figure 7.15: Phosphorus uptake ratio (mgPtot/gVSS) against the F/M ratio ($mgBOD_5$/gVSS) in mixed liquor for the B1 and B3 reactors.

As was mentioned above, as the C/P and F/M ratios were increased in the influent and mixed liquor, respectively, a gradual decrease of the P uptake ratio was observed in the B2 and B4 reactors. Similar results had already been observed in research by Liu et al. (2000) and Jenkins (2003). The results of the S reactors showed a similar behavior, with a decrease of the P uptake ratio as the C/P and F/M ratios were increased, although the S bacterial community was not a typical EBPR bacterial community.

Research by Jeon and Park (2000) may explain the good EBPR performance observed in the S reactors with bacterial communities that did not match a typical EBPR community. The metabolic pathway proposed by Jeon and Park (2000) suggests that there may be at least two groups of microorganisms involved in the EBPR process in reactors fed with glucose as carbon source. The first group of microorganisms would correspond to the Lactate Accumulating Organisms (LPO), with the following morphotype: perpendicular cuboidal bacteria of eight coccus-shaped cells. The LPO microorganisms would be able to assimilate glucose during the anaerobic phase and rapidly accumulate glycogen. The energy required for this process would be obtained by glycolysis, so that, the glucose would be transformed in lactate. Since the energy requirement would be met by glycolisis, the stored polyphosphate would not be used. Subsequently, the LPO would convert the stored glycogen into polymer storage and PHA. The second group of microorganisms would correspond to the PAO group, which would be able to uptake the lactate produced by the LPO and subsequently to transform it into PHA, meeting the energy demand of this process through the degradation of the stored polyphosphate and, therefore, releasing phosphate during the anaerobic phase. It is notable that the phosphorus release during the anaerobic phase in reactors fed with glucose seems not to correspond to the glucose consumed (Jeon and Park 2000; Hollender et al., 2002) and that the amount of phosphorus released is considerably less than the amount of phosphorus released during a typical EBPR process.

The release of phosphorus observed here during the anaerobic phase of the S (Glucose community), and B2 and B4 (EBPR community) reactors is shown in Figure 7.16. It is noticeable that the amount of phosphorus released in the B2 and B4 reactors is almost double of the amount released in the S reactors.

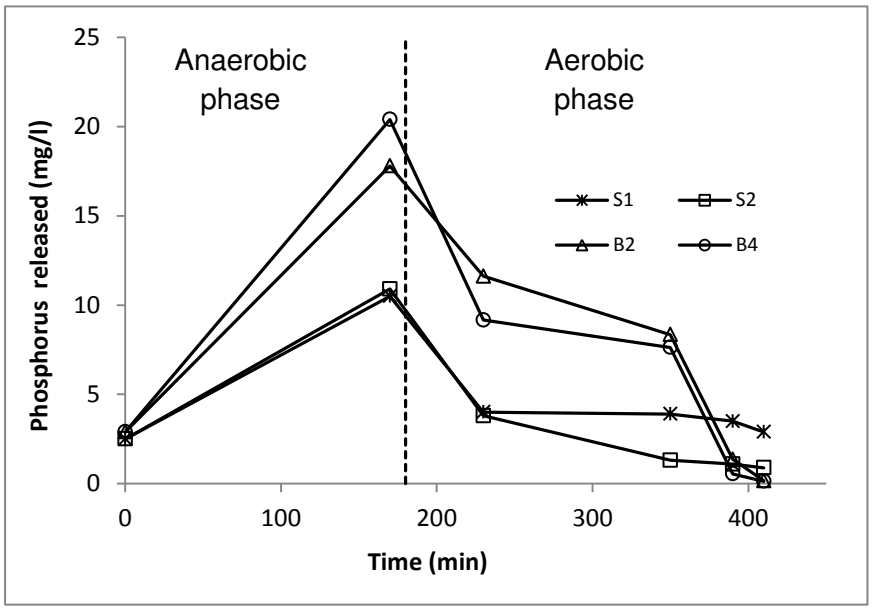


Figure 7.16: Total phosphorus concentration in mixed liquor during an operational cycle in the S1, S2 (Glucose community), B2 and B4 reactors (EBPR community).

Despite the different amount of phosphorus released during the anaerobic phase, all reactors recorded similar phosphorus removal performance at the end of the aerobic phase. Similar results were obtained in research by Hollender et al. (2002) using, respectively, glucose, acetate, and a mix of acetate and glucose as carbon sources, resulting in similar phosphorus content in the sludge samples at the end of the EBPR process.

As was already mentioned, the glucose bacterial community, in the B1 and B3 reactors, was not able to grow in the presence of VFA (Figure 7.5). The low phosphorus removal performance recorded for these reactors might be because the VFA was not assimilated by the LPO, and hence, these microorganisms could not produce lactic acid. Then, because of the absence of lactic acid, the PAO bacterial community would not be able to perform the EBPR process and a very low phosphorus removal performance would result. Although the VFAs are seen as the most suitable carbon source for EBPR processes, it seems that in these results, in the B1 and B3 reactors the PAO bacterial community was able to uptake lactic acid instead of VFA, and then, when the carbon source changed to VFA this bacterial community was not able to assimilate the organic acids immediately. This suggests that even PAO microorganisms can develop different metabolic pathways instead of only the Wentzel-Comeau or Mino model. Despite the low P uptake ratios achieved in the B1 and B3 reactors, they recorded a slow and steady increase of their phosphorus removal performance in relation to the increase of the C/P ratio in the influent and F/M ratio in the mixed liquor. In the light of the research by Jeon and Park (2000), the increase of

the phosphorus removal performance observed in the B1 and B3 reactors could be because of an adaptation process to a new carbon source (VFA) by a bacterial community (PAO) that was already adapted to uptake lactic acid as a carbon source.

Finally, other processes besides the EBPR may contribute to the observed phosphorus removal performance. Among these processes, the adsorption capacity of each bacterial community should be mentioned.

In research by Jenkins (2003), as the availability of COD in the influent increased, an increase in the concentration of carbohydrates to exocellular level was also observed; this effect may increase the adsorption capacity of these bacterial communities. The research results concerning the phosphorus adsorption capacity of the present bacterial communities is presented below (Chapter 8).

7.4 Conclusions

In summary, different types of wastewater were elaborated, varying in the carbon source and the C:N:P ratios used. The effects of these variations on the phosphorus removal percentage and on the phosphorus uptake ratio (mgPtot/gVSS) of different bacterial communities were assessed.

The results are presented in two big groups. The first group of results was obtained using three different C:N:P ratios chosen as adverse, medium and optimal for the EBPR process. The objective was to compare, within a specific C:N:P ratio, the difference of the phosphorus removal performance for the different carbon sources used and for different bacterial communities. The second group of results was obtained using different C/P and N/P ratios in the influent, maintaining optimal and constant conditions for other operational parameters that were identified as relevant for the EBPR process. The effects of the variations of the C/P and N/P ratios on the phosphorus removal performance were evaluated during the experimental process.

The conclusions are presented below:

1. Good EBPR performance is obtained with the glucose (TFO) and the EBPR bacterial communities when glucose and VFA are used as carbon sources, respectively.

It is possible to obtain good EBPR performance with different bacterial communities.

2. It is possible to obtain good EBPR performance in reactors fed with glucose as the sole carbon source, but the C/P ratio (BOD_5/Ptot) in the influent must be equal to or greater than 20.

3. The glucose bacterial community (TFO) obtained using glucose as carbon source recorded the lowest EBPR performance during the experimental process when supplied as a new carbon source to VFAs.

The VFAs are not suitable as a carbon source for the EBPR process in bacterial communities dominated by the TFO morphotype.

4. When the glucose bacterial community (B1 and B3 reactors) received VFA as a carbon source; initially, the results of phosphorus uptake and growth of biomass were the lowest observed during this research. Subsequently, a slow increase of their P uptake ratios was observed.

The glucose bacterial community can slowly adapt its metabolic pathways to use other carbon sources instead of glucose.

5. The EBPR bacterial community is able to perform efficient EBPR processes when supplied with appropriate concentrations of VFA. In this research, VFA concentrations greater than, approximately, 54 mg/l in the influent resulted in a decrease in the phosphorus uptake capacity.

6. In this research, N/P ratios in the influent of less than two impaired the phosphorus removal performance. This result agrees with the metabolic model proposed by Smolders et al. (1994) which explains the risk of nitrogen shortages during the aerobic phase.

N/P ratios (Total nitrogen/ Total phosphorus) of less than 2 in the influent can adversely affect the EBPR performance during the aerobic phase.

7. The benefit of using VFA as a carbon source in EBPR processes was evident when the C:N:P ratio in the influent was adverse, that is, when the system faced carbon shortages. Thereafter, when the C:N:P ratios became more appropriate for the EBPR process, the phosphorus removal percentage became similar across the reactors, regardless of the carbon source used as substrate. Likewise, the phosphorus uptake ratios in reactors fed with VFA became more similar as the C:N:P ratio in the influent was more appropriate for the EBPR process.

As the C:N:P ratio in the influent became more suitable for the EBPR process, the phosphorus removal performance between reactors became more similar regardless of the carbon source used.

8. When a C/P ratio of more than 40 was used in the influent, the amount of phosphorus released during the anaerobic phase was not directly related to the phosphorus removal performance in the EBPR processes, since the difference of the amount of phosphorus released between reactors were considerable but their phosphorus removal percentages were quite similar.

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CHAPTER 8: Assessment of the desorption and adsorption capacity of phosphorus in activated sludge samples

8.1 Introduction

The activated sludge system, with its specific configuration EBPR (Enhanced Biological Phosphorus Removal), is among the most efficient technologies for phosphorus removal from wastewater. However, this technology has been shown to be unstable (Hartley & Sickerdick 1994), which is why, generally, WWTPs have a complementary system which additionally achieves phosphorus removal by chemical precipitation (Blackall et al., 2002). Apparently, the stability of the EBPR process is strongly related to the composition of the influent wastewater, i.e. to obtain an efficient and stable EBPR performance it is necessary that the influent wastewater have appropriate C:N:P ratios and that these ratios be stable over time (Brdjanovic et al., 1998; Carucci et al., 1999).

Otherwise, research has suggested that phosphorus removal in EBPR systems is not only the result of metabolic processes but also occurs through adsorption processes (Cloete and Oosthuizen, 2001; Liu et al., 2006). Research by Cloete and Oosthuizen, (2001) using SEM/EDX technology, determined that, out of the total phosphorus content in the sludge biomass, between 27% and 30% would be located in the exopolymers (EPS).

The present research considered it of interest to evaluate the phosphorus sorption capacity of different activated sludge samples and simultaneously to evaluate the possibility of improving this capacity.

With regard to the phosphorus sorption capacities of different substrates, research by Wang and Li (2010) on the phosphorus sorption capacity of different sediments recorded the highest performance in estuarine sediments. Different physicochemical possibilities that might be responsible for this phosphorus sorption capacity were taken into account, including phosphorus precipitation because of the presence of metallic salts or lime in the aqueous phase. However, the concentration of iron and aluminium in the estuarine sediments were among the lowest in comparison to the other sediments evaluated.

In the present research, in order to explain the higher phosphorus sorption capacity recorded in estuarine sediments, the constant ion exchange process that the estuarine sediments would face during tidal changes is considered.

The ion exchange process that takes place in an estuary may be summarized as follows. During the low tide phase, the estuarine sediment is immersed in a freshwater environment where the principal dissolved ion is calcium bicarbonate, but most of the sediment free valences are compensated with calcium and, in a lower proportion, with magnesium. Then, during the flood-tide phase, the sodium ion in the incurrent seawater replaces the calcium ion in the sediment, with each calcium ion released to the aqueous phase being replaced by two sodium ions. Finally, the principal ion in the aqueous phase will be calcium chloride (Grupo de investigación de recursos hídricos – IUPA, 2009).

Additionally, research by Keiding and Nielsen (1997), and Muller (2006) mentioned the role of calcium ion in the structure of the sediments. These investigations suggested that the loss of calcium ion may destabilize the structure of the sediment. For estuarine sediments, the loss of calcium observed during the flood-tide phase may destabilize the sediment resulting in the desorption of organic molecules. Subsequently, during the next low tide phase a direct ion exchange process would be observed between the calcium ion dissolved in the incurrent freshwater and the sodium ions adsorbed in the sediment, with each calcium ion replacing two sodium ions, that would be released to the aqueous phase. Finally, the free valences of the sediment would be compensated again by calcium ions.

The present research aimed to apply the same ion exchange process, described for estuarine sediments, in activated sludge samples. With this purpose three different aqueous phases (solutions) are used: an eluent solution used during the desorption phase, a stabilization solution and finally a solution with a specific phosphorus concentration used during the sorption process.

At the end of the desorption-stabilization-sorption process, the phosphorus sequestration capacity of the activated sludge samples is evaluated, comparing the total phosphorus concentration in the sludge samples before and after the experimental process.

8.2 Material and methods

The following procedure was performed in order to evaluate the capacity of adsorption and desorption of phosphorus in the activated sludge samples.

8.2.1 Sludge sampling

The activated sludge samples were taken from the S1, S2, B2 and B4 reactors. Additional activated sludge samples were collected from the Uelzen, Steinhorst and Lüneburg WWTPs. Table 8.1 shows some characteristics of the sludge samples at the time of sampling.

Table 8.1: Characteristics of the activated sludge samples at the time of sampling.

Sludge source	Reactor phase	TSS (mg/l)	EBPR activity	VFA/Metalic salt	C:N:P in influent wastewater	Total Phosphorus in effluent (mg/l)
S1	Anaerobic	3.8 - 7.8	-	- /-	42:4.2:1	0.5
S2	Anaerobic	4.0 - 7.6	-	-/-	42:4.2:1	0.32
B2	Anaerobic	3 - 3.2	+	+/-	48:7.5:1	0.5
B4	Anaerobic	2.6 - 3.0	+	+/-	49:9.5:1	0.2
Uelzen						
UA	Aeration	4.2	-	-/+	----	-----
UF	Sludge thickener	24.1	-	-/+	-----	-----
Steinhorst						
BS	Aeration	3.8	-	-/-	24:6.5:1	14.3
RS	Sludge disposal line	8.1	-	-/-	24:6.5:1	-----
Lüneburg						
BS	Aeration	3.8	+	-/+	-----	-----
RS	Return sludge	7.8	+	-/+	-----	-----

The sludge samples had to meet certain characteristics before the experimental process could be started. These characteristics were important indicators of the

phosphorus sorption capacity, which showed it was possible to discard other physicochemical and biological processes as responsible for the phosphorus removal.

Among these characteristics, the reactor or reactor phase that the sludge sample came from provided information about the polyphosphate intracellular storage, when the sludge sample corresponded to an EBPR community.

The TSS concentration in the sludge sample showed the available amount of substrate.

The WWTPs reports about EBPR activity were confirmed in the laboratory using microscopic observation and staining techniques. The microscopic assessment served also to roughly characterize the morphotypes in each bacterial community.

The WWTPs reports about the addition of metallic salts to the phosphorus removal process were used to either approve or discard the application of the desorption-stabilization-sorption process in the sludge samples. The experimental process was approved, if, upon confirming the use of metallic salts, these did not present a concentration greater than 0.1 mg/l. This limit on the metallic salts concentration was calculated considering that to remove 1 g of phosphorus, it is necessary to employ 9.6 g of aluminium sulfate as $Al_2(SO_4)_3.14H_2O$ or 5.2 g of ferric chloride as $FeCl_3$ (WERF-Tertiary Phosphorus Removal).

The reports of the C:N:P ratio in the influent and the phosphorus concentration in the effluent served as indicators of the efficiency of the phosphorus removal process.

8.2.2 Pre-stabilization of the activated sludge samples using solutions of $CaCl_2$ and $MgCl_2$

It is important to mention that the sludge samples were evaluated in different sessions. For each sludge sample tested, before starting the desorption-stabilization-sorption process, the following samples were taken:

- Total phosphorus content in mixed liquor (mg/l)
- Total phosphorus content in supernatant (mg/l)
- Total phosphorus content in settled sludge (mg/l)

Subsequently, each sludge sample was distributed into glass beakers with a capacity of one liter (reactors) in duplicate and for each eluent evaluated. The number of reactors used per eluent is shown below (Table 8.2).

Table 8.2: Distribution of the sludge samples and the number of reactors used per eluent.

Sludge sample	Control	Eluents			
		Citric acid	Sulphuric acid	Hydrochloric acid	Potassium chloride
S1	2	2	2	2	2
S2	2	2	2	2	2
B2	2	2	-	-	2
B4	2	2	-	-	2
Uelzen	2	2	-	-	2
UA	2	2	-	-	2
UF	2	2	-	-	2
Steinhorst	2	2	-	-	2
BS	2	2	-	-	2
BR	2	2	-	-	2
Lüneburg	2	2	-	-	2
BS	2	2	-	-	2
BR	2	2	-	-	2

The sludge samples were allowed to precipitate for 45 minutes. The volumes of the precipitated sludge samples were recorded and the supernatants were decanted. The settled sludge samples were stabilized with a solution of $CaCl_2$ (5 mg/l) and $MgCl_2$ (10 mg/l) in deionized water. This solution was added to the settled sludge until a volume of 800 ml was reached.

8.2.3 Desorption process

The desorption-stabilization-sorption process was performed for the S1 and S2 sludge samples, using the four eluents mentioned. Samples of each of the three acid eluents were used with pHs of desorption of 2, 3, 4 and 5 in the case of acid eluents and with potassium chloride concentrations of 20, 30, 40 and 50 mmol/l in mixed liquor. As aforementioned, the stabilization process was performed using a solution of $CaCl_2$ and $MgCl_2$ in concentrations of 5 mg/l and 10 mg/l, respectively. On the basis of the results obtained with the S1 and S2 sludge samples regarding desorption performance, survival of bacterial communities (Chapter 9), and sorption performance, it was decided

to perform the desorption-stabilization-sorption process for the remaining sludge samples. Some characteristics of the eluents used are shown in Table 8.3.

Table 8.3: Characteristics of the eluents used in the desorption process.

Eluent	Concentration	Function
Citric acid	1 molar	Organic acid (weak acid)
Sulphuric acid	1 molar	Mineral acid (strong
Hydrochloric acid	1 molar	acid)
Potassium chloride	1 molar	Ion exchanger

The desorption process started with a pH of 2. The volumes of acid eluents used were recorded. For potassium chloride, in the first test a volume of KCl (1 M) was added until a concentration of 20 mmol/l in mixed liquor was reached. Control reactors maintained their own supernatant and no eluents were added.

The beakers were stirred at 60 rpm and maintained at room temperature (18 - 22°C). Mixed liquor samples of approximately 15 ml were taken at the end of the 1st, 3rd, 6th and 24th hours of the desorption process. Each sample was filtered in folding filters (Macherey-Nagel MN 615) and the filtrate was deposited in polyethylene bottles and stored at -20°C until analysis.

Upon completion of the desorption process, the sludge samples were allowed to settle for 45 minutes and the supernatants were decanted. The volumes of the precipitated sludge samples were recorded and samples of the precipitated were also taken.

8.2.4 Stabilization of the activated sludge samples using a solution of $CaCl_2$ and $MgCl_2$

Subsequently, the settled sludge samples were resuspended in a solution of $CaCl_2$ (5 mg/l) and $MgCl_2$ (10 mg/l) reaching a volume of one liter.

Each reactor was stirred at 60 rpm for 15 minutes at room temperature (18 - 22°C). The sludge samples were allowed to settle for 45 minutes, then samples of supernatant were taken and filtered in folding filters and the filtrates were deposited in polyethylene bottles and stored at -20°C until analysis. Finally, the remaining supernatants were decanted.

8.2.5 Sorption process

This experimental phase sought to determine the sorption capacity of phosphorus (adsorbate) of a substrate (adsorbent). The sludge samples previously stabilized were used as adsorbent and a specific concentration of phosphorus diluted in synthetic wastewater was used as adsorbate.

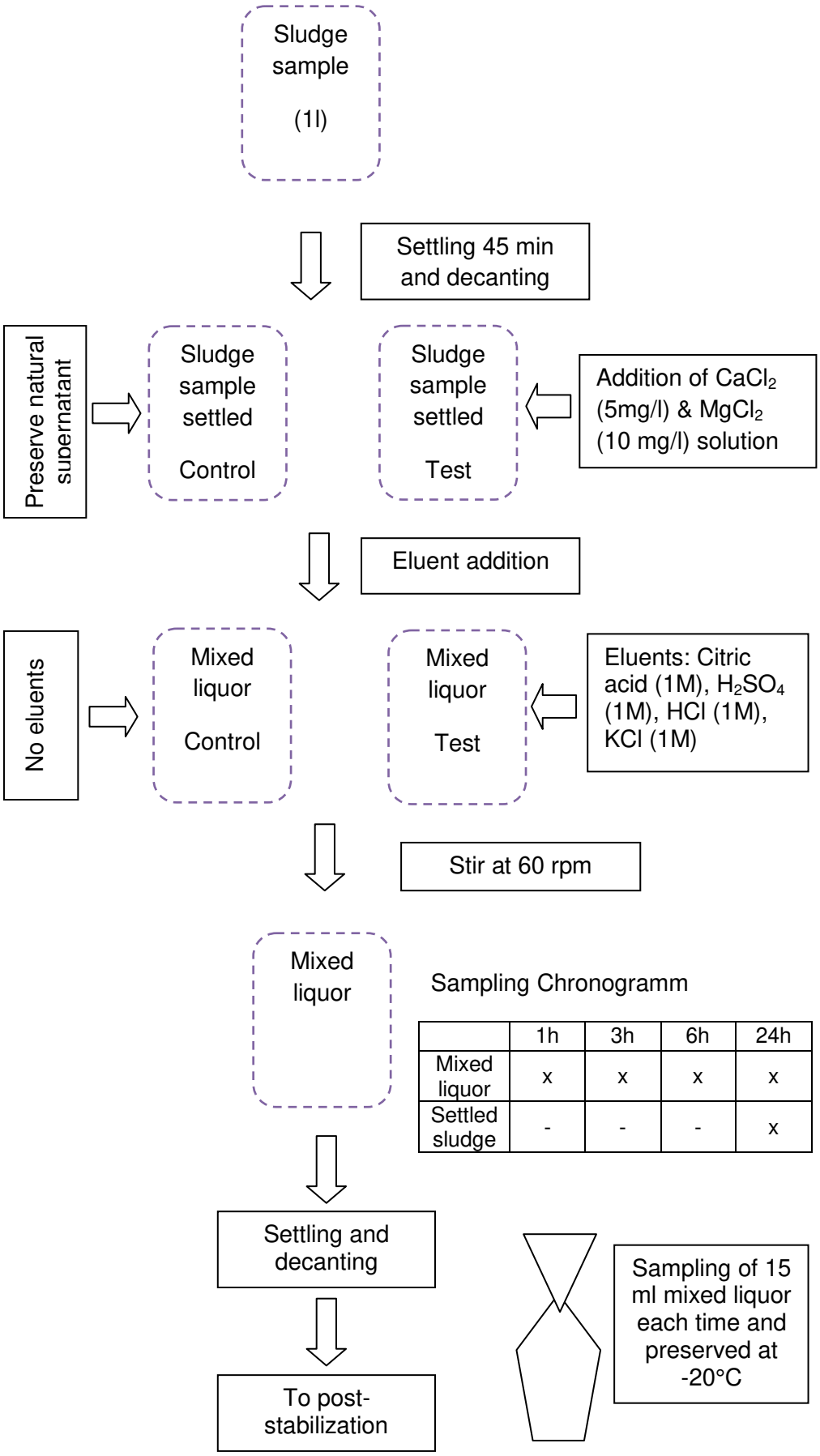
The C:N:P ratio in the influent synthetic wastewater was not the most suitable for EBPR processes (Yu et al., 2007). The total phosphorus concentration (7.5 mg/l) was within the middle range for domestic wastewater, according to Metcalf and Eddy (1995).

Table 8.4: C:N:P ratio in the influent synthetic wastewater.

Test solution	CSB (mg/l)	BSB_5 (mg/l)	Total nitrogen (mg/l)	Total phosphorus (mg/l)	C:N:P
WWp	375	156	31	7.5	20.8:4.2:1

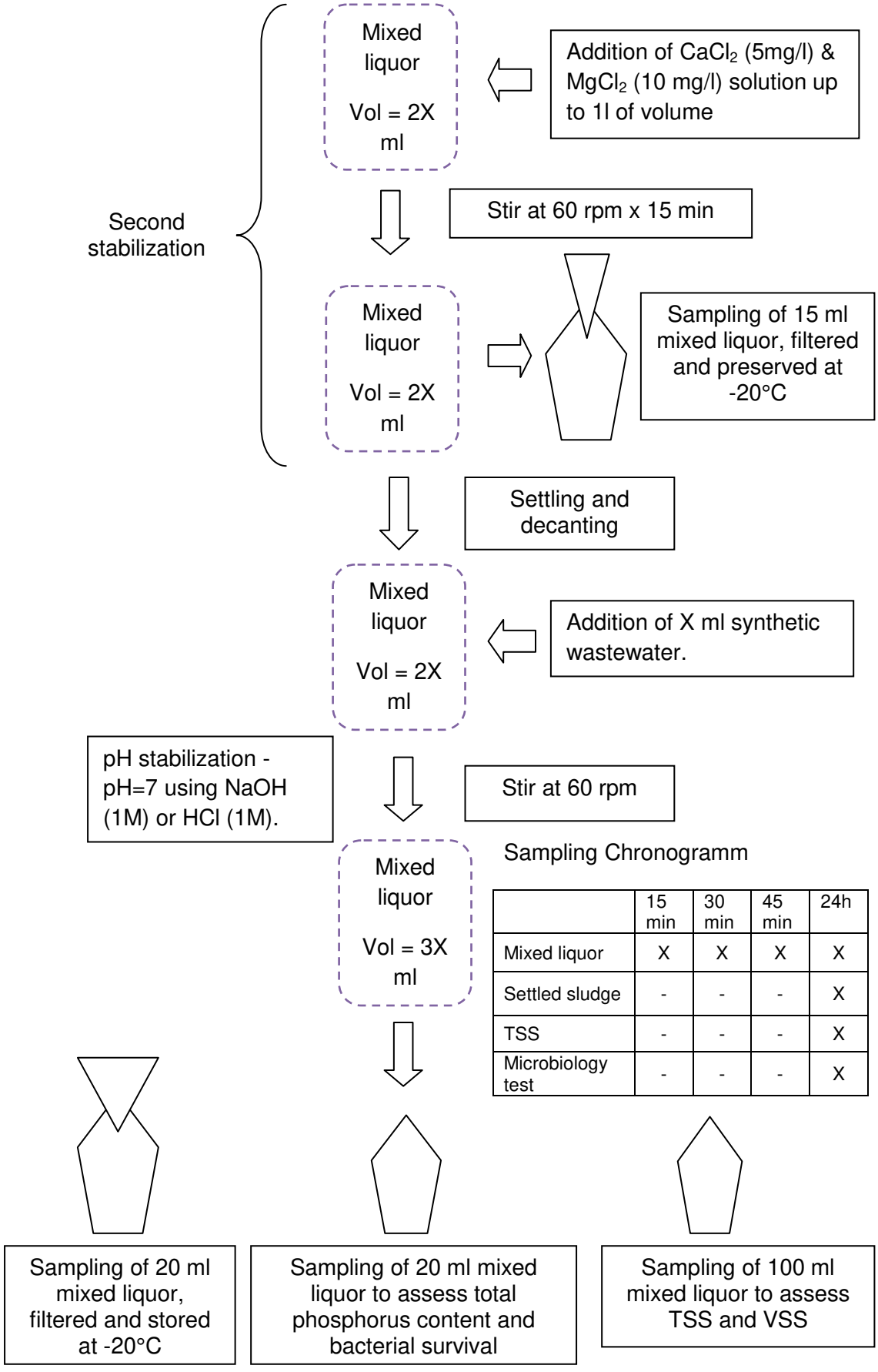
The volume of settled sludge in each reactor (adsorbent) accounted for 67% of the total sorption volume; thus, the synthetic wastewater accounted for the remaining 33% of this volume. Each beaker was stirred at 60 rpm and maintained at room temperature (18°C - 22°C). Mixed liquor samples of approximately 15 ml were taken at the end of the 15th, 30th and 45th minutes and at the end of the 24th hour of the sorption process. Each sample was filtered using folding filters (Macherey-Nagel MN 615) and the filtrate was deposited in polyethylene bottles and stored at -20°C until analysis.

At the end of the sorption process, two samples of mixed liquor (20 ml) were taken: one of them was stored at -20°C to assess the total phosphorus concentration and the second was stored at 4°C for 24 hours to analyze bacterial survival rates. Finally, a mixed liquor sample of about 100 ml was taken and immediately analyzed to assess the TSS and VSS concentrations.



	1h	3h	6h	24h
Mixed liquor	x	x	x	x
Settled sludge	-	-	-	x

Figure 8.1: Flowchart of the first stabilization and desorption processes.



	15 min	30 min	45 min	24h
Mixed liquor	X	X	X	X
Settled sludge	-	-	-	X
TSS	-	-	-	X
Microbiology test	-	-	-	X

Figure 8.2: Flowchart of the second stabilization and adsorption process.

8.3 Results and discussion – Desorption process

Initially, the desorption-stabilization-sorption processes were performed with the S1 and S2 sludge samples using the eluents citric acid (1M), sulphuric acid (1M) and hydrochloric acid (1M) with reaction pHs between 2 and 5. Likewise, potassium chloride (1M) was used as eluent with concentrations ranging between 20 and 50 mmol/l in mixed liquor.

The results of the S1 and S2 sludge samples were used to determine the most appropiate eluents and pH values to obtain good phosphorus desorption performances, considerable bacterial survival rates, and good phosphorus sorption performances. Table 8.2 shows the sludge samples used and the pHs and concentrations of the eluents in each case. The choice of the eluents was based on previous investigations such as research by Ye et al. (2011) where citric acid was identified as the best eluent for copper in granular activated sludge. The capacity of citric acid to weaken specific and electric adsorption was also noted by Ye et al. (2011). In the same research, sulphuric acid was recorded as the second best eluent in terms of desorption efficiency. Research by Kuczajowska-Zadrozna et al. (2004) on cadmium desorption processes in activated sludge recorded that the eluents HCl (1M) and H_2SO_4 (1M) showed the same desorption efficiency. Finally, research by Wang and Li (2010) using KCl (1M) as the eluent in desorption processes on sediments of different origins recorded a low desorption capacity for phosphorus in comparison to the results obtained using acid eluents in the other research just mentioned.

8.3.1 Phosphorus desorption percentage vs reaction time

The results of the desorption processes for the S1 and S2 sludge samples at a pH of 2 are shown in Figure 8.3. Basic characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before desorption process, and the eluent concentration used in each reactor, are shown in Table 8.5.

Table 8.5: Characteristics of the S1 and S2 sludge samples, and concentrations of the eluents used to reach a pH of 2 or KCl concentration of 20 mmol/l during the desorption process.

				Eluent concentration			
Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Citric Ac. (mmol/l)	H_2SO_4 (mmol/l)	HCl (mmol/l)	KCl (mmol/l)
S1	13.4	120.8	9	300	9.5	6.5	20
S2	16.4	264.2	16.1	250	7	8	20

As shown in Figure 8.3, the phosphorus release into the supernatant was recorded from the first hour of the desorption process for all reactors. The percentages of phosphorus release varied between 22 % (7.6 mg/l) recorded in the S1-HCl reactor at the end of the first hour, and 47.2 % (16.3 mg/l) recorded in the S1-SO reactor at the overall end of the reaction time (24 hours). Likewise, for the S2 sludge sample, the percentage of phosphorus release varied between 27.5 % (24.2 mg/l) recorded in the S2-HCl reactor at the end of the first hour and 55.5 % (48.9 mg/l) recorded in the S2-SO reactor at the overall end of the reaction time.

The desorption equilibrium was not reached in any of the S1 reactors but it was observed in the S2 reactors at the end of the sixth hour of the desorption process. Significant differences in desorption performance between reactors was not observed. For both sludge samples, the highest percentages of phosphorus desorption were achieved using sulphuric acid as the eluent.

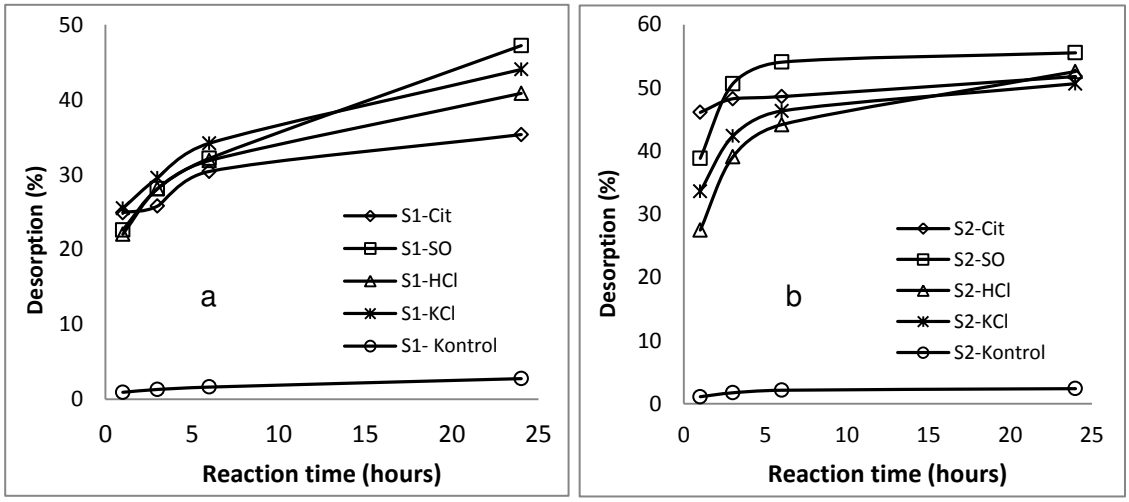


Figure 8.3: Phosphorus desorption percentages at a pH of 2 for the S1 (a) and S2 (b) sludge samples.

The results of desorption process for the S1 and S2 sludge samples at pH 3 are shown in Figure 8.4. Basic characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before the desorption process, and the eluent concentration used in each reactor are shown in Table 8.6.

Table 8.6: Characteristics of the S1 and S2 sludge samples, and concentration of the eluents used to reach a pH of 3 or KCl concentration of 30 mmol/l during the desorption process.

				Eluent concentration			
Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Citric Ac. (mmol/l)	H_2SO_4 (mmol/l)	HCl (mmol/l)	KCl (mmol/l)
S1	18.6	212.1	11.4	5.5	1.5	1.8	30
S2	15.3	196.8	12.8	7.5	2	2.5	30

Figure 8.4 shows that phosphorus desorption was observed from the first 15 minutes of reaction for all reactors.

For the S1 sludge sample, the phosphorus desorption percentages ranged between 1.3 % (0.8 mg/l) recorded in the S1-KCl reactor at the end of the first hour, and 54.6 % (33.1 mg/l) recorded in the S1-Cit reactor at the end of the overall reaction time. For the S2 sludge sample the phosphorus desorption percentages ranged between 3.4 % (2.2 mg/l) recorded in the S2-KCl reactor at the end of the first hour, and 66.3 % (43.5 mg/l) recorded in the S2-Cit reactor at the end of the overall reaction time.

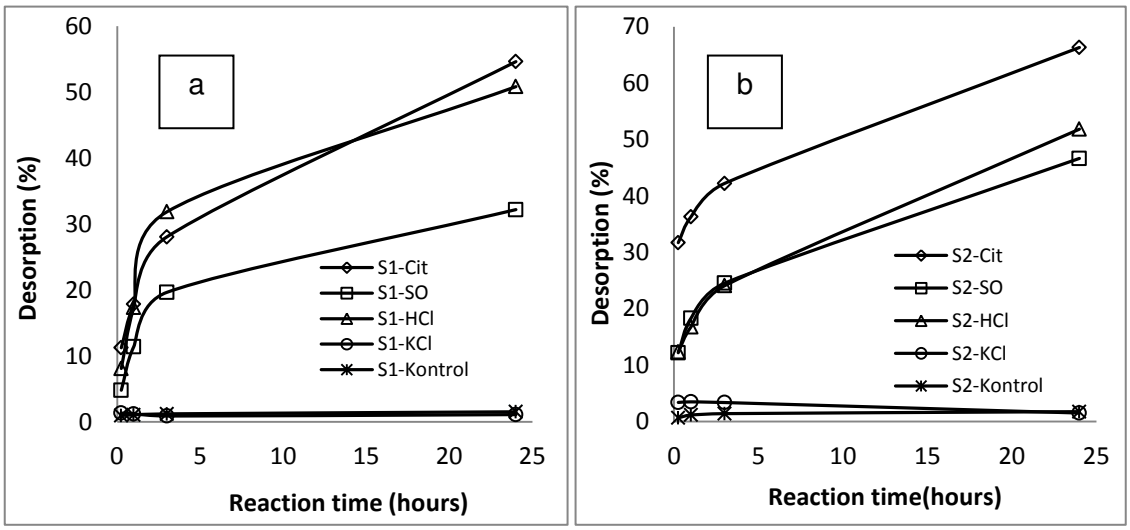


Figure 8.4: Phosphorus desorption percentages at a pH of 3 for the S1 (a) and S2 (b) sludge samples.

At a pH of 3, the best desorption percentages were recorded using citric acid as the eluent. Otherwise, the desorption percentages achieved for the S1-KCl and S2-KCl reactors were similar to the low values achieved for the control reactors. The desorption equilibrium was not reached in any reactor by the end of the reaction time.

The results of the S1 and S2 sludge samples at a pH of 4 are shown in Figure 8.5. Basic characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before the desorption process, and the eluent concentration used in each reactor are shown in Table 8.7.

Table 8.7: Characteristics of the S1 and S2 sludge samples, and concentrations of the eluents used to reach a pH of 4 or KCl concentration of 40 mmol/l during the desorption process.

				Eluent concentration			
Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Citric Ac. (mmol/l)	H_2SO_4 (mmol/l)	HCl (mmol/l)	KCl (mmol/l)
S1	23.4	218.6	9.4	1	0.5	0.5	40
S2	22.7	278	12.2	1.75	0.8	1.5	40

Low percentages of phosphorus desorption were recorded for the S1 sludge sample from the first hour of reaction. The same results were recorded for the S2 sludge sample with the exception that, for the S2-Cit reactor, a desorption percentage of 22.7% (21 mg/l) was recorded. The phosphorus desorption percentages ranged between 1.6 % (1 mg/l) recorded for the S1-KCl reactor at the end of the first hour, and 25.1 % (15.7 mg/l) recorded for the S1-Cit reactor at the overall end of the reaction time. Likewise, for the S2 sludge sample the desorption percentages ranged between 0.7 % (0.7 mg/l) recorded for the S2-KCl reactor during the third hour, and 27.4 % (25.4 mg/l) recorded for the S2-Cit reactor at the overall end of the reaction time. In no case was a considerable amount of phosphorus released.

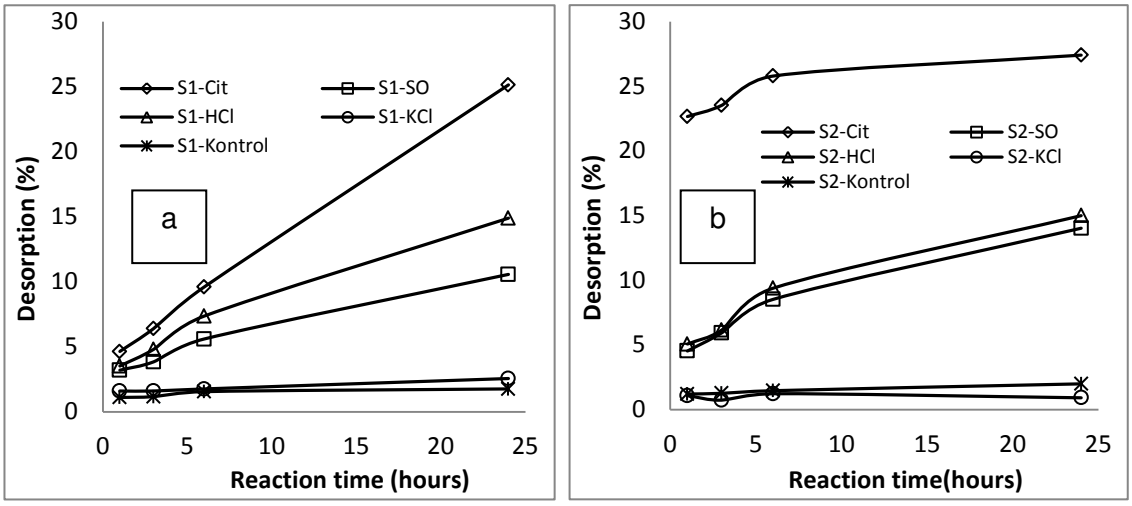


Figure 8.5: Phosphorus desorption percentages at a pH of 4 for the S1 (a) and S2 (b) sludge samples.

Again, the lowest desorption percentages were recorded in the S1-KCl and S2-KCl reactors and these percentages were similar to the values obtained for the control reactors. The desorption equilibrium was not achieved for either sludge sample by the end of the reaction time.

The results of the S1 and S2 sludge samples at a pH of 5 are shown in Figure 8.6. Characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before the desorption process, and the eluent concentration used in each reactor, are shown in Table 8.8.

Table 8.8: Characteristics of the S1 and S2 sludge samples, and concentrations of the eluents used to reach a pH of 5 or KCl concentration of 50 mmol/l during the desorption process.

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				Eluent concentration			
Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Citric Ac. (mmol/l)	H_2SO_4 (mmol/l)	HCl (mmol/l)	KCl (mmol/l)
S1	27.2	284	10.5	0.5	0.3	0.3	50
S2	12	208.5	17.4	0.6	0.5	0.7	50

Figure 8.6 shows the results of the S1 sludge sample which recorded the lowest phosphorus desorption percentages in the experimental process. Likewise, the results of the S2 sludge samples recorded desorption percentages that ranged between 2.3 % (1.6 mg/l) in the S2-KCl reactor, and 39 % (27.1 mg/l) in the S2-Cit reactor.

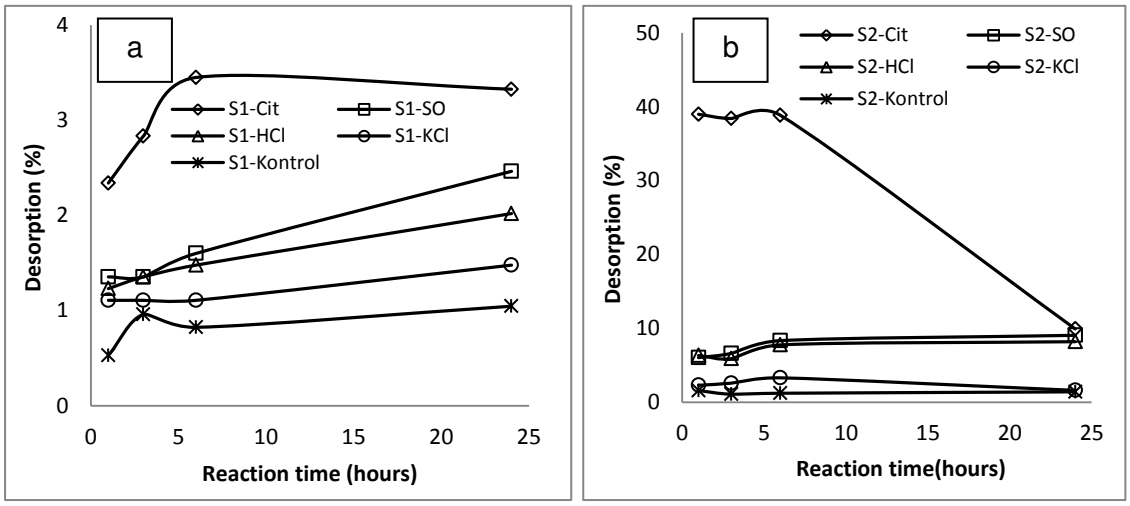

Figure 8.6: Phosphorus desorption percentages at a pH of of 5 for the S1 (a) and S2 (b) sludge samples.

The results noted thus far recorded the highest desorption percentages for the S2-Cit, S2-HCl and S1-Cit reactors at a pH of 3, with phosphorus desorption percentages that ranged between 54.6% (33.1 mg/l) and 66.3% (43.5 mg/l). Good desorption percentages were also recorded for the S2-SO, S2-HCl, S2-Cit reactors at a pH of 2 and for the S2-KCl reactor (eluent concentration = 20 mmol/l) with percentages ranging between 50.6% (44.6 mg/l) and 55.5% (48.9 mg/l). These results were obtained at the end of the reaction time (24 hours).

The results obtained for the S1 and S2 reactors were used to choose the pH and eluents for the remaining desorption-stabilization-sorption processes. The selection criteria used to choose the type and concentration of eluents were: the phosphorus desorption and sorption performance, the phosphorus release during the stabilization process, and the bacterial survival rates after completion of the desorption-stabilization-sorption process.

The phosphorus release during the stabilization process was assessed in order to determine the finalization of the desorption process. In the stabilization process the sludge samples were diluted in a $CaCl_2$ (5mg/l) and $MgCl_2$ (10 mg/l) solution with the aim of stabilizing the sludge biomass after the desorption process. Once the stabilization process had been completed, the total phosphorus content in the supernatant was evaluated. The amount of phosphorus released was considered an

indicator of incomplete desorption processes, and hence, a sludge sample which continued releasing phosphorus to the supernatant would not be used in a subsequent sorption process. Another criterion was the phosphorus sorption capacity of the sludge samples. The sorption results of each sludge sample are presented in Chapter 8.3.2; however, as they are selection criteria, the sorption results of the S1 and S2 sludge samples at pH 2 and 3 are shown in Table 8.9.

Finally, the last selection criterion was the bacterial survival rate in each sludge sample once the desorption-stabilization-sorption process was completed. The survival capacity of each bacterial community was evaluated after completion of the sorption process using the Agar Pour Plate technique. The results of the bacterial survival rates are shown in Chapter 9; however, the results of the S1 and S2 sludge samples are shown in Table 8.9, again, as they are selection criteria.

Table 8.9 shows that the S1-KCl and S2-KCl were the only reactors with sorption capacity. Likewise, upon completion of the stabilization process, the amount of phosphorus released for the S1-KCl and S2-KCl reactors was not high when the previous desorption process used an eluent concentration of 30 mmol/l. Finally, good bacterial survival percentages were recorded for the S1-KCl-pH3 reactor (152%), while the S2-KCl-pH3 reactor reached 14% survival.

Considering the low bacterial survival percentages achieved upon completion of the desorption-stabilization-sorption processes at pH 2, and also considering the low sorption percentages obtained in all reactors which underwent previous desorption processes at this pH, the desorption process at pH 2 was dismissed for the remaining experimental processes.

Finally, the eluents citric acid at pH 3 and potassium chloride with concentration of 30 mmol/l were selected as desorption eluents. These eluents were choosen considering the best desorption results using citric acid as eluents, and also because the best sorption performances were achieved after completion of a desorption process using potassium chloride as eluent with a concentration of 30 mmol/l. The results of the phosphorus release during the stabilization process and the bacterial survival percentages were also considered.

Table 8.9: Selection criteria of the eluents for the remaining desorption-stabilization-sorption processes.

Reactor	Eluent-acid / Eluent KCl	Stabilization process: Ptot in supernatant (mg/l) pH 2 (20 mmol/l)	Stabilization process: Ptot in supernatant (mg/l) pH 3 (30 mmol/l)	Sorption process (%) pH 2 (20 mmol/l)	Sorption process (%) pH 3 (30 mmol/l)	Survival test (%) pH 2 (20 mmol/l)	Survival test (%) pH 3 (30 mmol/l)
S1	Cit	3.8	15.2	-128	-1890.9	4.5	3
	SO	3	10.2	13.4	-1175.3	3	112
	HCl	3.1	14.5	-11.5	-1040.2	3	42
	KCl	3.5	1.6	64.4	74.2	6.1	152
	Control	0.9	1.1	20.8	7.3	45	55
S2	Cit	16.6	14.1	-78.3	-494.1	13.4	87
	SO	17.4	10.8	-36.9	-600	12.6	152
	HCl	16.2	12.2	-28.8	-604	10.5	70
	KCl	14.9	0.3	-55.6	78.6	29.8	14
	Control	0.8	0.7	20.2	24	51.3	49

The remaining processes of desorption-stabilization-sorption were perfomed with the B2 and B4 sludge samples, and with the Lüneburg WWTP and Steinhorst WWTP sludge samples.

The results of the phosphorus desorption percentages for the B2 and B4 reactors are shown in Figure 8.7. Basic characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before the desorption process, as well as the eluent concentration used in each reactor, are shown in Table 8.10.

Table 8.10: Characteristics of the B2 and B4 sludge samples and eluent concentrations used to achieve a reaction pH of 3 during the desorption process.

Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Eluent concentration: Citric Ac. (mmol/l)	Eluent concentration: KCl (mmol/l)
B2	9.6	252.5	26.3	5	30
B4	7.7	248.5	32.4	5.5	30

Figure 8.7 shows that for the B2-Cit and B4-Cit reactors, phosphorus release into the supernatant was recorded from the first hour of desorption. For these reactors, the phosphorus desorption percentages increased gradually, achieving 45.9% (38.6 mg/l) for the B2-Cit reactor and 26.1% (21.6 mg/l) for the B4-Cit reactor. The B2-KCl and B4-KCl reactors achieved very low desorption percentages, similar to the values achieved in their control reactors. In no reactor was desorption equilibrium reached by the end of the reaction time.

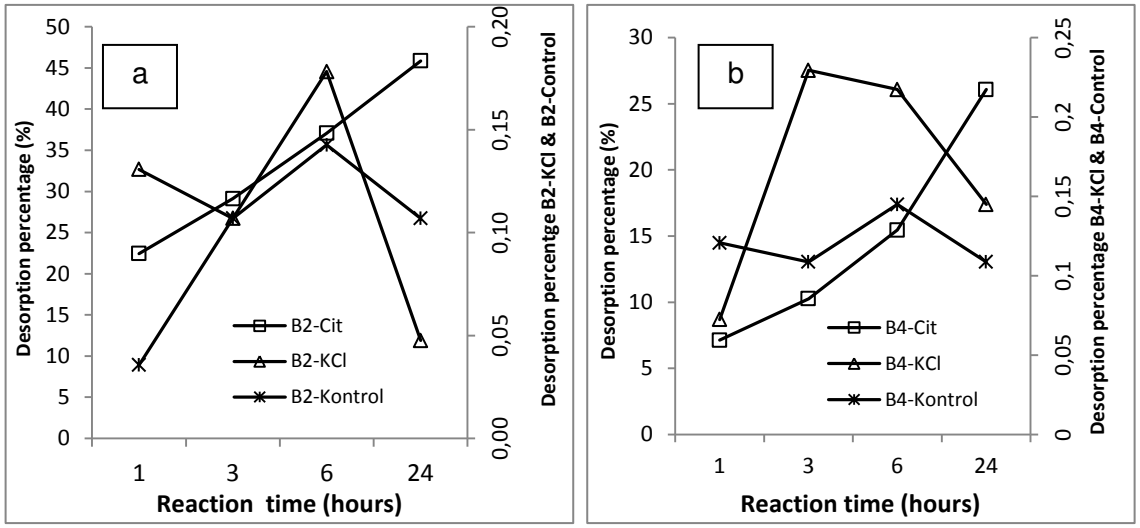

Figure 8.7: Phosphorus desorption percentages at a pH of 3 in the B2 (a) and B4 (b) sludge samples.

The phosphorus desorption percentages for the Lüneburg sludge sample are shown in Figure 8.8. Basic characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before the desorption process, as well as the eluent concentration used in each reactor, are shown in Table 8.11. The Lüneburg-BS sludge sample was taken from the aeration tank and the Lüneburg-RS sludge sample was taken from the Return Activated Sludge line (RAS) of the Lüneburg WWTP.

Table 8.11: Characteristics of the Lüneburg-BS and Lüneburg-RS sludge samples and eluent concentrations used to achieve a reaction pH of 3 during the desorption process.

				Eluent concentration	
Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Citric Ac. (mmol/l)	KCl (mmol/l)
BS	19.2	452.9	23.6	6.3	30
RS	19.4	549.1	28.3	7.5	30

As shown in Figure 8.8, phosphorus release into supernatant for the BS-Cit and RS-Cit reactors was recorded from the first reaction hour. The phosphorus desorption percentages ranged between 1.3% (1.2 mg/l) for the BS-KCl reactor and 70.3% (63.7 mg/l) for the BS-Cit reactor. Meanwhile, for the RS sludge samples, the phosphorus desorption percentages ranged between 1.7% (3.7 mg/l) for the RS-KCl reactor and 59.3% (130.3 mg/l) for the RS-Cit reactor. The latter values were achieved at the end of the reaction time.

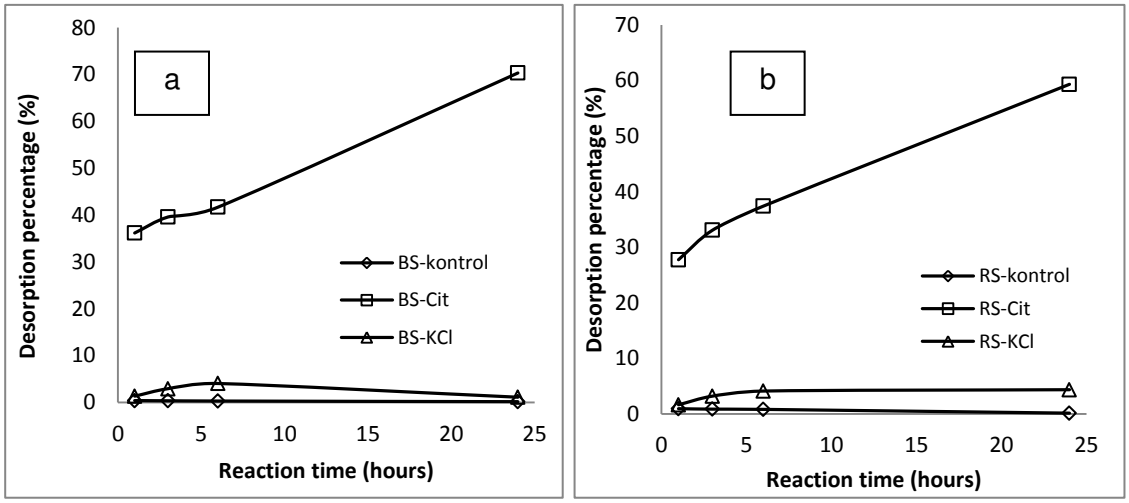

Figure 8.8: Phosphorus desorption percentages at a pH of 3 for the BS (a) and RS (b) sludge samples of the Lüneburg WWTP.

The highest desorption percentage achieved in the BS-KCl reactor was 4% (3.7 mg/l) and in the RS-KCl reactor was 4.4% (9.6 mg/l). These results were slightly higher than those obtained in the control reactors. Apparently, the desorption equilibrium was achieved in the RS-KCl reactor during the sixth hour of reaction.

The phosphorus desorption percentages of the Steinhorst sludge sample are shown in Figure 8.9. Basic characteristics of the TSS concentration in mixed liquor, phosphorus content per sludge mass unit before the desorption process, as well as the eluent concentration used in each reactor, are shown in Table 8.12. The Steinhorst-BS sludge sample was taken from the aeration tank and the Steinhorst-RS sludge sample was taken from the sludge stabilization line of the Steinhorst WWTP.

Table 8.12: Characteristics of the Steinhorst-BS and Steinhorst-RS sludge samples and eluent concentrations used to achieve a reaction pH of 3 during the desorption process.

				Eluent concentration	
Reactor	TSS (g/l)	Ptot initial (mg/l)	Ptot/TSS (mg/g)	Citric Ac. (mmol/l)	KCl (mmol/l)
BS	9.5	158.2	16.8	6	30
RS	13.4	253.4	18.9	7.5	30

As shown in Figure 8.9, the phosphorus release was recorded from the first hour of desorption for the BS-Cit and RS-Cit reactors. The phosphorus desorption percentages ranged between 2.4% (1.5 mg/l) for the BS-KCl reactor and 53.6% (33.9 mg/l) for the BS-Cit reactor. Meanwhile, for the RS sludge sample, the phosphorus desorption percentages ranged between 2.9% (4.4 mg/l) and 47.9% (72.7 mg/l) for the RS-Cit reactor. It should be mentioned that at the end of the reaction time, the BS-Control and RS-Control reactors achieved considerable desorption percentages of 35.3% (22.3 mg/l) and 25.6% (38.8 mg/l), respectively.

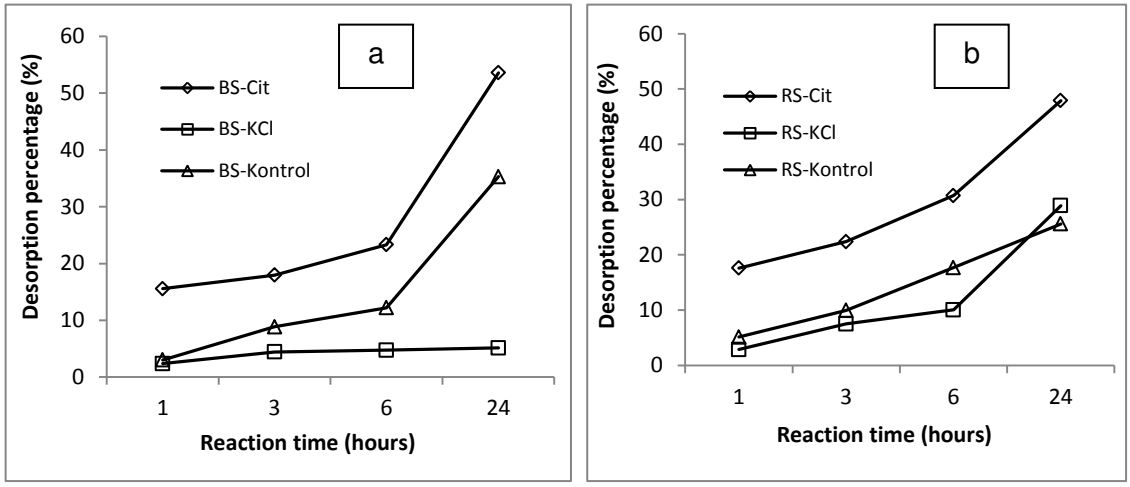

Figure 8.9: Phosphorus desorption percentages at a pH of 3 for the BS (a) and RS (b) sludge samples of the Steinhorst WWTP.

As can be seen, the greatest release of phosphorus was recorded when citric acid was used as eluent (RS-Lun-Cit 130.3 mg/l; RS-Stein-Cit 89.3 mg/l; BS-Lun-Cit 63.7 mg/l; S2-Cit 43.5 mg/l; B2 38.6 mg/l; BS-Stein-Cit 38.5 mg/l; S1-Cit 33.1 mg/l). Additionally, the consumption of citric acid as carbon source was confirmed by the results of the bacterial survival test (Chapter 9). Apparently, the EBPR bacterial community was able to assimilate the citric acid during the desorption process. Therefore, the phosphorus release during the desorption process may partly be caused by the phosphorus release

observed in an EBPR process during the anaerobic phase in presence of a carbon source.

Likewise, it is unlikely that the bacterial communities in reactors S1 and S2 were able to use citric acid as a carbon source since the results of bacterial survival recorded the decrease of their initial bacterial concentration. Therefore, it can be confirmed that the phosphorus release during the desorption processes for the S1 and S2 sludge samples was caused by the eluent effect. For the B2, B4 and Lüneburg sludge samples, it was not possible to differentiate the percentage of phosphorus released due to the eluent effect and due to the phosphorus release during the EBPR processes.

In order to achieve a successful desorption process, the eluent used should be able to abate the forces that bind the chemical compound (sorbat) with the flock structure (adsorbent). Among the binding forces which were involved in sorption processes are the electrostatic forces, van der Waals forces and chemical attractions (Grupo de investigación de recursos hídricos - IUPA, 2009). Depending on the eluent used it would be possible to weaken one or all these binding forces, reaching a partial or total desorption.

The present research shows that the citric acid was able to abate more binding forces than the KCl. The only binding forces that the ion exchanger would be able to abate are the electrostatic forces, hence the low phosphorus desorption percentages obtained when potassium chloride was used as eluent. Similar results were observed in research by Wang and Li (2010) using potassium chloride as eluent. This research recorded phosphorus retention potentials up to 95% for estuarine sediments and up to 85% for sediments from a lake and a canal. In the same research it was proposed that the low desorption potentials recorded using potassium chloride as eluent might be due to the slightly acidic environmental conditions (pH 5 - 6) that increase the amount of positive charges in the sediment surface. In turn, these positives charges would maintain the attachment of the phosphorus to the sediment surface. Finally, the research recorded that the estuarine sediment had a high phosphorus adsorption capacity but a low desorption potential. Additionally, Wang and Li (2010) mentioned that the phosphorus retention capacity of a substrate is related to the density of vacant sites on the substrate surface.

Other research has proposed that the availability of vacant sites might decrease if the substrate was exposed over a long period of time to an aqueous media with high phosphorus concentration. Research by Lin et al. (2009) on phosphorus sorption and desorption capacity of Daliao River sediments (China) concluded that, because of the

long history of pollution in the river, the phosphorus sorption capacity of the sediment decreased and its phosphorus desorption potential increased. Similar results were obtained by Lin and Banin (2005) but with sediments from a WWTP.

In the present study, the phosphorus sorption capacity of the sludge samples will be evaluated in Chapter 8.3.2. An aspect of the sorption research will seek to clarify if the sludge samples with less phosphorus desorption potential will be those that, at the same time, recorded the highest sorption performance.

8.3.2 Determination of the apparent desorption coefficient (Kdes)

The apparent desorption coefficient expresses the desorption behavior of substrates. This coefficient represents the mobility of the chemical compounds in substrates (OECD, 2000). The Kdes relates the content of the chemical substance (sorbat) that remains sorbed in the soil and the mass concentration of the chemical substance desorbed in the aqueous phase. Therefore, its having a lower value would be an indicator that the amount of chemical compound desorbed was greater.

A further objective of the sorption-desorption investigations is to determine the reversibility of the sorption process, i.e. to determine which percentage of the chemical compound previously sorbed will be released to the aqueous phase during the desorption process; therefore, the difference between the coefficients Kdes and Kd (sorption distribution coefficient) is evaluated. According to Wang and Li (2010), wide differences between both coefficients are caused by the large sorption capacity of the substrate and by the availability of vacant sites on its surface that impede the desorption of the chemical compound. Significant differences between both coefficients are called adsorption/desorption hysteresis.

The equation of the Kdes coefficient (Formula 8.1) is used in research of adsorption-desorption when a batch equilibrium method is applied (OECD, 2000).

$$\text{Kdes} = \frac{m_s^{ads}\,(eq) - m_{aq}^{des}\,(eq)}{m_{aq}^{des}\,(eq)} * \left(\frac{Vt}{msoil}\right) \quad \ldots\ldots\ldots (8.1)$$

where:

- K_{des} : apparent desorption coefficient (ml/g)
- $m_s^{ads}\ (eq)$: mass of the chemical substance adsorbed by the soil under adsorption equilibrium (mg).
- $m_{aq}^{des}\ (eq)$: total mass of chemical substance desorbed during desorption equilibrium (mg)
- V_t: total volume of aqueous phase in contact with the substrate during kinetic desorption tests by serial method (ml).
- M_{soil}: amount of substrate expressed as dry mass (g).

Normally, the sorption-desorption investigations in sediments (Wang and Li, 2010), soils (OECD, 2000) and activated sludge (EPA, 1998) are developed taking into consideration a sequential process of sampling and pretreatment of the sludge sample followed by the sorption and desorption process.

The experimental process in the present research considered an initial desorption phase where different eluents were used to simultaneously, recover the phosphorus previously sorbed and to release active sites on the surface of the floc. After the desorption process, each sludge sample was stabilized using a solution of $CaCl_2$ and $MgCl_2$.Determining the amount of phosphorus previously sorbed by the substrate was not possible since the initial experimental process corresponded to a desorption process. Therefore, the total phosphorus content in the settled sludge before the desorption–sorption process may represent the amount of phosphorus previously sorbed in the biomass. From this value, the amount of phosphorus released into supernatant during the desorption tests was calculated. The amount of phosphorus desorbed at the end of the reaction time (24 hours) was also used to calculate the Kdes coefficient, although the desorption equilibrium was not reached (Appendix 1).

The results of the S1 and S2 reactors are shown in Figure 8.10. In these sludge samples, the lowest Kdes coefficients were recorded in the S1-Cit and S2-Cit reactors at a pH of 3. These results confirm that the best desorption performance is achieved using as eluent citric acid at the desorption pH of 3.

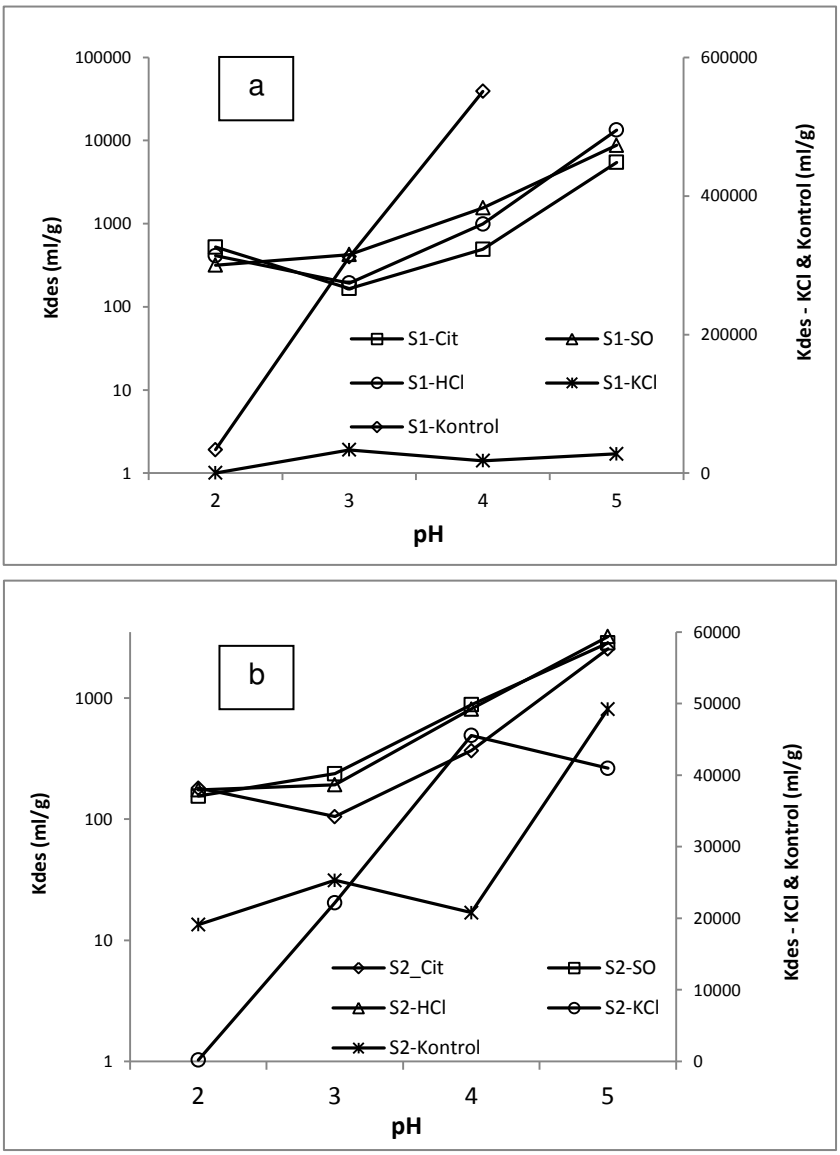

Figure 8.10: Apparent desorption coefficients (Kdes) vs the pH of desorption for the S1 (a) and S2 (b) sludge samples.

The highest Kdes coefficients, between reactors which underwent desorption processes, were recorded in the S1-KCl (33692.4 ml/g) and S2-KCl (45566.9 ml/g) reactors. This result indicates that potassium chloride is an inefficient eluent for phosphorus desorption and also suggests the high potential of the S1 and S2 sludge samples to adsorb phosphorus and to retain it.

The desorption processes for the B2, B4, Lüneburg and Steinhorst sludge samples were performed only at a pH of 3; therefore, the resulting Kdes coefficients can be compared.

As shown in Figure 8.11, the lowest Kdes values were obtained in the S1-Cit (165.9 ml/g), S2-Cit (104.9 ml/g), BS-Cit-Lun (110.5 ml/g), RS-Cit-Lun (146.8 ml/g) and RS-Cit-Stein (120.9 ml/g) reactors. It should be noted here that the Lüneburg sludge samples corresponded to an EBPR bacterial community; therefore, the amount of

phosphorus released by these samples during the desorption process may be partly due to the phosphorus commonly released in an EBPR process.

With respect to the low Kdes values obtained using acid eluents, a research by Hongthanat, (2010) on phosphorus sorption-desorption of soils and sediments reported that the lower the PEBC (phosphorus equilibration buffering concentration in l/kg), more susceptible the substrate sample to loss of phosphorus. At this point, it is possible to estimate the phosphorus sorption capacity of sludge samples which were shown to have low Kdes values during the previous desorption process.

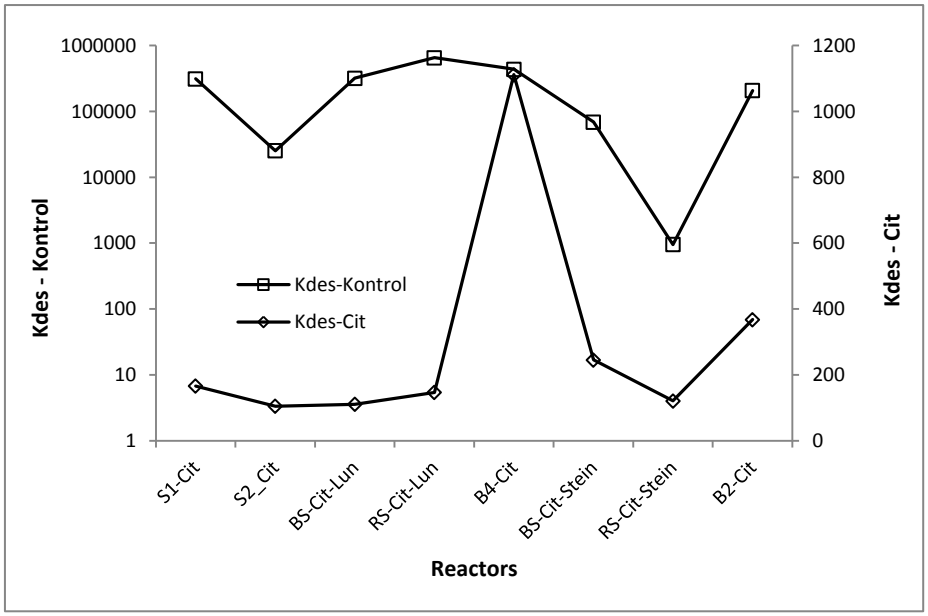

Figure 8.11: Kdes coefficients at the end of the reaction time. Citric acid was used as eluent during the desorption process.

The results of the desorption process using potassium chloride with a concentration of 30 mmol/l as eluents are shown in Figure 8.12. As can be noted, the Kdes coefficients were so high that in some cases (S2-KCl and B4-KCl), they were similar to the Kdes coefficients recorded for the control reactors. It is important to note here that the lowest Kdes coefficients recorded using potassium chloride as eluent were obtained in the RS-KCl-Lun and RS-KCl-Stein reactors. This result may be due to the effect of the application of the eluent to sludge samples that were already destabilized since these samples came from anaerobic and static environments (Return Activated Sludge line and sludge stabilization line).

The Kdes coefficients of the control reactors serve as a reference point allowing for a comparison of the efficiency of the desorption process. These Kdes coefficients are also indicators of phosphorus retention capacity: The higher the Kdes coefficient during the desorption process, the higher the phosphorus sorption potential during the sorption process.

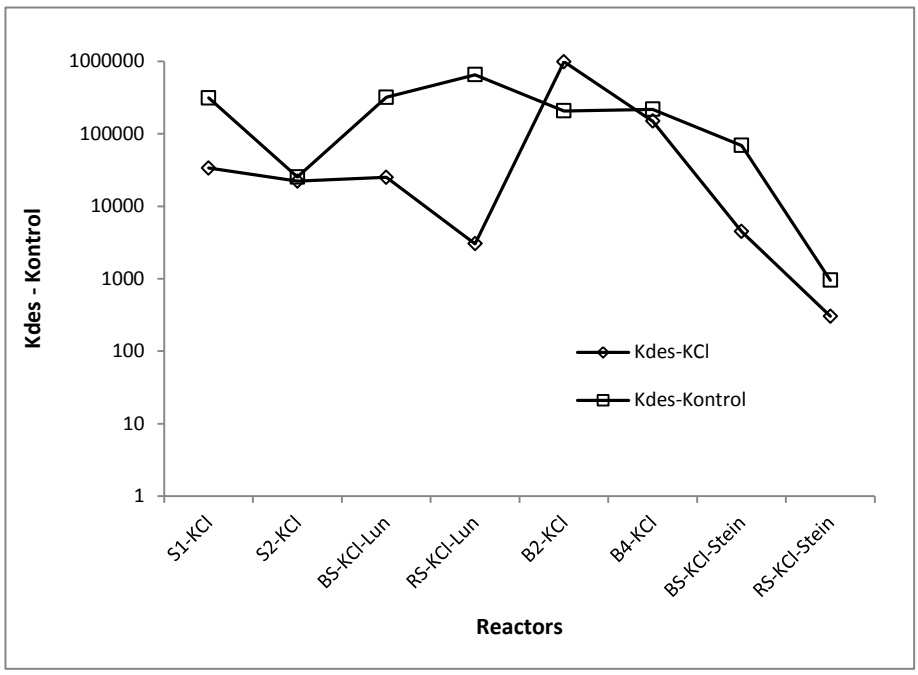

Figure 8.12: Kdes coefficients obtained at the end of the desorption process using potassium chloride as eluent.

8.3.3 Relationship between the Soil/Solution ratio and the apparent desorption coefficient (Kdes)

The soil/solution ratios were plotted against the apparent desorption coefficients (Kdes) to determine, using these graphical representations, the soil/solution ratios, that is needed if a particular desorption percentage were sought (OECD, 2000).

The relation between the Kdes coefficient and the soil/solution ratio may be deduced from the Equation 8.1, considering that this is a linear equation only in this case:

$$K_{des} = \frac{m_s^{ads}(eq) - m_{aq}^{des}(eq)}{m_{aq}^{des}(eq)} * (V_t/m_{soil}) \quad \text{..... (8.1)}$$

From Equation 8.1, the logarithmic equation (Appendix 2) that link the soil/solution ratio, the desorption percentage and the apparent desorption coefficient (Kdes) is deduced:

$$\frac{V_t}{m_{soil}} = K_{des}\left(\frac{m_{aq}^{des}(eq)}{m_s^{ads}\,(eq) - m_{aq}^{des}\,(eq)}\right) \text{..... (8.2)}$$

where:

$$R = \frac{m_{soil}}{V_{tot}} \text{..... (8.3)}$$

$$D_{eq} = \left(\frac{m_{aq}^{des}\,(eq)}{m_s^{ads}\,(eq)}\right) * 100 \text{..... (8.4)}$$

$$\frac{m_{soil}}{V_{tot}} = \left(\frac{1}{K_{des}}\right) * \left(\frac{m_s^{ads}\,(eq) - m_{aq}^{des}\,(eq)}{m_{aq}^{des}\,(eq)}\right)$$

$$R = \left(\frac{1}{K_{des}}\right) * \left(\frac{1 - D_{eq}\,\%100}{D_{eq}\,\%100}\right)$$

finally:

$$Log\ R = -Log\ K_{des} + Log\ \left[\frac{1 - D_{eq}\%100}{D_{eq}\%100}\right] \text{... (8.5)}$$

In the figure describing the relationship between the soil/solution vs Kdes coefficients (Figure 8.13), the diagonal lines correspond to specific desorption percentages. Thus, for a specific Kdes, it is possible to estimate the desorption percentage that corresponds to a specific soil/solution ratio. More specifically, it is possible to determine the highest desorption percentage considering a specific Kdes coefficient and specific soil/solution ratios.

The soil/solution vs Kdes graphic for the S1 sludge sample is shown in Figure 8.13. The highest desorption percentages (Deq) were recorded in the S1-pH3 reactors with a Kdes coefficient of 165.9 ml/g and soil/solution ratio of 0.00532 g/ml using citric acid as eluent. Although the best desorption performance was obtained using citric acid as eluent at a pH of 3, a higher soil/solution ratio compared to the soil/solution ratio required for the S1-pH2 reactors was required.

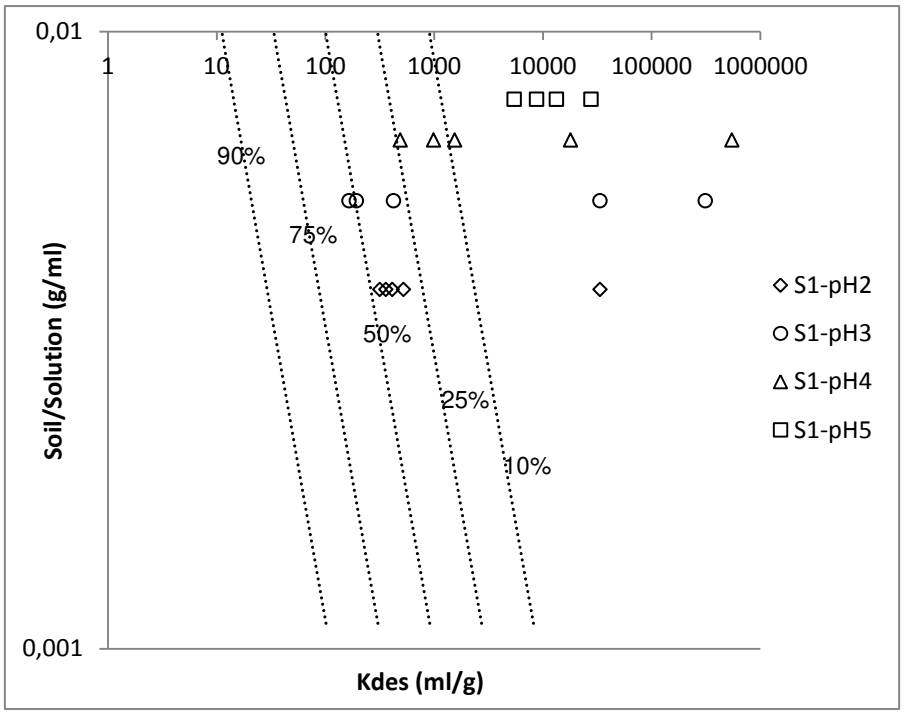

Figure 8.13: Soil/solution ratios vs Kdes coefficients for the S1 sludge sample.

For the S2 sludge sample (Figure 8.14), the best desorption percentage was obtained in the S2-Cit reactor (65%) at a pH of 3. It is noteworthy that the S1 sludge sample at a pH of 2 had similar soil/solution ratios but the desorption percentages reached were lower (50% S1-Cit).

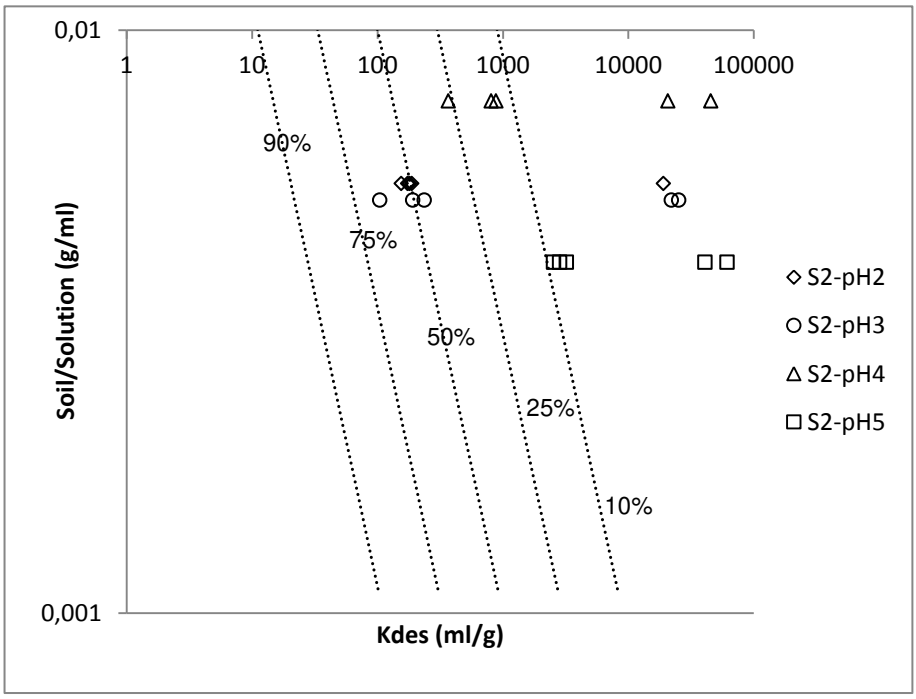

Figure 8.14: Soil/solution ratios vs Kdes coefficients for the S2 sludge sample.

The desorption percentages of the S1 and S2 sludge samples at a pH of 4 and 5 were very low even when higher soil/solution ratios were used; therefore, these results are not considered in this analysis.

So far, it can be concluded that the type of eluent used during desorption processes and the pH of desorption are factors determinig the phosphorus desorption performance. The soil/solution ratio does not exert the same influence on this performance, as even with very high ratios, high desorption percentages were not achieved if the pH and the eluent were not appropiate.

Other factor influencing the phosphorus desorption performance is the origin of the activated sludge sample. In this study, the origin of a sludge sample is determined by: the wastewater treatment, the regular composition of the influent wastewater, the use of trivalent metallic salts in phosphorus precipitation processes and the microbial composition of the activated sludge.

Considering these factors, it is possible to explain the similarity between the results obtained in the S1 and S2 sludge samples since both sludge samples underwent similar wastewater treatment processes resulting in similar bacterial communities (Chapter 6).

Additionally, the sludge samples of the B2 and B4 reactors and from Lüneburg and Steinhorst WWTPs underwent different wastewater treatments and received influent wastewater characterized by different compositions. In the present study, the possibility that the microbial composition affected the phosphorus desorption-sorption capacity of the activated sludge samples was considered. The results of the B2 and B4 reactors (Figure 8.15) show that the soil/solution ratios used in both reactors were similar (0.0032 g/ml for B2 and 0.00256 g/ml for B4); however, the Kdes coefficient for the B2-Cit reactor (367.9 ml/g) was lower than the coefficient for the B4-Cit reactor (1111.7 ml/g). With these Kdes values, the B2 and B4 reactors reached phosphorus desorption percentages of 46% and 26%, respectively.

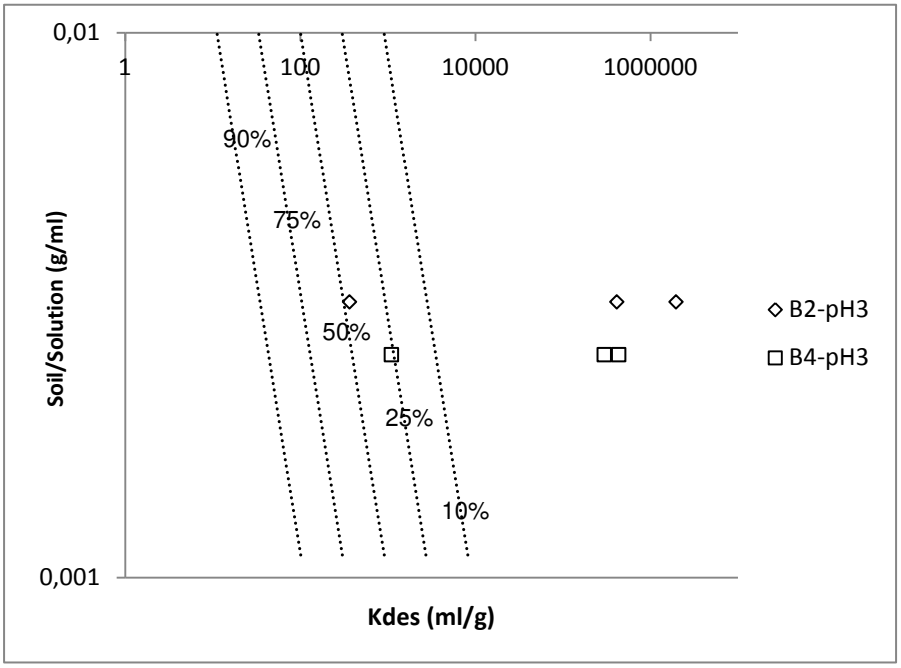

Figure 8.15: Soil/solution ratios vs Kdes coefficients for the B2 and B4 sludge samples.

From these groups of results, the B2 sludge sample was characterized by higher desorption percentages than the B4 sludge sample even though both samples were obtained with similar operational parameters and both bacterial communities were described as EBPR communities. The only difference between both reactors was the carbon source used in the influent: a VFA mixture for the B2 reactor and propionic acid for the B4 reactor. Seviour et al. (2003) suggested that if a mix of VFA is used as substrate, the B2 bacterial community might develop not only a normal EBPR bacterial community but it might also further the development of the GAO community.

In this study, the presence of the GAO community was not determined, but a TFO morphotype in high concentrations was identified in the S1, S2 and B2 reactors. Similar concentrations of the TFO morphotype were not observed in the B4 reactor, where this bacterial group was almost absent. The difference in the phosphorus release capacity between the B2 and B4 reactors might then be related to their different microbial composition.

As was mentioned above, the B2 and B4 sludge samples corresponded to EBPR bacterial communities; hence, the phosphorus release observed during the desorption process using citric acid as eluent might be due to the effect of the eluent and to the phosphorus release by the EBPR process under anaerobic conditions. Research by Lopez-Vasquez et al. (2009) showed that the increase of propionic acid in the influent contributes to the development of the PAO bacterial group. Similar growth of the PAO bacterial group was also expected in the B4 reactor and, thus, improved the EBPR

performance. However, in the present study, the results obtained in the bacterial survival test (Chapter 9) showed that the B4 bacterial community using citric acid as carbon source was more efficient than the B2 bacterial community. For this reason, it was expected that the B4 bacterial community might release more phosphorus than the B2 bacterial community through the EBPR process. However, the B2 bacterial community released more phosphorus during the desorption process than the B4 bacterial community. This difference might be due to the effect of the eluent on different bacterial communities.

The results of the Lüneburg sludge samples are shown in Figure 8.16. The BS-Lun and RS-Lun sludge samples used different soil/solution ratios of approximately 0.0038 g/ml and 0.0078 g/ml, respectively. Despite the higher mass concentration in the RS-Lun reactors, the best Kdes value was recorded in the BS-Lun-Cit reactor (110.5 ml/g), achieving also a desorption percentage of approximately 70%. Additionally, the RS-Lun-Cit reactor recorded a Kdes value of 146.8 ml/g with a desorption percentage of approximately 47%. Because they released a considerable amount of phosphorus during the desorption process, the BS-Lun and RS-Lun sludge samples can be considered potential sources of phosphorus recovery (63.7 mg/l for BS-Lun-Cit and 130.3 mg/l for RS-Lun-Cit reactors).

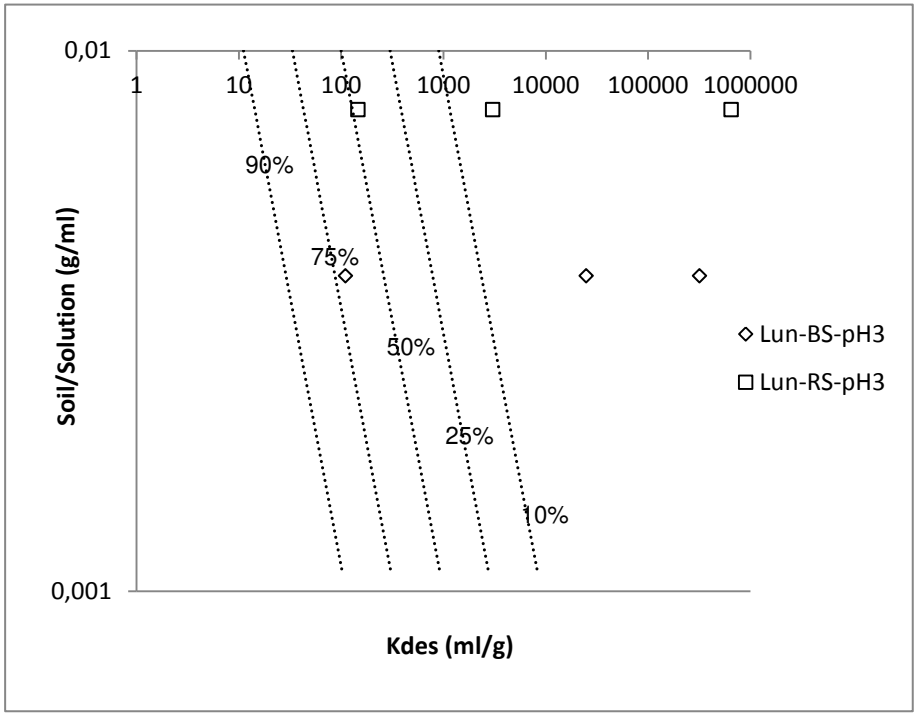

Figure 8.16: Soil/solution ratios vs Kdes coefficients for the Lüneburg sludge sample.

Finally, the results of the Steinhorst sludge sample are shown in Figure 8.17. Again, a considerable difference between the soil/solution ratio used in the BS-Stein ((0.0037

g/ml) and the RS-Stein (0.0089 g/ml) sludge samples was observed. Despite the higher sludge mass concentration recorded in the RS-Stein sludge sample, the phosphorus desorption percentages for the BS-Stein-Cit and RS-Stein-Cit reactors at the end of the desorption process were similar (approximately 40%).

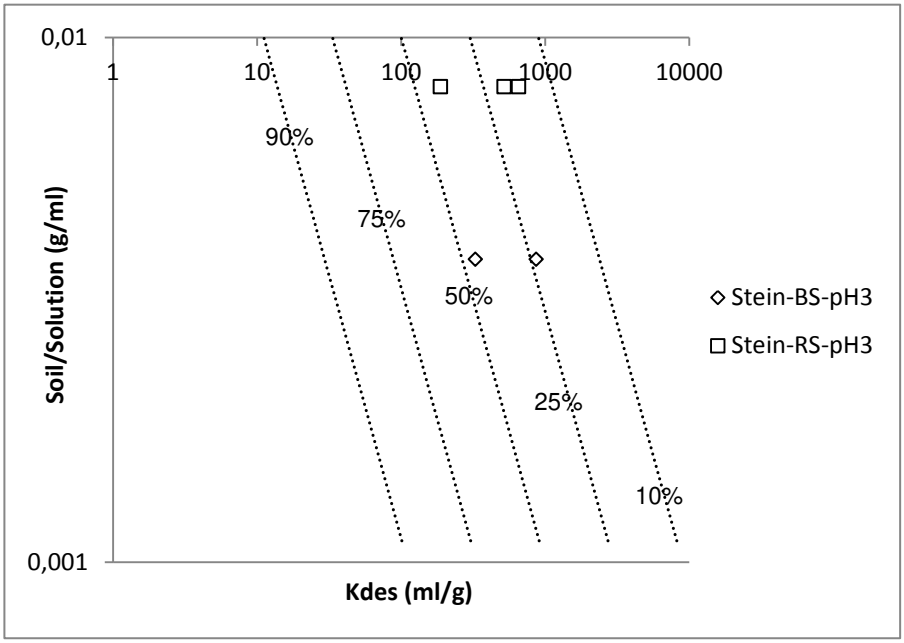

Figure 8.17: Soil/solution ratios vs Kdes coefficients for the Steinhorst sludge sample.

For the Steinhorst sludge sample, the phosphorus release may be due only to the phosphorus desorption process, as the Steinhorst WWTP was not designed to perform EBPR processes. This inability was reflected in the results: The comparison of the phosphorus content of the influent (13.5 mg/l) and of the effluent (14.3 mg/l) at the moment of the sludge sampling.

It is important to note here that the Steinhorst sludge samples may be considered a potential source of phosphorus recovery as suggested by the phosphorus desorption performance achieved in the RS-Stein-Cit reactor (72.7 mg/l).

8.4 Results and discussion – Sorption process

The sorption capacity of substrates, more specifically, of soils, sludge and sediments is closely related to the structure and composition of the mineral and organic compounds that they contain.

For the specific structure of activated sludge, which contains organic and mineral compounds, in made up of flocs, which, in optimal conditions, shows a rounded shape and an average diameter between 150 and 500 µm. (Kunst, *et al.*, 2000). The characteristics of the floc determine the ease with which the aqueous phase is separated from the solid phase during sedimentation. There are some physicochemical characteristics that define the floc structure and provide its cohesion ability and stability. One of the characteristics that influence the cohesion strength is the chemical composition of the extracellular polymeric substances (EPS). Thus, the floc's composition of carbohydrates, lipids, proteins and divalent calcium will provide the cohesion capacity between the individual molecules of the EPS and the cohesion between the EPS and the cell surface. The chemical or physical forces that hold together the floc components are the same forces that are involved in the sorption process of chemical substances to the floc (Muller, 2006). These binding forces are weak physicochemical interactions such as electrostatic interactions, and Van der Waals forces, as stipulated by the theory of Derjaugin, Landau, Verwey, and Overbeek (DLVO theory), and hydrogen bonds (Zita and Hermansson, 1994; Mayer et al., 1999; Muller, 2006).

Regarding the EPS composition, studies show that a high concentration of polysaccharides and hydrophobic substances (lipids and proteins) might be favorable for sludge flocculation and sedimentability (Goodwin and Forster, 1985; Higgins and Novak, 1997). Additionally, Wilen et al. (2003) mentioned that the protein content in the EPS is directly related to the amount of superficial negative charges in the floc and to the floc's ability to flocculate. Finally, other studies suggests that it is more likely that flocculation are related to the hydrophobicity, EPS composition and the superficial charges in the floc than to the amount of EPS in the floc (Keiding and Nielsen, 1997).

An additional factor that defines the floc structure is the content of calcium ions and its influence on the floc stability (cohesion). Previous research identified divalent calcium ion (Ca^{+2}) as an important bridging ion that links the negative charges in the cellular surface and the EPS polymers (Bruus et al., 1992; Keiding and Nielsen, 1997). Additionally, Keiding and Nielsen (1997) mentioned that even small changes in calcium

ion concentration might result in the desorption of organic molecules and a floc's disintegration.

As mentioned above, the constant ion exchange process in an estuarine ecosystem would be observed between the calcium ion on the substrate's surface and the sodium ion in the aqueous phase. Therefore, the estuarine sediment would be subject to constant processes of loss and recovery of calcium ion between tides (Grupo de Investigación de Recursos Hídricos - IUPA, 2009).

In this study, it is proposed that the loss of calcium by ion exchange may result in sediment destabilization and, as a consequence, the desorption of other molecular components this time not due to ion exchange but to the same sediment destabilization. Subsequently, this sediment destabilization might result in the release of active sites, previously saturated in the sediment. If the physicochemical processes described above occur in an estuarine ecosystem, it may be expected that the sorption capacity of estuarine sediments would be greater than the sorption capacity of other natural sediments. Research by Wang and Li (2010) on phosphorus desorption-sorption capacity of different sediments indicated that estuarine sediment showed the highest phosphorus sorption capacity. The possibility of phosphorus precipitation was also considered, but the calcium carbonate, iron and aluminium concentrations in the sediment were among the lowest of all sediments examined in this article.

In this study, each sludge sample went through a desorption-stabilization-sorption process. During the desorption phase, acids and an ion exchanger were used as eluents. The goal of the desorption process was not only to assess the phosphorus desorption capacity of each sludge sample but also to desorb calcium and, as a consequence, to expose active sites on the surface of the flocs. Potassium chloride was used as one of the eluents in the desorption process, to obtain the same effect exerted by sodium chloride on estuarine sediments. Thus, during the first reaction phase (desorption process), the activated sludge samples were diluted in different concentrations of potassium chloride (20, 30, 40 and 50 mmol/l), in which the potassium ion was expected to replace the available calcium ion on the surface of the floc (Wang and Li, 2010). The substitution of sodium ion with potassium ion was performed because of the high sorption affinity of calcium ion for the substrate, a quality that due to its position in the Hofmeister liotropic series represents an obstacle for its desorption. Therefore, the potassium ion was chosen since this ion has a higher sorption priority than sodium ion; to the extent this cation could be irreversibly fixed in clays due to the strong chemical interactions that it may build with the substrate (Grupo de Investigación de Recursos Hídricos - IUPA, 2009).

Upon completion of the desorption process, the floc structure in particular and the sludge sample in general were destabilized. This destabilization phase resulted in an increase of the desorption potential, the release of some colloidal material to the supernatant and a decrease of sludge settleability. To avoid the problems of a destabilized sludge mass, a solution of $CaCl_2$ (1M) and $MgCl_2$ (1M) in concentrations normally reported in domestic wastewater (Liu et al., 2006) was used to re-stabilize the sludge samples. At the end of this process, the sludge samples were considered ready for a sorption process. It was then tested whether its phosphorus sorption capacity had improved by comparing the total phosphorus content in the sludge samples before and after the desorption-stabilization-sorption process.

8.4.1 Phosphorus sorption percentage (A%) vs reaction time.

The sludge samples that previously underwent a desorption process using acid eluents did not show phosphorus sorption capacity. Furthermore, the sorption percentages in these sludge samples were negative, that is, the sludge samples released phosphorus into the aqueous phase even during sorption process.

The results concerning the phosphorus sorption capacity of the S1 sludge sample are shown in Figure 8.18. Negative sorption percentages were recorded in all reactors that used acid eluents regardless of the pH of desorption. Among the sludge samples that underwent desorption pH of 2 or KCl concentration of 20 mmol/l (Figure 8.18.a), the S1-KCl-20 mmol/l and S1-SO-pH2 reactors reported positive sorption percentages at the end of the reaction time of approximately 64.4% and 13.4%, respectively. The highest negative sorption percentage was observed in the S1-Cit reactor (-128%).

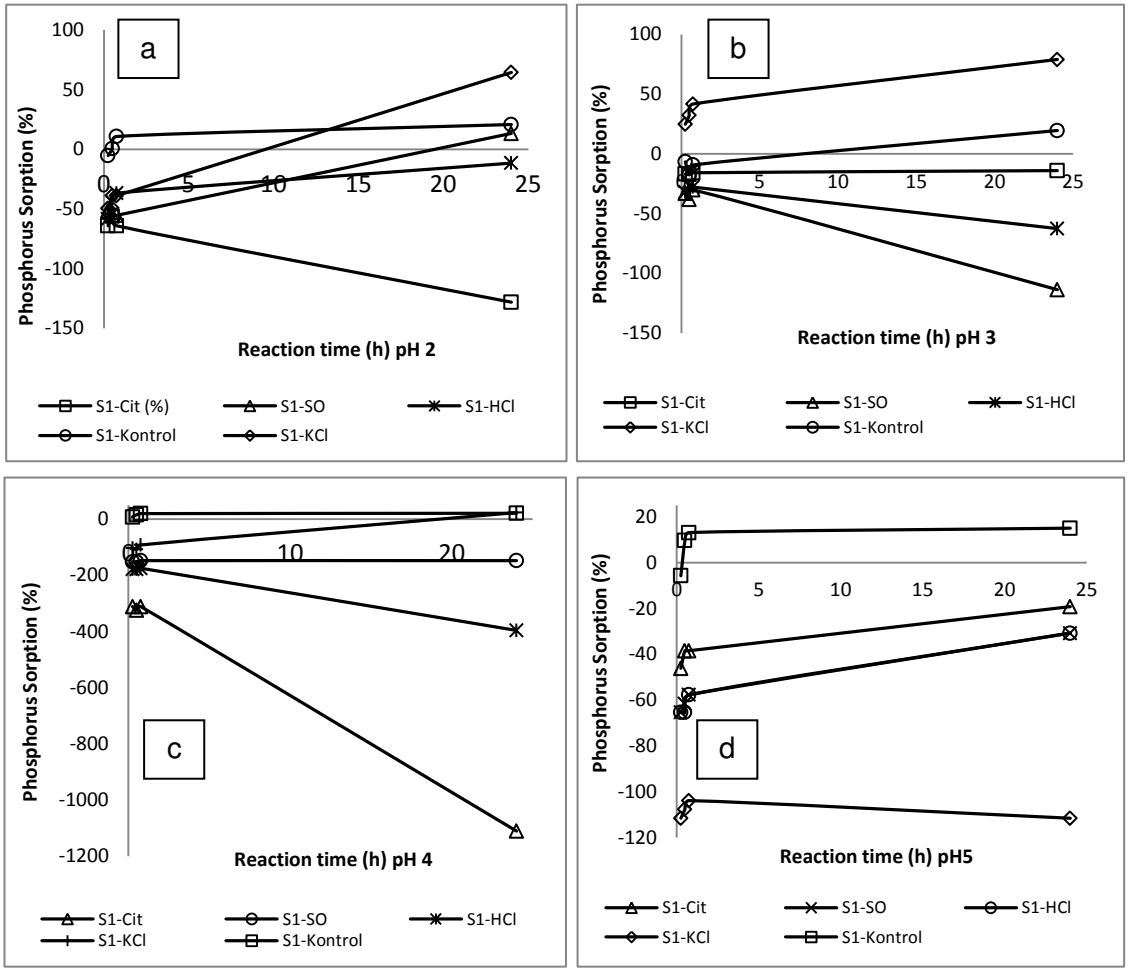

Figure 8.18: Phosphorus sorption percentages vs reaction time for the S1 sludge sample.

Of the results of the S1 sludge sample that underwent a desorption pH of 3 or KCl concentration of 30 mmol/l (Figure 18.b), the S1-KCl and S1-Control reactors recorded positive sorption percentages of approximately 78.9% and 19.5%, respectively, at the end of the reaction time. The S1-Cit, S1-SO and S1-HCl reactors recorded negative sorption percentages, with the lowest value observed in the S1-SO reactor (-113.8). For the sludge sample that underwent a desorption process at a pH of 4 or KCl concentration of 40 mmol/l (Figure 8.18.c), positive sorption percentages were reached in the S1-Control reactor, while for the S1-KCl reactor, the sorption percentage increased gradually until 20.4% at the end of the reaction time. The remaining reactors recorded negative sorption percentages, with the lowest value of -1110.7% for the S1-Cit reactor. From the results of the S1 sludge sample that underwent a desorption process at a pH of 5 or KCl concentration of 50 mmol/l (Figure 8.18.d), the only positive sorption percentage was obtained in the S1-Control reactor (15%), while the remaining reactors recorded negative sorption percentages, with the lowest value in the S1-KCl reactor (-111.5%).

The results of the S2 sludge sample are shown in Figure 8.19. In general, positive sorption percentages were recorded in the S2-KCl-pH3 and S2-KCl-pH4 reactors and in all the S2-Control reactors.

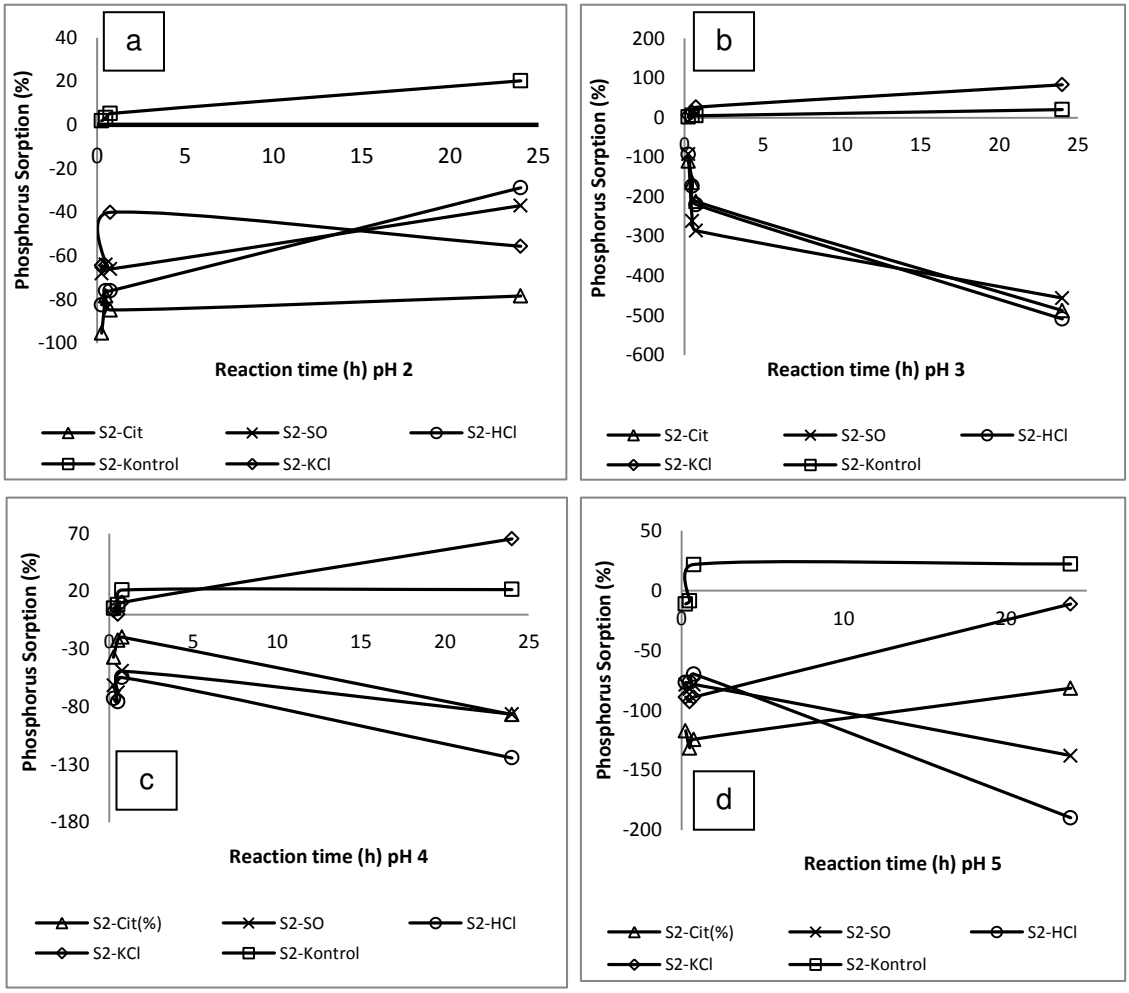

Figure 8.19: Phosphorus sorption percentages vs reaction time for the S2 sludge sample.

For the sludge samples that underwent a previous desorption process at a pH of 2 or KCl concentration of 20 mmol/l, the S2-KCl reactor was the only one that recorded a positive sorption percentage (20.2%). The remaining reactors recorded negative sorption percentages, with the lowest value achieved in the S2-Cit reactor during the first 15 minutes of the sorption process (-95.3%). Among the sludge samples that underwent a previous desorption process at a pH of 3 or KCl concentration of 30 mmol/l, the results concerning the phosphorus sorption percentage recorded positive values for the S2-KCl and S2-Control reactors with 83.2% and 20.5%, respectively. The remaining reactors recorded negative sorption percentages, with the lowest value of approximately -500%, which was recorded for the S2-HCl and S2-Cit reactors. The results corresponding to the sludge samples that underwent a previous desorption process at a pH of 4 or KCl concentration of 40 mmol/l reported positive sorption percentages in the S2-KCl (65.5%) and S2-Control (21.6%) reactors. The remaining

reactors recorded negative sorption percentages, with the lowest value obtained in the S2-HCl reactor (-124.2%). Among the sludge samples that underwent a previous desorption process at a pH of 5 or KCl concentration of 50 mmol/l, the only reactor that recorded positive sorption percentages was obtained in the S2-Control reactor. Among the remaining reactors, the lowest sorption percentage was recorded in the S2-HCl reactor (-189.8%). The results obtained in the S1-Control and S2-Control reactors were similar, with an average phosphorus sorption percentage of approximately 20%.

The negative sorption percentages may be due to an incomplete previous desorption process and/or to the phosphorus commonly released in EBPR processes during the anaerobic phase and in the presence of a carbon source. As mentioned above, the adsorbate (phosphorus) was diluted in a solution of synthetic wastewater that used glucose as the carbon source. Furthermore, the bacterial communities in the S1 and S2 reactors corresponded to a TFO bacterial community. The capacity of this community to release phosphorus during the anaerobic phase in comparison to a normal EBPR bacterial community is limited. According to Jeon and Park (2000), the phosphorus release in this case might involve two bacterial groups: The TFO bacterial community, that would be able to uptake the glucose during the anaerobic phase and to produce lactate from it, and the PAO bacterial community that would be able to uptake the lactate released by the TFO and subsequently release phosphorus to the aqueous phase. Additionally, the S1-Control and S2-Control reactors did not record a considerable phosphorus release but phosphorus removal during the sorption process. Considering the results obtained in the control reactors, the phosphorus release during the sorption process because of the EBPR process was discarded. One possible explanation for the phosphorus release during the sorption process in the S1 and S2 sludge samples could be the destabilizing effect caused by the use of eluents during the previous desorption process. An indication of sludge destabilization was the release of color into the supernatant, which was observed at the end of the desorption process. In some reactors, the release of color continued to the end of the stabilization and sorption processes. Another indication of sludge destabilisation was the constant release of phosphorus during the desorption, stabilisation, and sorption processes (Table 8.13).

Table 8.13: Phosphorus concentration (mg/l) in the aqueous phase at the end of the desorption, stabilisation and sorption processes.

			Reactors		
Desorption pH	Sludge sample	Process	Cit	SO	HCl
2	S1	Desorp	12.2	16.3	14.1
		Stabilizat	3.8	3.0	3.1
		Sorption	5.7	2.4	3.1
	S2	Desorp	45.6	48.9	46.3
		Stabilizat	16.6	17.4	16.2
		Sorption	8.4	6.6	6.0
3	S1	Desorp	33.1	19.5	30.8
		Stabilizat	15.2	10.2	14.5
		Sorption	43.8	22.7	24.4
	S2	Desorp	43.5	30.6	34.0
		Stabilizat	14.1	10.8	12.2
		Sorption	16.1	16.1	17.6
4	S1	Desorp	15.7	6.6	9.3
		Stabilizat	5.6	2.2	3.0
		Sorption	36.2	6.7	13.9
	S2	Desorp	25.4	13.0	13.9
		Stabilizat	7.0	5.4	5.8
		Sorption	6.4	6.0	7.4
5	S1	Desorp	2.7	2.0	1.6
		Stabilizat	1.0	0.8	0.6
		Sorption	3.1	3.4	3.4
	S2	Desorpt	6.9	6.3	5.7
		Stabilizat	2.3	2.7	2.5
		Sorption	5.1	6.8	8.2

In summary, despite the desorption pH and the type of eluent used during the desorption process, it were recorded negative sorption percentages in most of the S1 and S2 reactors. From these results it may be said that using acid eluents at a pH of 2 and 3 during the desorption process a constant phosphorus release will be observed throughout the experimental process. At the same time a great destabilization was observed in the sludge samples, in such a way that the re-stabilization of the floc using a solution of $CaCl_2$ and $MgCl_2$ was not possible. Because of this instability, the sludge samples that underwent a previous desorption process using acid eluents were discarded for the remaining sorption tests.

In contrast, positive sorption percentages were recorded for the sludge samples that underwent a previous desorption process using as eluent to potassium chloride with concentrations of 20, 30 and 40 mmol/l in mixed liquor (Figure 8.20).

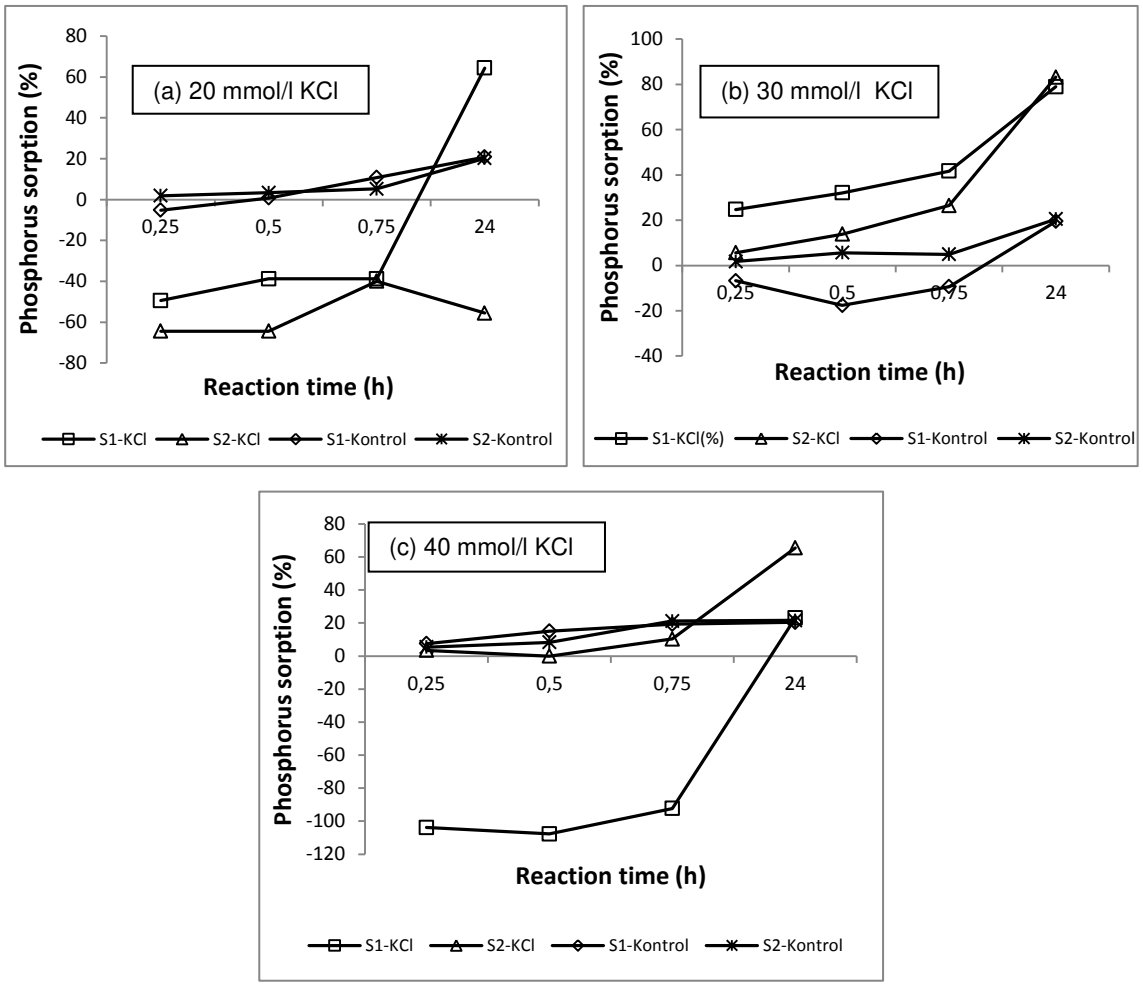

Figure 8.20: Phosphorus sorption percentages vs reaction time for the S1-KCl, S2-KCl and control reactors. The sludge samples underwent a previous desorption process using 20 mmol/l (a), 30 mmol/l (b), and 40 mmol/l (c) of KCl as eluent.

Figure 8.20 shows that good sorption percentages were recorded in the S1-KCl-20mmol/l, S1-KCl-30mmol/l, S2-KCl-30mmol/l, and S2-KCl-40mmol/l reactors. The best phosphorus sorption percentages were recorded in the S2-KCl-30 mmol/l (83.2%) and S1-KCl-30 mmol/l (78.9%) reactors.

Because of the good phosphorus sorption percentages obtained in the S1-KCl-30mmol/l and S2-KCl-30mmol/l reactors, the test of desorption-stabilization-sorption was repeated for the S1 and S2 sludge samples. In the repeated test during the sorption process a phosphorus concentration of 2.5 mg/l was used in the synthetic wastewater (Figure 8.21).

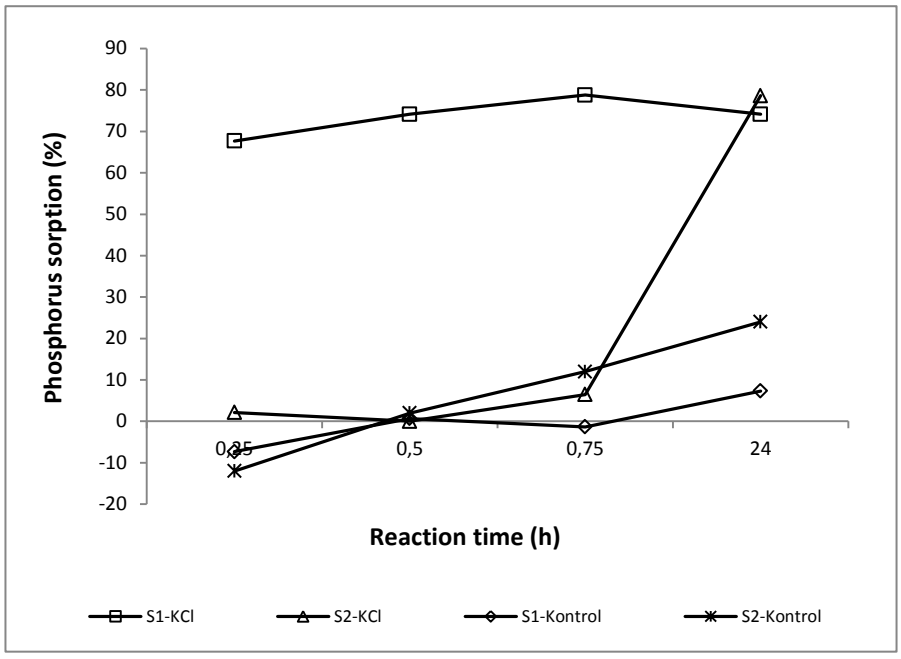

Figure 8.21: Phosphorus sorption percentages vs reaction time for the S1-KCl, S2-KCl and control reactors. The sludge samples underwent a previous desorption process using KCl (30mmol/l) as eluent. The total phosphorus concentration in the influent during sorption process was 2.5 mg/l.

Figure 8.21 shows that the S1-KCl and S1-Control reactors recorded positive sorption percentages of 74.2% and 7.3%, respectively. Meanwhile, the S2-KCl and S2-Control reactors achieved sorption percentages of 78.6% and 24%, respectively. The S1-KCl and S2-KCl reactors showed similar sorption percentages at the end of the reaction time, but for the S1-KCl reactor high sorption percentages were recorded from the first 15 minutes of the sorption process, while for the S2-KCl reactor the increase of the sorption percentage was gradual. In summary, the phosphorus sorption percentages of the S1-KCl-30mmol/l and S2-KCl-30mmol/l reactors were very similar no matter if the phosphorus concentration in the influent wastewater was 7.5 mg/l (Figure 8.20.b) or 2.5 mg/l (Figure 8.21).

The remaining sorption tests were developed with the sludge samples from the Lüneburg WWTP (Lun-BS and Lun-RS). These sludge samples underwent previous desorption processes using citric acid as eluents at a pH of 3, and potassium chloride with a concentration of 30 mmol/l. The results of the Lun-BS sludge sample are shown in Figure 8.22. As can be seen, negative sorption percentages were observed in all reactors during the first hours of reaction. The concentrations of phosphorus released were 12.2 mg/l for the BS-Kontrol, 103.8 mg/l for the BS-Cit and 30.4 mg/l for the BS-KCl reactors. The BS-Cit reactor showed a constant release of phosphorus during the sorption process, while the BS-KCl and BS-Control reactors also released phosphorus

but only during the first hour of reaction. At the end of the reaction time, the BS-KCl and BS-Kontrol reactors recorded positive phosphorus sorption percentages of 44.1% and 12.7%, respectively.

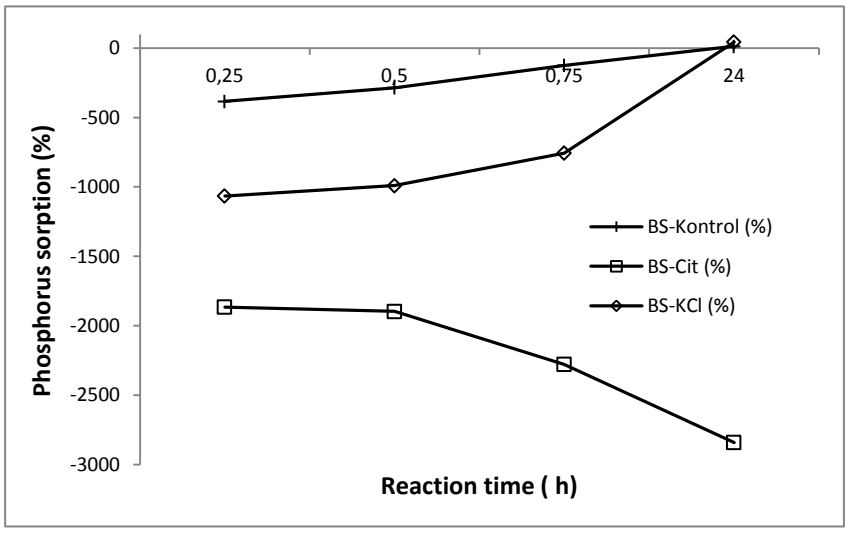

Figure 8.22: Phosphorus sorption percentages vs reaction time for the sludge sample from the aeration tank of the Lüneburg WWTP.

The results of the Lun-RS sludge sample are shown in Figure 8.23. As can be seen, all sludge samples released phosphorus from the first hour of reaction. Negative sorption percentages were recorded in reactors RS-Control with -443.3% (14.7 mg/l), RS-Cit with -806.1% (63.4 mg/l), and RS-KCl with 864.1% (35.9 mg/l). Finally, at the end of the reaction time, the RS-Cit reactor achieved the highest percentage of phosphorus released of -1160.9% (83.3 mg/l), while the RS-KCl and RS-Control reactors recorded positive sorption percentages of 7.2% and 76.7%, respectively.

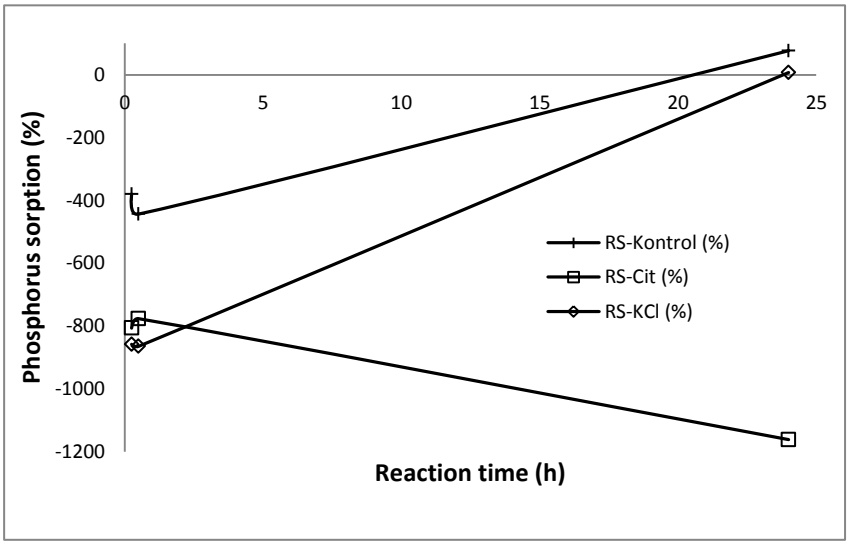

Figure 8.23: Phosphorus sorption percentages vs reaction time for the sludge sample from the return sludge line (RS) of the Luneburg WWTP.

As already observed, the phosphorus release was recorded from the first hour of sorption reaction in all reactors of the BS-Lun and RS-Lun sludge samples. This release of phosphorus may be partly due to the phosphorus release process observed in EBPR systems during anaerobic phase and in presence of a carbon source. As was already mentioned, the Luneburg WWTP recorded a functional EBPR process during the sludge sampling. Another reason that might explain the phosphorus release is the destabilization of the sludge samples during the previous desorption process using as eluents to citric acid and potassium chloride. The phosphorus release due to the EBPR process, should be discussed, since the bacterial community in the Lüneburg sludge sample is a typical EBPR community (PAO bacterial community); that means, that this bacterial community is able to uptake VFA as carbon source during the anaerobic phase but not to glucose directly (Kong et al ., 2004). Additionally, research by Wentzel et al. (1991) and Oehmen et al. (2007) mentioned that sugars among other carbon sources should be previously fermented before being assimilated by PAO communities. For the Lüneburg sludge samples, the phosphorus release was recorded from the first hour of the sorption process. Therefore, if previous glucose fermentation has been considered, this fermentation process should happen during this first hour of the sorption process. Additionally, the highest concentrations of phosphorus released for control reactors were recorded during the first hour of the sorption process with 12.2 mg/l of phosphorus released for the BS-Control and 14.7 mg/l for the RS-Control reactors. For the BS-Lun sludge sample, if the phosphorus release due to EBPR processes is considered, this phosphorus release may correspond to the amount of phosphorus released in control reactors, since this sludge sample did not underwent a previous desorption process. Therefore, the amount of phosphorus released in control reactors may be considered as result of the usual phosphorus release observed during the anaerobic phase in EBPR processes. In contrast, the high concentrations of phosphorus released in the BS-Cit, BS-KCl, RS-Cit and RS-KCl reactors may be due to the eluent effect during the previous desorption process and also to the usual phosphorus release observed in EBPR processes.

The differences of the phosphorus sorption percentages between the BS-KCl and RS-KCl reactors may be explained when considering the origin of these sludge samples. As was aforementioned, the sludge floc stability is influenced for several factors. Among these factors, the pH and the ionic strength in mixed liquor may be influenced by operational conditions as the TSS and DO concentrations. The BS-Lun and RS-Lun sludge samples have undergone different operational conditions in their reactors of origin. The origin of the BS-Lun sludge sample was the aeration tank of a WWTP with an almost neutral pH and a TSS concentration up to 4 g/l. Meanwhile, the RS-Lun

sludge sample has its origin in the return sludge line of a WWTP where the sample was exposed to anaerobic conditions in the secondary clarifier and the TSS concentration in mixed liquor was up to 8 g/l. The anaerobic environment and the high TSS concentration in mixed liquor may be appropriate conditions to promote a hydrolysis process which may result in the decrease of the pH and hence destabilization of the floc. In this study, the destabilization of the sludge samples from anaerobic environments (return activated sludge and sludge disposal line) was observed previous to start the experimental process. These sludge samples during the desorption process released high amounts of phosphorus, but subsequently to re-stabilize the sludge samples was not possible since the phosphorus release was recorded throughout the experimental process (Table 8.14).

Table 8.14: Phosphorus concentration in the aqueous phase for the sludge samples from the disposal sludge line of the Steinhorst WWTP (RS-Steinhorst) and from the return sludge line of the Lüneburg WWTP (RS-Lüneburg).

		Phosphorus release (mg/l)			
WWTP	Reactor	Desorption	Stabilizat.	Sorption	EBPR
Steinhorst	RS-Cit	89.3	25.4	63.1	No
	RS-KCl	58.7	10.2	24.7	
Lüneburg	RS-Cit	103.3	33.7	88.3	Yes
	RS-KCl	9.6	6.9	3.5	

In contrast, the DO and TSS concentrations in aeration reactors are appropriated to maintain a stable floc structure. In consequence, the Lun-BS sludge sample was composed for stable flocs, able to support a previous desorption process using as eluent to potassium chloride and subsequently it was possible to re-stabilize this sludge sample using a solution of $CaCl_2$ and $MgCl_2$. Finally, during the sorption process the Lun-BS-KCl sludge sample recorded an increase of its phosphorus sorption capacity and also an increase on the amount of phosphorus sequestered in the sludge biomass in comparison to the initial phosphorus amount per sludge mass unit recorded (Table 8.15). The results presented below (Table 8.15) consider only the reactors which recorded positive sorption percentages after to undergo a previous desorption process using potassium chloride as eluents with a concentration of 30 mmol/l.

Table 8.15: Phosphorus sequestration capacity in mg of total phosphorus per gram of TSS (mg/g) for the sludge samples before the desorption process (Initial P content) and at the end of the sorption process (Final P content).

Reactor	Initial P content	Phosphorus release	Final P content
	(mg/g)	(mg/l)	(mg/g)
Lun-BS-Control	23.6	0.3	24.5
Lun-BS-KCl		1.7	28.2
S1-Control	11.4	0.7	11.8
S1-KCl		0.7	15.2
S2-Control	12.8	0.4	11.9
S2-KCl		0.3	18.7

As can be seen in Table 8.15, the sludge samples in control reactors did not increase significantly their phosphorus sequestration capacity. In contrast, the reactors which underwent a previous desorption process using potassium chloride as eluent with concentration of 30 mmol/l, increased their phosphorus sequestration capacity in all cases. The S2-KCl reactor increased its phosphorus sequestration capacity in 46.1% while an increase of about 33.3% was recorded for the S1-KCl reactor and of 19.5% for the Lun-BS-KCl reactor.

The special case of the Lun-RS-Control reactor should be mentioned as this reactor recorded an increase of 28.6% of its phosphorus sequestration capacity, and this increase was not observed for the Lun-RS-KCl reactor. To explain these results it is necessary to mention the purpose of using potassium chloride as eluent. Potassium chloride was not used only as an eluent but also as a light destabilizer of the floc. As is proposed in this study, this destabilization process might release, through desorption process, some active sites on the floc surface that were previously saturated. The increase of the free active sites on the sludge samples might increase its sorption capacity. Another process that might destabilize the floc structure is the light hydrolysis process observed in an anaerobic environment as for example the environment of the RAS line (Lun-RS). In summary, the increase of the phosphorus sequestration capacity recorded in the Lun-RS-Control reactor might be due to the fact that this sludge sample was already slightly destabilized.

As mentioned above, the high sorption capacity of phosphorus observed in estuarine sediments in comparison to other sediments from rivers, lakes, sea or channels (Wang

and Li, 2010), might be explained considering the constant and permanent ion exchange process observed between the Ca^{+2} ion present in the sediment and the Na^{+} ion present in the aqueous phase during the tidal change. This foundament was applied in the present study using instead of sodium chloride to potassium chloride. Thus, using a potassium chloride concentration in mixed liquor of 30 mmol/l during the desorption process it was possible to observe during the sorption process, positive sorption percentages and an increase of the phosphorus sequestration capacity of the sludge samples. It is also important to highlight that the concentration of potassium chloride used in the desorption process was within the range of the sodium chloride concentrations recorded in estuarine ecosystems. In contrast, the sludge samples which underwent a desorption process using potassium chloride concentrations of 40 and 50 mmol/l in mixed liquor recorded a slight higher phosphorus desorption capacity than using other KCl concentrations. However, these sludge samples recorded negative sorption percentages due to the constant release of phosphorus throughout the experimental process.

8.4.2 Relationship between the distribution coefficient (Kd) and the soil to solution ratio

The Kd coefficient relates the distribution of a chemical compound between a solid and an aqueous phase (EPA, 1999). This coefficient may be obtained through a study of adsorption kinetic, and in this case the coefficient is known as distribution coefficient, or through a study of adsorption isotherms in which case the coefficient may take different names according to the best mathematical model that explain the distribution of the data. The aim of a study of adsorption kinetic or adsorption isotherms is to determine the sorption potential of a substrate to remove specific chemical compounds (EPA, 1998). In the present study, the activated sludge biomass was considered as the substrate and the total phosphorus content in a sample of synthetic wastewater was considered the adsorbate. In this study, a linear distribution coefficient (Kd) was calculated with the aim to perform an adsorption kinetic investigation (OECD, 2000). The linear Kd coefficient used in this investigation, accurately describe the adsorption behavior in soils and represents an expression of the inherent mobility of the chemical compound in the soil. The basic equation for the linear Kd coefficient is shown below (Equation 8.6) as the result of the ratio between the content of the chemical substance in the solid phase and the concentration in mass units of the chemical substance in the aqueous phase when the adsorption equilibrium was reached (Appendix 3).

$$Kd = \frac{C_s^{ads}(eq)}{C_{aq}^{ads}(eq)} = (m_s^{ads}(eq))/m_{aq}^{ads}(eq)) * \left(\frac{Vo}{m_{soil}}\right) \quad \ldots\ldots\ldots\ldots (8.6)$$

where:

- $C_s^{ads}(eq)$: Content of the chemical substance adsorbed on substrate at adsorption equilibrium (mg/g).
- $C_{aq}^{ads}(eq)$: Mass concentration of the chemical substance in the aqueous phase at adsorption equilibrium (mg/ml).
- $m_s^{ads}(eq)$: Mass of the chemical substance adsorbed on substrate at adsorption equilibrium (mg).
- $m_{aq}^{ads}(eq)$: Mass of the chemical substance in the aqueous phase at adsorption equilibrium (mg).
- m_{soil} : Amount of solid phase expressed in dry mass of substrate (g).
- V_o: Initial volume of the aqueous phase in contact with the substrate during the adsorption test (ml).

To develop an adsorption isotherm, it is required an experimental design to evaluate the adsorption capacity of a substrate respect to a chemical compound. This experimental design may use various substrate concentrations with a stable concentration of the chemical compound in the aqueous phase, or may use various concentrations of the chemical substance in the aqueous phase with a stable concentration of substrate. Among the mathematical models that describe the adsorption isotherms, the Freundlich model may be considered as the most widely used to describe the adsorption processes in diluted wastewater systems (EPA, 1998). The Kd coefficients obtained for each sludge sample and their adsorption potential may be compared.

The Kd coefficients obtained in the adsorption kinetic study are shown in Figure 8.24, where is possible to observe that as the reaction time passed the Kd values also increased. As was aforementioned, upon completion the reaction time the sorption equilibrium was not achieved since the sorption percentages continued to increase.

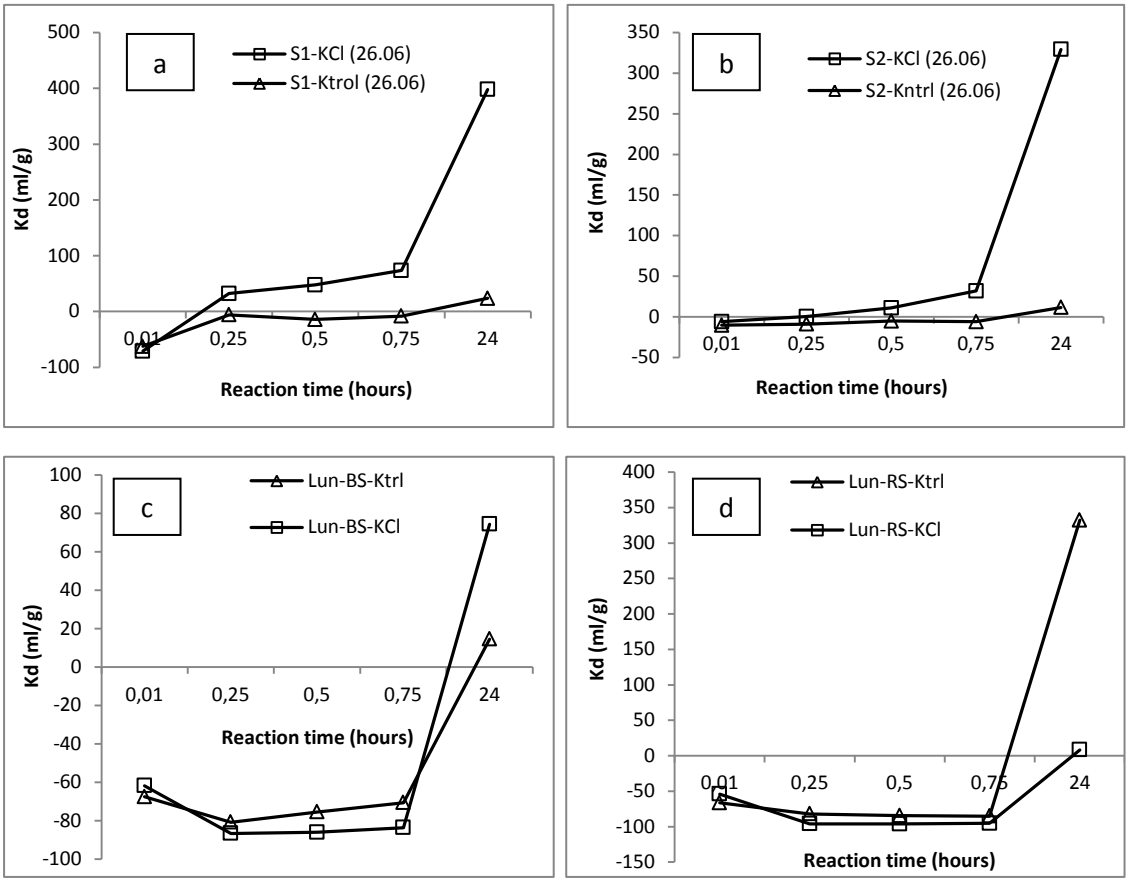

Figure 8.24: Kd coefficients vs reaction time for the S1 (a), S2 (b), Lun-BS (c), and Lun-RS (d) sludge samples.

The sludge samples which underwent a previous desorption process using KCl as eluent with a concentration in mixed liquor of 30 mmol/l recorded generally an increase in their phosphorus sequestration capacity during the adsorption process in comparison to the control reactors. An exception to these results was observed in the Lun-RS-KCl reactor which recorded a very low Kd coefficient of 8.3 ml/g in comparison to the Lun-RS-Control reactor which achieved a Kd coefficient of 332.1 ml/g.

According to the Kd coefficients obtained, the sludge samples can be arranged as follows:

S2-KCl	>	Lun-RS-Control	>	S1-KCl	>	Lun-BS-KCl
471.5 ml/g		332.1 ml/g		254.8 ml/g		74.4 ml/g

In a research by Wang and Li (2010) on the phosphorus sorption capacity of different sediments, the estuarine sediment recorded the highest phosphorus sorption capacity; however, the Kd value achieved with this sediment was not the highest among the substrate samples.

8.4.3 Determination of the optimum soil to solution ratio:

The possibility to use the activated sludge biomasses as potential phosphorus adsorbents and simultaneously to increase its phosphorus sequestration capacity was considered. Once determined the sorption potential capacity of a sludge sample, it might be possible to determine also the sorption percentage that may be reached if a specific soil to solution ratio is applied. In this case, the graphics that show the relation between the soil to solution ratios and the Kd coefficients can be used to predict the phosphorus sorption percentages.

In a WWTP, specific soil to solution ratios should be considered since these ratios correspond to specific TSS concentrations in each reactor. Thus, the TSS concentration varies between 1.5 and 5 g/l during the aeration phase if the sludge sample of a Sequencing Batch Reactor (SBR) is used and between 8 and 13 g/l for the settled sludge of a secondary clarifier that is purged as excess sludge (Metcalf and Eddy, 1985).

In the present study, according to the TSS concentrations it was possible to calculate graphically what might be the maximal sorption percentages for each sludge sample. Figure 8.25 shows the relation between the soil to solution ratios and the Kd coefficients for the S1, S2, Lun-BS and Lun-RS sludge samples as well as their respective control reactors.

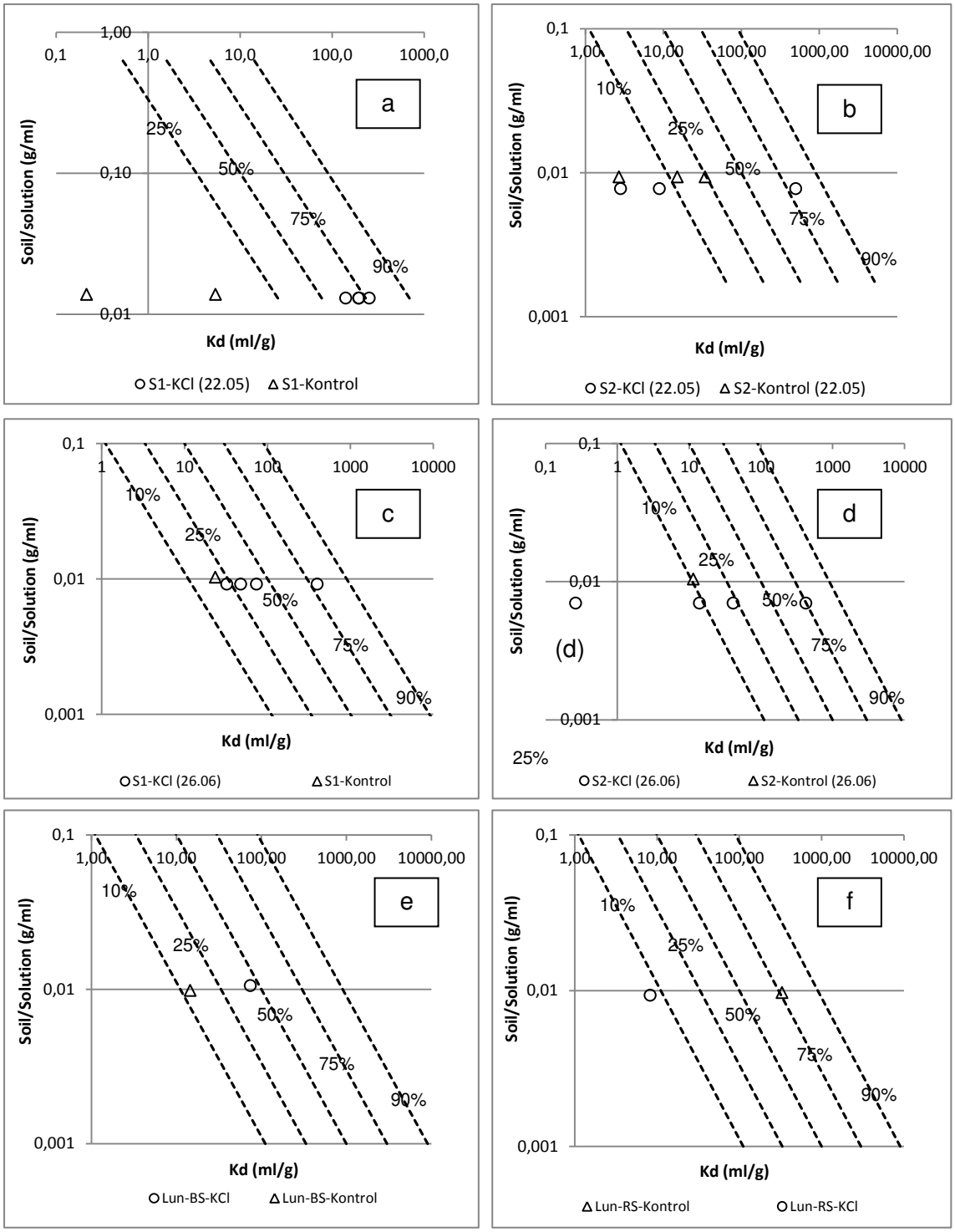

Figure 8.25: Soil/solution ratios vs Kd coefficients considering the potential phosphorus sorption percentages for the S1-KCl-2.5 (a), S2-KCl-2.5 (b), S1-KCl-7.5 (c), S2-KCl-7.5 (d), Lun-BS-KCl (e) and Lun-RS-KCl (f) reactors.

For the S1-KCl-2.5 and S1-Control-2.5 reactors the TSS concentration in the origin SBR reactor was up to 5 g/l and before to undergo the desorption-stabilization-sorption process the sludge sample was settled and decanted achieving a TSS concentration of

13 g/l or 0.013g/ml (Figure 8.25.a). Whit this amount of substrate a Kd coefficient of 254.8 ml/g and a sorption percentage of 77% was achieved for the S1-KCl reactor. Additionally, a Kd coefficient of 5.4 ml/g and a sorption percentage of 7% was achieved for the S1-Control reactor. These sorption percentages were the highest that may be achieved with this sludge sample since the substrate concentration in the origin reactor (SBR) was the highest according to Metcalf and Eddy (1995).

The difference between the Kd coefficients obtained for the S1-KCl and S2-KCl reactors may be due to the higher phosphorus adsorption capacity of the S2-KCl sludge sample. This higher phosphorus sorption capacity was observed when the S2-KCl sludge sample with a TSS concentration in mixed liquor of 7.8 mg/l achieved a phosphorus removal percentage of 78%. Similar phosphorus removal percentage was recorded in the S1-KCl reactor but with a TSS concentration of 13 g/l. The reason why the S1-KCl and S2-KCl sludge samples recorded different Kd coefficients may be based in the differences observed between both bacterial communities. In both bacterial communities the dominance of the TFO morphotype (Glucose community) was observed. However, the S1 bacterial community showed always a considerable amount of filamentous bacteria, generally of the gender Nostocoida (Chapter 6). The results of the amount of phosphorus accumulated in filamentous bacteria obtained by the SEM-EDX technology (Chapter 6.3.2) showed that this morphotype recorded the lowest amount of phosphorus accumulated in comparison to the other morphotypes identified i.e. TFO and cocobacillus. Therefore, if the biomass of the S1-KCl reactor showed an important amount of filamentous bacteria then the phosphorus sequestration capacity in this reactor might decrease in comparison to the S2-KCl reactor.

Additionally, to increase the phosphorus sorption percentage of the control reactors until reach the same percentage (approximately 78%) achieved in the S1-KCl or S2-KCl reactors, it would be necessary to increase the soil to solution ratios until 0.6 g/ml (600 g/l of TSS) for the S1-Control reactor and until 0.1 g/ml (102.9 g/l of TSS) for the S2-Control reactor.

Figures 8.25.e and 8.25.f show the results of the sludge samples from the aeration tank (Lun-BS) and the return sludge line (Lun-RS) of the Luneburg WWTP, respectively. The TSS concentration in the Lun-BS sludge sample was up to 3.8 g/l and for the Lun-RS sludge sample up to 7.8 g/l. These sludge samples were allowed to settle to concentrate the sludge biomasses to finally obtain TSS concentrations of 9.8 g/l for the BS-Control, 10.6 g/l for the BS-KCl, 9.7 g/l for the RS-Control, and 9.3 g/l for the RS-KCl reactors.

With these TSS concentrations, Kd coefficients of 14.6 ml/g for the BS-Control and 74.4 ml/g for the BS-KCl reactors were obtained. The phosphorus sorption percentages recorded for both reactors may be considered as the maximal reached percentages since the TSS concentration in the origin reactor was 3.8 g/l that is close to the maximal value of TSS described for aeration tanks in complete mixing activated sludge systems (Metclaf and Eddy, 1995). The BS-Control reactor might reach a similar phosphorus sorption percentage than the BS-KCl reactor when increase its soil to solution ratio to more than 0.08 g/ml or 80 g/l.

Figure 8.25.f shows the results for the sludge sample from the return sludge line of the Luneburg WWTP. With a Kd coefficient of 332.1 ml/g and a soil to solution ratio of 0.0097 g/ml (9.7 g/l) the sorption percentage achieved for the RS-Control reactor was of 76.7% at the end of the reaction time. The maximal TSS concentration for the return sludge line is 13 g/l according to Metcalf and Eddy (1995) therefore, it is possible to determine graphically that when increasing the soil to solution ratio to 0.0013 g/ml, the new phosphorus sorption percentage may be greater than 80% considering that the Kd is the same.

8.5 Conclusions:

1. In desorption processes of phosphorus in activated sludge samples, good desorption results may be obtained when citric acid is used as the eluent at a desorption pH of 3.

2. In desorption processes of phosphorus in activated sludge samples able to perform EBPR processes, the phosphorus release to supernatant may be due to the effect of the eluent during the desorption process and/or due to the phosphorus release observed in EBPR bacterial communities under anaerobic conditions and when a carbon source is present.

3. The most influential factors on the phosphorus release during the desorption processes are the type of eluent applied, the pH of desorption, and the type of microbial community in the sludge samples.

4. Desorption processes that use potassium chloride as eluent may increase the desorption performance when the sludge samples are previously destabilized, for example, samples from static, anaerobic and slightly acid environments (e.g. sludge samples from the RAS line).

5. The most influential factors on the phosphorus sorption capacity of activated sludge samples are related to the characteristics of the source of the sludge biomass. In this research, the following characteristics of the source were taken into account: the type of wastewater treatment, the origin reactor and the general characteristics of the influent wastewater.

6. The phosphorus sorption percentages and the Kd coefficients achieved were higher in the S2 and S1 bacterial communities which were dominated by the TFO morphotype.

   The phosphorus sorption capacity of a sludge sample may be related also to the abundance of the TFO morphotype in the bacterial community.

7. Other factor influencing the phosphorus sorption capacity of activated sludge samples is related to the structural stability of the floc. In this research, the capacity of a sludge sample to bear a desorption process and subsequently be

used successfully as sorbent was related with the presence of stable flocs in the sludge sample.

8. The phosphorus sorption capacity of activated sludge samples able to perform EBPR processes, may be improved when previously the sludge sample is subjected to desorption processes using potassium chloride as eluent with a concentration of 30 mmol/l in mixed liquor and subsequently being subjected to stabilization processes using a solution of $CaCl_2$ and $MgCl_2$ with concentrations of 5 mg/l and 10 mg/l, respectively.

8.6 References

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CHAPTER 9: Bacterial survival assessment in activated sludge samples subjected to desorption – sorption processes

9.1 Introduction

The desorption-stabilization-sorption tests (Chapter 8) were performed with the aim of determining the desorption and sorption capacity of phosphorus of different sludge samples.

It was considered the possibility to reuse sludge samples with high phosphorus sorption capacity. Therefore, the assessment of the bacterial survival rates in the activated sludge samples was also of interest.

With the aim of evaluating the survival capacity of the bacterial communities in the activated sludge samples, the count of viable microorganisms by the Plate Count - Agar Pour Plate technique was used. This technique was recommended for water samples and wastewater analysis (US FDA, 2014). Using this method, the number of colonies is counted as bacterial colony forming units (CFU/ml) after to inoculate a given volume of diluted or undiluted sample in a determined nutrient medium. Then, this bacterial culture will be incubated at a given temperature during a specific time period. The results are presented as colony forming units per milliliter of initial sample (CFU / ml).

The current methodology for estimating viable biomass parameters, including the concentration of viable microorganisms, comprise microbiological techniques such as culture media (Plate Count, NMP-Most Probable Number), staining techniques - counting of viable microorganism (Trypan Blue), biomolecular tests as DNA analysis, Fluorescence in Situ Hybridization (FISH), probes of fluorescents sequences for ribosomal RNA and ribosomal DNA (Oosthuizen, 2001).

The results of these techniques are not conclusive. For example, for a sample of activated sludge, high percentages (> 90%) of *Acinetobacter*, using the technique of Plate Count, were reported, while for the same type of sample, FISH technique showed only small amounts of this gender (3 - 6%) (Atkinson, 1999).

The Plate Count Technique may underestimate the concentration of viable microoganisms, because several factors such as the selectivity of the culture medium, the temperature of application of the medium (45°C) and because the difficulties to homogenize the sludge sample.

Despite these constraints, the Plate Count – Agar Pour Plate technique may be used to count viable microorganisms in activated sludge samples as the results will be comparable.

To assess the bacterial survival rates of the sludge samples, the concentration of microorganisms (CFU/ml) before and after the desorption-stabilization-sorption process are compared.

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9.2 Materials and methods

9.2.1 Sampling

The sludge samples were taken at the end of the desorption-stabilization-sorption process (Chapter 8) and immediately transported to the microbiology laboratory to be evaluated using the Plate Count – Agar Pour Plate technique.

Each survival test was labeled with the initials of the eluents used in the previous desorption process, and the name of the source reactor. Table 9.1 shows the sludge samples or reactors and the eluents used for each sample.

During the desorption process, the following eluents were used: citric acid (Cit), sulphuric acid (SO), hydrochloric acid (HCl), and potassium chloride (KCl).

Table 9.1: Number of repetitions in the Plate Count test according to the eluents and reactors used during the desorption process.

Desorption pH		pH = 2,3,4	pH = 2,3,4	pH = 2,3,4	20,30,40 mmol/l of KCl
Eluent / Sludge sample	Control	Acetic acid	Sulphuric acid	Hydrochloric acid	Potassium chloride
Reactors					
S1	2	2	2	2	2
S2	2	2	2	2	2
B2	2	2	-	-	2
B4	2	2	-	-	2
Steinhorst WWTP	2	2	-	-	2
BS	2	2	-	-	2
BR	2	2	-	-	2
Lüneburg WWTP	2	2	-	-	2
BS	2	2	-	-	2
BR	2	2	-	-	2

9.2.2 Plate Count – Agar Pour Plate method

The culture medium was the Agar Plate Count (APHA, ISO 4833:2003 - Karl Roth). The dilutions were performed using sterile water until a dilution factor of 10^{-5} for each sludge sample. Subsequently, 1 ml of the dilutions 10^{-4} and 10^{-5} were plated separately with about 15 ml of the fluidized medium. Then, the sample and the fluidized medium were mixed by gentle agitation of the plate. Each sample dilution was plated by duplicate (US FDA, 2014).

The culture medium was left to solidify at room temperature and then incubated at 37°C. The counting of the colony forming units (CFU) was performed at the end of 24 hours and 48 hours after starting the incubation.

For colony counting, the plates with 25-250 colonies were selected and counted including those of pinpoint size.

- If the plates from all dilutions exceed the 250 colonies, count colonies in representative portions of the plates and estimate the total.
- If plates from all dilutions reported less than 25 colonies, report the colonies number with the lowest dilution.

The results are obtained multiplying the number of colonies by the dilution factor. If consecutive dilutions yield 25-250 colonies, the counting should be performed using the Formula 9.1 (US FDA, 2014).

$$N = \frac{(\Sigma\, C)}{((1*n1)+ (0.1*n2))\, d} \quad \text{..... (9.1)}$$

where:

N: Number of colonies per milliliter

Σ C: Sum of all colonies in all plates counted

n 1: Plates number in the lowest dilution counted

n 2 : Plates number in the next highest dilution counted.

d: Dilution in the first count obtained.

The survival percentage was calculated considering the initial microorganism concentration in the sludge samples, i.e. the concentration before the desorption-stabilization-sorption process, and comparing these results with the concentration obtained at the end of the sorption process.

9.3. Results and discussion

9.3.1 Survival percentage of bacterial communities at the end of the desorption-stabilization-sorption processes.

The results of viable microorganisms concentration (CFU/ml) and survival percentage before starting the desorption process (control sample) and after the sorption process are shown below.

The S1 reactor showed an initial concentration of 33 x 10^5 CFU/ml (Table 9.2). After the desorption-stabilization-sorption process at a pH of 2, the concentration of microorganisms showed a survival rate of about 4% when acid eluents were used. For the sludge samples that underwent desorption process using KCl as eluent, the survival percentage was 6%. At the end of the experimental process at a pH of 3, an increase in the survival rate was recorded, and even percentages of bacterial growth in the S1-SO (112 %) and S1-KCl (152%) reactors. At the end of the experimental process at a pH of 4, some bacterial populations showed a gradual increase of the survival percentages according to the increase of the pH (S1-Cit and S1-KCl). It was expected the increase of the bacterial concentration according to the pH increase, but for the S1- SO and S1- HCl reactors the highest percentages of survival were obtained at a pH of 3. In order to explain this fact, the different TSS concentrations of the sludge samples at the end of the sorption process should be considered. Additionally, for the S1-SO reactor, the use of sulphuric acid as eluent may result in the development of sulphur oxidising bacteria, as this microorganisms are able to oxidize reduced inorganic sulphur compounds (H_2S, $S_2O_3^{2-}$) or elemental sulphur (Seviour and Nielsen, 2010).

Table 9.2: Bacterial survival results in CFU/ml and in percentage for the S1 sludge sample.

			M.O concentration (CFU/ml) at dilution 10^{-5}				
Sludge sample	Initial concent.	pH	Citric acid	H_2SO_4	HCl	KCl	Control
S1	$33x10^5$	2	1.5	1	1	2	15
		% survival	4.5	3.0	3.0	6.1	45.0
		3	1	37	14	50	18
		% survival	3.0	112.0	42.0	152.0	55.0
		4	9	3	6	42	16
		% survival	27.0	9.0	18.0	127.0	48.0

The S2 sludge sample recorded an initial concentration of 238 x 10^5 CFU/ml (Table 9.3). After the desorption(pH=2)-stabilization-sorption process survival percentages ranging between 10.5% and 13.4% were recorded in case of reactors that used acid eluents. For the S2-KCl reactor the survival percentage was about 30% at the end of the experimental process. At the end of the desorption(pH=3)-stabilization-sorption process the sludge samples showed an increase in the survival percentages, recording population growth percentages up to 152% for the S2-SO reactor. Finally, at the end of the desorption(pH=4)-stabilization-sorption process, a gradual increase in the survival percentage of the S2-Cit reactor was observed, while the S2-SO and S2-HCl reactors showed the highest survival percentages at a pH of 3. The S2-KCl reactors showed low survival percentages in all cases. The survival percentages for the S1-Control and S2-Control reactors ranged between 42% and 55%.

Table 9.3: Bacterial survival results in CFU/ml and in percentage for the S2 sludge sample.

			M.O concentration (CFU/ml) at Dilution 10^{-5}				
Sludge sample	Initial concent.	pH	Citric acid	H_2SO_4	HCl	KCl	Control
S2	$238x10^5$	2	32	30	25	71	122
		% survival	13.4	12.6	10.5	29.8	51.3
		3	206	362	166	34	116
		%survival	87.0	152.0	70.0	14.0	49.0
		4	196	60	115	57	99
		%survival	82.0	25.0	48.0	24.0	42.0

The remaining processes of desorption-stabilization-sorption were performed for the B2 and B4 sludge samples, using as eluents to citric acid at desorption pH of 3 and 4, and potassium chloride with eluent concentrations of 30 mmol/l and 40 mmol/l. For the sludge samples from Steinhorst and Lüneburg WWTPs the desorption-stabilization-sorption processes were performed using as eluents to citric acid with a desorption pH of 3 and potassium chloride concentration of 30 mmol/l.

The B2 sludge sample recorded an initial concentration of 28 x 10^5 CFU/ml. After the desorption(pH=3)-stabilization-sorption process, the survival percentages recorded bacterial growth up to 204% for the B2-Cit reactor.

Finally, at the end of the desorption(pH=4)-stabilization-sorption process no bacterial growth was recorded in any case.

Table 9.4: Bacterial survival results in CFU/ml and in percentage for the B2 sludge sample.

		M.O concentration (CFU/ml) at Dilution 10^{-5}			
Sludge sample	Initial concent.	pH	Citric acid	KCl	Control
B2	$28x10^5$	3	57	3	16
		%survival	204	11	57
		4	14	3	15
		%survival	50	11	54

The B4 sludge sample recorded an initial concentration of 164 x 10^5 CFU / ml (Table 9.5). As for the B2 sludge sample, after the desorption(pH=3)-stabilization-sorption process the B4 sludge sample recorded a considerable bacterial growth up to 1234% for the B4-Cit reactor, while for the B4-KCl reactor the survival percentage was 2%.

At the end of the desorption(pH=4)-stabilization-sorption process the bacterial community in the B4-Cit reactor continued showing population growth but this time of only 183%, while for the B4-KCl reactor a survival rate of 2% was recorded. For the B2-Control and B4-Control sludge samples, the survival percentages ranged between 43% and 57%.

Table 9.5: Bacterial survival results in CFU/ml and in percentage for the B4 sludge sample.

		M.O concentration (CFU/ml) at Dilution 10^{-5}			
Sludge sample	Initial concent.	pH	Citric acid	KCl	Control
B4	$164x10^5$	3	2024	4	79
		%survival	1234	2	48
		4	300	4	71
		%survival	183	2	43

The bacterial survival rates were also evaluated in samples from the aeration tank (BS) and sludge disposal line (RS) of the Steinhorst WWTP and from the aeration tank (BS) and return sludge line (RS) of the Lüneburg WWTP.

The sludge sample from the Steinhorst WWTP recorded an initial concentration in the aeration tank of about 11 x 10^5 CFU/ml and in the sludge disposal line of about $19x10^5$ CFU/ml (Table 9.6). After the desorption(pH=3)-stabilization-sorption process, the St-BS-Cit reactor recorded a bacterial growth up to 1045%, while the St-BS-KCl reactor showed a survival rate of 64%. Meanwhile, the return sludge sample (RS) recorded bacterial growth rate up to 4342% for the St-RS-Cit reactor and of 132% for the St-RS-KCl reactor. The control reactors recorded around 50% of bacterial survival.

Table 9.6: Bacterial survival results in CFU/ml and in percentage for the Steinhorst WWTP sludge sample.

		M.O concentration (CFU/ml) at dilution 10^{-5}			
Sludge sample	Initial concent.	Reactor	Citric acid	KCl	Control
Steinhorst (St)	$11x10^5$	BS	126	7	5
		%survival	1145	64	47
	$19x10^5$	RS	844	44	10
		%survival	4442	232	53

The sludge sample from the Lüneburg WWTP recorded an initial concentration in the aeration tank of about $8x10^5$ CFU/ml and in the return sludge line of about $12x10^5$ CFU/ml (Table 9.7).

After the desorption(pH=3)-stabilization-sorption process, bacterial growth of approximately 688% was recorded for the Lu-BS-Cit reactor and a survival percentage of 75% for the Lu-BS-KCl reactor. Similar percentage of bacterial growth was recorded

for the Lu-RS-Cit reactor (500%) while for the Lu-RS-KCl reactor a survival rate of 75% was recorded. The survival percentage for the control reactors ranged between 42% and 50%.

Table 9.7: Bacterial survival results in CFU/ml and in percentage for the Lüneburg WWTP sludge samples.

		M.O concentration (CFU/ml) at dilution 10^{-5}			
Sludge sample	Initial concent	Reactor	Citric acid	KCl	Control
Lüneburg	$8x10^5$	BS	63	6	4
		%survival	788	75	50
	$12x10^5$	RS	72	9	5
		%survival	600	75	42

Figure 9.1 shows the survival percentages in relation to the desorption pH. For the sludge samples that underwent a desorption process using citric, sulphuric and hydrochloric acids at pH of 3 were recorded the highest values of survival percentages. After the desorption(pH=3)-stabilization-sorption process with citric acid, the S1 and S2 sludge samples recorded the lowest survival percentages (S1 = 3% and S2 =87%). However, with this eluent, other biomasses showed bacterial growth as for example the St-RS-Cit reactor (4342%). The high survival percentages and bacterial growth obtained using citric acid as eluent was one of the reasons to use this eluent in the remaining desorption-stabilization-sorption processes.

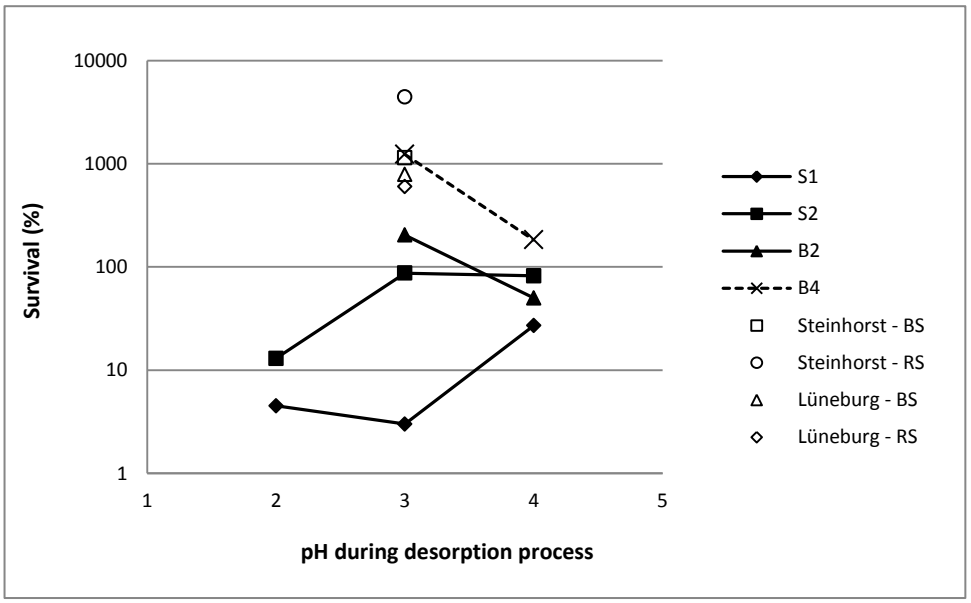

Figure 9.1: Bacterial survival percentages after the desorption-stabilization-sorption process using citric acid as eluent during the desorption process.

The desorption-stabilization-sorption processes that used hydrochloric and sulphuric acid as eluents were performed only with the S1 and S2 sludge samples at pH 2, 3 and 4.

At the end of the desorption(pH=3)-stabilization-sorption process using sulphuric acid as eluent (Figure 9.2.a), the S1 and S2 reactors recorded bacterial growth of about 12% and 52%, respectively. Meanwhile, at the end of the experimental process using hydrochloric acid as eluent (Figure 9.2.b), none of the two reactors recorded bacterial growth, but survival rates of 41% and 70% for the S1 and S2 reactors, respectively.

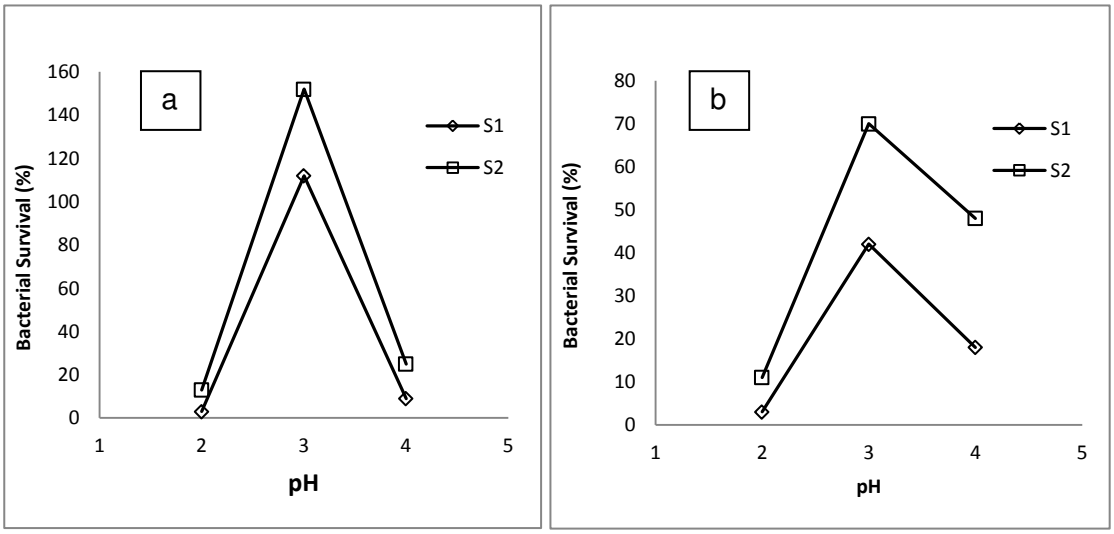

Figure 9.2: Bacterial survival percentages for the S1 and S2 sludge samples after the desorption-stabilization-sorption process using sulphuric acid (a) and hydrochloric acid (b) as eluents during the desorption process.

At the end of the experimental process using potassium chloride as eluent during the desorption process (Figure 9.3.a) bacterial growth was not recorded in any case, but with an eluent concentration of 30 mmol/l the survival percentages remained almost constant for the Lu-BS-KCl and Lu-RS-KCl reactors in comparison to their initial bacterial concentration.

For the control reactors (Figure 9.3.b) the survival percentages in all cases showed values of approximately 50%, considering that these reactors did not receive eluents solutions.

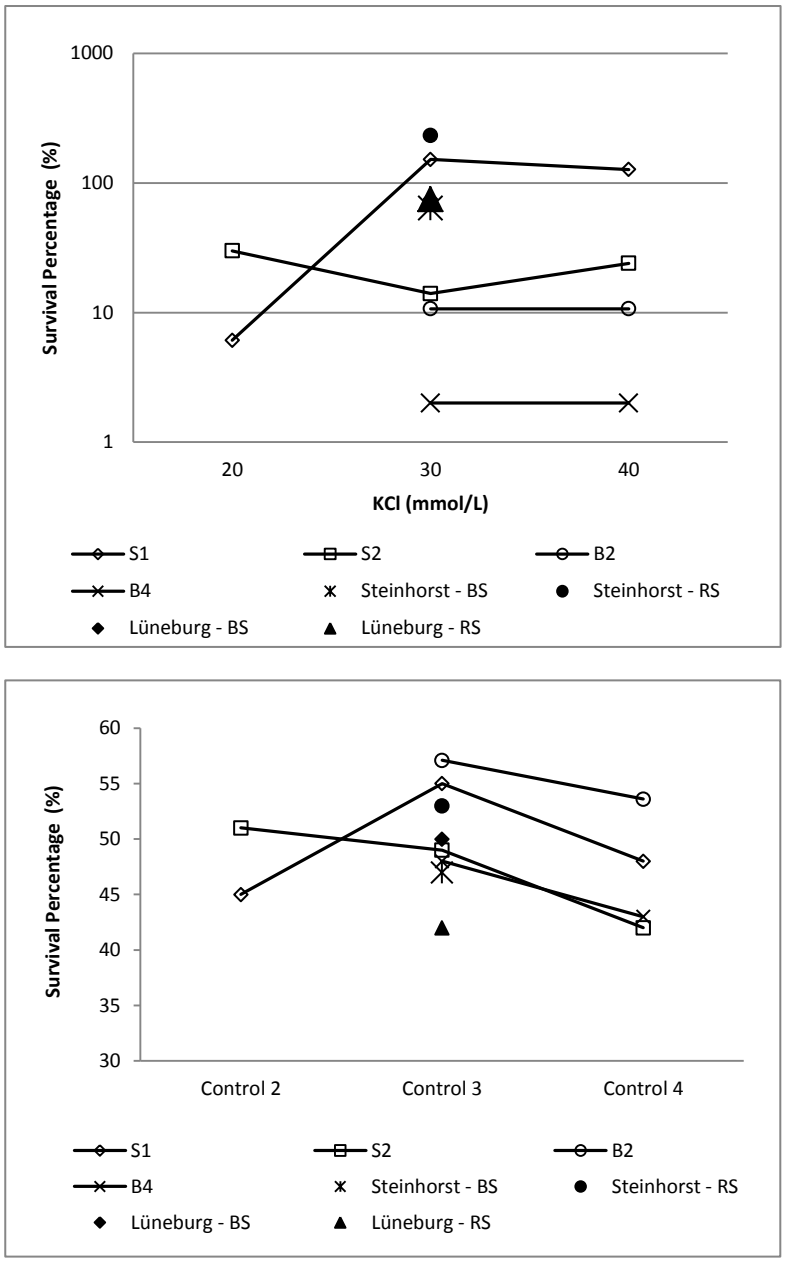

Figure 9.3: Bacterial survival percentage for control reactors and after the desorption-stabilization-sorption process using potassium chloride as eluent

In summary, after the completion of the desorption-stabilization-sorption processes, the best bacterial survival results were obtained using citric acid and sulphuric acid as eluents at a pH of 3.

9.4 Conclusions

1. The highest survival percentages and bacterial growth rates at the end of the desorption-stabilization-sorption processes were reached for sludge samples with typical EBPR bacterial communities that underwent desorption processes using citric acid as eluent at a pH of 3.

The mentioned bacterial communities might be able to uptake citric acid as carbon source during the desorption process resulting in a considerable bacterial growth increase. The highest survival and growing rates observed at a pH of 3 indicate that this pH resulted not so aggressive to stop the bacterial metabolism and, at the same time, that this concentration of citric acid covered the metabolic requirements of the bacterial communities to increase their concentrations.

2. The S1 and S2 sludge samples did not show bacterial growth after the desorption-stabilization-sorption process at any pH or with any eluent.

3. The average survival percentages reached in control reactors were approximately 50%. The decrease of the bacterial concentration may be due to the exponential death phase observed when the bacterial communities underwent long periods (24-36 hours) without a carbon source.

9.5 References

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CHAPTER 10: Conclusions

1. Good and stable VFA production (328 mg/l) may be obtained in glucose fermentation reactors when the HRT (12 hours) is controlled to avoid the development of methanogenic bacteria and when the F/M ratio is maintained under values (0.4 gCOD/gTSS.cycle) that not promote the overdevelopment of the bacterial community and therefore avoid the consumption of the VFA as carbon source.

   This conclusion considered the amount of VFA produced per gram of COD consumed that recorded a maximum value of 0.722 gVFA/gCOD.

2. The bacterial communities TFO and EBPR showed different bacterial structure. While the TFO community was dominated by the TFO morphotype, the EBPR bacterial community showed a mixed microbial composition without dominance of a specific morphotype. However, both bacterial communities recorded good and stable EBPR processes. The effluents obtained with both bacterial communities recorded total phosphorus concentrations of less than 1 mg/l and the results of the staining techniques confirmed the storing and release of polymers typical of the EBPR process during the anaerobic and aerobic phases. The highest differences of phosphorus removal performance between the bacterial communities, were recorded when the C: N: P ratio in the influent was adverse for the EBPR process (18.1:1.9:1) to subsequently the more suitable was the C: N: P ratio in the influent (45.2:4:1), the phosphorus removal performance between reactors became more similar regardless the carbon source used. At this point all the reactors reached a total phosphorus concentration of less than 0.5 mg/l in the effluent.

   It is possible to obtain bacterial communities in activated sludge samples with different microbiological characteristics able to perform efficient and stable EBPR processes.

3. During the phosphorus desorption test the best desorption performance were obtained with the sludge samples corresponding to the EBPR bacterial community and using to citric acid as eluent with a desorption pH of 3. The highest phosphorus desorption performance (70.3%) was reached with the Lüneburg-WWTP sludge sample with a maximum phosphorus release up to 130.3 mg/l. Additionally, with this sludge sample the highest concentration of

phosphorus released per gram of sludge dry mass was recorded (16.8 mgP/gTSS). However, the phosphorus release to the aqueous phase observed during the desorption process would correspond not only to the eluent effect of the citric acid but also to the typical phosphorus release observed in the EBPR process under anaerobic conditions and in presence of a carbon source.

During desorption processes of phosphorus in activated sludge samples, the release of phosphorus to the aqueous phase due to the EBPR process should be also considered combined to the phosphorus release due to the eluents effect.

4. The most influential factors on the phosphorus desorption process of an activated sludge sample are the type of bacterial community present, the eluent used, the desorption pH and the environmental conditions of the origin reactor.

   In summary, the phosphorus release performance may be increased when the activated sludge mass corresponds to an EBPR bacterial community, using to citric acid as eluent, with a desorption pH of 3 and when the sludge sample previously underwent anaerobic, slightly acid and static conditions in its origin reactor.

5. At the end of the desorption-stabilization-sorption processes the sludge samples underwent a survival test. The highest survival rates and even bacterial growth rates were recorded in the EBPR bacterial communities which underwent previous desorption processes using to citric acid as eluent.

   From this result it is possible to conclude that the EBPR bacterial community is able to use the citric acid as carbon source under anaerobic conditions but this capacity is not reported for the glucose-TFO bacterial community.

6. The highest phosphorus sorption capacity were recorded in the S1, S2 and Lüneburg-BS activated sludge samples which correspond to the TFO, TFO + filamentous and mixed EBPR bacterial communities, respectively. The highest phosphorus sorption percentage (78.6%) was reached in the S2-KCl reactor (TFO bacterial community) and considering the initial phosphorus concentration in the mixed liquor. The phosphorus adsorbed per mass of total solids was up to 0.23 mgPtot/gTSS. Apparently, the phosphorus sorption capacity of an

activated sludge sample would be directly related to the abundance of the TFO morphotype.

7. The phosphorus sorption capacity of an EBPR - activated sludge sample may be improved when previously to the sorption process the sludge sample undergoes a desorption process using to potassium chloride as eluent with a concentration of 30 mmol/l and subsequently a stabilization process using a $MgCl_2$ and $CaCl_2$ solution with 10 and 5 mg/l respectively. Thus, considering the initial phosphorus concentration (before the desorption process) the Lüneburg-KCl sludge sample increased in 19.5% its phosphorus concentration while the S2-KCl sludge sample increased its phosphorus concentration in 46.1% at the end of the sorption process.

8. The phosphorus sorption capacity of an EBPR - activated sludge sample would be influenced by:
    - The dominant bacterial community in the sludge sample since the highest phosphorus sorption performance was recorded when the dominant bacterial morphotype was the TFO.
    - The floc stability in the sludge sample which was influenced by the environmental conditions of the origin reactor. Apparently, sludge samples from anaerobic, static and slightly acid environments would present destabilized flocs that cannot be used as substrate in sorption processes.

APPENDIX

APPENDIX 1: Calculation of the apparent desorption coefficient (Kdes) using the batch equilibrium method in activated sludge samples

pH	Reactor	m_w^{aq} (eq) (mg)	m_{soil}^{aq} (eq) (mg)	Vt (ml)	msoil (g)	Kdes (ml/g)
2	S1-Cit	34.549	11.516	1000	3.819	523.715
	S1-SO	34.549	15.616	1000	3.819	317.465
	S1-HCl	34.549	13.416	1000	3.819	412.462
	S1-KCl	34.549	14.516	1000	3.819	361.364
	S1-Control	34.549	0.266	1000	3.819	33747.763
	S2_Cit	87.979	44.335	1000	5.46	180.297
	S2-SO	87.979	47.635	1000	5.46	155.119
	S2-HCl	87.979	45.035	1000	5.46	174.648
	S2-KCl	87.979	43.335	1000	5.46	188.684
	S2-Control	87.979	0.835	1000	5.46	19123.460
3	S1-Cit	60.652	32.217	1000	5.32	165.909
	S1-SO	60.652	18.617	1000	5.32	424.431
	S1-HCl	60.652	29.917	1000	5.32	193.116
	S1-KCl	60.652	0.337	1000	5.32	33692.433
	S1-Control	60.652	0.037	1000	5.32	312161.500
	S2-Cit	64.944	42.268	1000	5.113	104.926
	S2-SO	64.944	29.368	1000	5.113	236.924
	S2-HCl	64.944	32.768	1000	5.113	192.048
	S2-KCl	64.944	0.568	1000	5.113	22170.577
	S2-Control	64.944	0.498	1000	5.113	25315.046
Lüneburg	BS-Control	90.580	0.074	1000	3.831	319251.907
3	BS-Cit	90.580	63.644	1000	3.831	110.475
	BS-KCl	90.580	0.934	1000	3.831	25053.701
	RS-Control	219.624	0.043	1000	7.773	653915.977
	RS-Cit	219.624	102.573	1000	7.773	146.809
	RS-KCl	219.624	8.923	1000	7.773	3037.784
3	B2-Control	84.083	0.063	200	0.64	414852.317
	B2-Cit	84.083	38.616	200	0.64	367.933
	B2-KCl	84.083	0.013	200	0.64	1976796.699
	B4-Control	82.825	0.074	200	0.512	436819.046
	B4-Cit	82.825	21.536	200	0.512	1111.674
	B4-KCl	82.825	0.108	200	0.512	299178.964
Steinhorst	BS-Control	63.280	14.772	1000	3.778	869.185
3	BS-Cit	63.280	28.242	1000	3.778	328.394
	RS-Control	152.052	24.281	1000	8.047	653.931
	RS-Cit	152.052	60.460	1000	8.047	188.257
	RS-KCl	152.052	29.301	1000	8.047	520.605

pH	Reactor	m_{Cu}^{ads} (eq) (mg)	m_{Cu}^{des} (eq) (mg)	Vt (ml)	msoil (g)	Kdes (ml/g)
4	S1-Cit	62.520	14.617	1000	6.672	491.185
	S1-SO	62.520	5.517	1000	6.672	1548.587
	S1-HCl	62.520	8.217	1000	6.672	990.493
	S1-KCl	62.520	0.517	1000	6.672	17974.769
	S1-Cntrl	62.520	0.017	1000	6.672	551052.687
	S2-Cit	92.574	24.468	1000	7.57	367.706
	S2-SO	92.574	12.068	1000	7.57	881.279
	S2-HCl	92.574	12.968	1000	7.57	810.947
	S2-KCl	92.574	0.268	1000	7.57	45566.936
	S2-Cntrl	92.574	0.585	1000	7.57	20786.583
5	S1-Cit	81.235	1.874	1000	7.766	5454.578
	S1-SO	81.235	1.174	1000	7.766	8785.078
	S1-HCl	81.235	0.774	1000	7.766	13394.694
	S1-KCl	81.235	0.374	1000	7.766	27877.650
	S2-Cit	69.431	6.201	1000	4.003	2547.390
	S2-SO	69.431	5.601	1000	4.003	2847.053
	S2-HCl	69.431	5.001	1000	4.003	3218.625
	S2-KCl	69.431	0.421	1000	4.003	40978.180
	S2-Cntrl	69.431	0.281	1000	5.003	49239.996

APPENDIX 2: RELATIONSHIP BETWEEN THE SOIL/SOLUTION RATIO, DESORPTION PERCENTAGE (Deq) AND APPARENT DESORPTION COEFFICIENT (Kdes) IN A LOGARITHMIC LINEAR EQUATION

pH	Reactor	$\frac{m_{soil}^{ini}(aq) - m_{soil}^{eq}(aq)}{m_{soil}^{eq}(aq)}$	Vt/msoil (ml/g)	Deq (%)	Msoil/Vtot (g/ml)	Log (1-Deq/Deq)	Log R	Log Kdes
2	S1-Cit	2.000	261.849	0.333	0.004	0.301	-2.418	2.719
	S1-SO	1.212	261.849	0.452	0.004	0.084	-2.418	2.502
	S1-HCl	1.575	261.849	0.388	0.004	0.197	-2.418	2.615
	S1-KCl	1.380	261.849	0.420	0.004	0.140	-2.418	2.558
	S1-Control	128.883	261.849	0.008	0.004	2.110	-2.418	4.528
	S2_Cit	0.984	183.150	0.504	0.005	-0.007	-2.263	2.256
	S2-SO	0.847	183.150	0.541	0.005	-0.072	-2.263	2.191
	S2-HCl	0.954	183.150	0.512	0.005	-0.021	-2.263	2.242
	S2-KCl	1.030	183.150	0.493	0.005	0.013	-2.263	2.276
	S2-Control	104.414	183.150	0.009	0.005	2.019	-2.263	4.282
3	S1-Cit	0.883	187.970	0.531	0.005	-0.054	-2.274	2.220
	S1-SO	2.258	187.970	0.307	0.005	0.354	-2.274	2.628
	S1-HCl	1.027	187.970	0.493	0.005	0.012	-2.274	2.286
	S1-KCl	179.244	187.970	0.006	0.005	2.253	-2.274	4.528
	S1-Control	1660.699	187.970	0.001	0.005	3.220	-2.274	5.494
	S2-Cit	0.536	195.580	0.651	0.005	-0.270	-2.291	2.021
	S2-SO	1.211	195.580	0.452	0.005	0.083	-2.291	2.375
	S2-HCl	0.982	195.580	0.505	0.005	-0.008	-2.291	2.283
	S2-KCl	113.358	195.580	0.009	0.005	2.054	-2.291	4.346
	S2-Control	129.436	195.580	0.008	0.005	2.112	-2.291	4.403

pH	Reactor	$\frac{C_{eq}(s) - C_{eq}(s)}{C_{eq}(s)}$	Vt/msoil (ml/g)	Deq (%)	Msoil/Vtot (g/ml)	Log (1-Deq/Deq)	Log R	Log Kdes
Lüneburg	BS-Control	1223.054	261.028	0.001	0.004	3.087	-2.417	5.504
	BS-Cit	0.423	261.028	0.703	0.004	-0.373	-2.417	2.043
	BS-KCl	95.981	261.028	0.010	0.004	1.982	-2.417	4.399
3								
	RS-Control	5082.889	128.650	0.000	0.008	3.706	-2.109	5.816
	RS-Cit	1.141	128.650	0.467	0.008	0.057	-2.109	2.167
	RS-KCl	23.613	128.650	0.041	0.008	1.373	-2.109	3.483
	B2-Control	1327.527	312.500	0.001	0.003	3.123	-2.495	5.618
	B2-Cit	1.177	312.500	0.459	0.003	0.071	-2.495	2.566
	B2-KCl	6325.749	312.500	0.000	0.003	3.801	-2.495	6.296
3								
	B4-Control	1118.257	390.625	0.001	0.003	3.049	-2.592	5.640
	B4-Cit	2.846	390.625	0.260	0.003	0.454	-2.592	3.046
	B4-KCl	765.898	390.625	0.001	0.003	2.884	-2.592	5.476
Steinhorst	BS-Control	3.284	264.690	0.233	0.004	0.516	-2.423	2.939
	BS-Cit	1.241	264.690	0.446	0.004	0.094	-2.423	2.516
3								
	RS-Control	5.262	124.270	0.160	0.008	0.721	-2.094	2.816
	RS-Cit	1.515	124.270	0.398	0.008	0.180	-2.094	2.275
	RS-KCl	4.189	124.270	0.193	0.008	0.622	-2.094	2.717

pH	Reactor	$\frac{C_{in}(aq) - C_{eq}(aq)}{C_{in}(aq)}$	Vt/msoil (ml/g)	Deq (%)	Msoil/Vtot (g/ml)	Log (1-Deq/Deq)	Log R	Log Kdes
4	S1-Cit	3.277	149.880	0.234	0.007	0.516	-2.176	2.691
	S1-SO	10.332	149.880	0.088	0.007	1.014	-2.176	3.190
	S1-HCl	6.609	149.880	0.131	0.007	0.820	-2.176	2.996
	S1-KCl	119.928	149.880	0.008	0.007	2.079	-2.176	4.255
	S1-Cntrl	3676.624	149.880	0.000	0.007	3.565	-2.176	5.741
	S2-Cit	2.784	132.100	0.264	0.008	0.445	-2.121	2.566
	S2-SO	6.671	132.100	0.130	0.008	0.824	-2.121	2.945
	S2-HCl	6.139	132.100	0.140	0.008	0.788	-2.121	2.909
	S2-KCl	344.942	132.100	0.003	0.008	2.538	-2.121	4.659
	S2-Cntrl	157.354	132.100	0.006	0.008	2.197	-2.121	4.318
5	S1-Cit	42.360	128.766	0.023	0.008	1.627	-2.110	3.737
	S1-SO	68.225	128.766	0.014	0.008	1.834	-2.110	3.944
	S1-HCl	104.023	128.766	0.010	0.008	2.017	-2.110	4.127
	S1-KCl	216.498	128.766	0.005	0.008	2.335	-2.110	4.445
	S2-Cit	10.197	249.813	0.089	0.004	1.008	-2.398	3.406
	S2-SO	11.397	249.813	0.081	0.004	1.057	-2.398	3.454
	S2-HCl	12.884	249.813	0.072	0.004	1.110	-2.398	3.508
	S2-KCl	164.036	249.813	0.006	0.004	2.215	-2.398	4.613
	S2-Cntrl	246.348	199.880	0.004	0.005	2.392	-2.301	4.692

APPENDIX 3: Calculation of the distribution coefficient (Kd) using the batch equilibrium method in activated sludge samples.

22-jun-13	Time	$C_{eq}^{soln}(eq)$	$C_{s}^{soln}(eq)$	V_o	m_{soil}	$m_{s}^{soln}(eq)$	$m_{eq}^{soln}(eq)$	Kd
pH 2	(h)	(mg/ml)	(mg/g)	(l)	(g)	(mg/l)	(mg/l)	(ml/g)
S1-Cit	0.01	0.0075	-0.32567	300	4.6056	-4.99966	7.5	-43.42246
	0.25	0.0041	-0.10420	300	4.6056	-1.59966	4.1	-25.41438
	0.5	0.0039	-0.09118	285	4.37532	-1.39966	3.9	-23.37726
	0.75	0.0041	-0.10420	270	4.14504	-1.59966	4.1	-25.41438
	24	0.0057	-0.20843	255	3.91476	-3.19966	5.7	-36.56489
S1-SO	0.01	0.0075	-0.24813	300	5.562	-4.73333	7.5	-34.04051
	0.25	0.0042	-0.07013	300	5.562	-1.43333	4.2	-18.40722
	0.5	0.0043	-0.07553	285	5.2839	-1.53333	4.3	-19.23350
	0.75	0.0043	-0.07553	270	5.0058	-1.53333	4.3	-19.23350
	24	0.0024	0.02695	255	4.7277	0.36667	2.4	8.24044
S1-HCl	0.01	0.0075	-0.33718	300	4.0812	-4.72444	7.5	-46.30446
	0.25	0.0044	-0.10930	300	4.0812	-1.62444	4.4	-27.13848
	0.5	0.004	-0.07990	285	3.87714	-1.22444	4	-22.50155
	0.75	0.0038	-0.06520	270	3.67308	-1.02444	3.8	-19.81701
	24	0.0031	-0.01374	255	3.46902	-0.32444	3.1	-7.69329
S1-KCl	0.01	0.0075	-0.34294	300	3.966	-4.68889	7.5	-47.29086
	0.25	0.0042	-0.09332	300	3.966	-1.38889	4.2	-25.01421
	0.5	0.0039	-0.07062	285	3.7677	-1.08889	3.9	-21.11969
	0.75	0.0039	-0.07062	270	3.5694	-1.08889	3.9	-21.11969
	24	0.001	0.14874	255	3.3711	1.81111	1	136.99781
S1-Control	0.01	0.0075	-0.34390	300	4.266	-4.92667	7.5	-46.19472
	0.25	0.0042	-0.11183	300	4.266	-0.05667	2.63	-1.51521
	0.5	0.0039	-0.09074	285	4.0527	0.09333	2.48	2.64658
	0.75	0.0039	-0.09074	270	3.8394	0.34333	2.23	10.82708
	24	0.001	0.11320	255	3.6261	0.59333	1.98	21.07337

22. Jun 13	Time	$C_{aq}^{ads}(eq)$	$C_{s}^{ads}(eq)$	V_o	m_{soil}	$m_{s}^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d
pH 2	(h)	(mg/l)	(mg/g)	(l)	(g)	(mg/l)	(mg/l)	(ml/g)
S2-Cit	0,01	7,5	-195,21874	450	6,42465	-2,787	7,5	-26,02
	0,25	9,2	-314,29138	450	6,42465	-4,487	9,2	-34,16
	0,5	8,4	-258,25720	435	6,210495	-3,687	8,4	-30,74
	0,75	8,7	-279,27001	420	5,99634	-3,987	8,7	-32,10
	24	8,4	-258,25720	405	5,782185	-3,687	8,4	-30,74
S2-SO	0,01	7,5	-288,37891	450	4,18275	-2,68	7,5	-38,44
	0,25	8,1	-352,92975	450	4,18275	-3,28	8,1	-43,57
	0,5	7,9	-331,41280	435	4,043325	-3,08	7,9	-41,94
	0,75	8	-342,17127	420	3,9039	-3,18	8	-42,76
	24	6,6	-191,55266	405	3,764475	-1,78	6,6	-29,02
S2-HCl	0,01	7,5	-320,37740	450	3,9897	-2,84	7,5	-42,71
	0,25	8,5	-433,16783	450	3,9897	-3,84	8,5	-50,95
	0,5	8,2	-399,33070	435	3,85671	-3,54	8,2	-48,69
	0,75	8,2	-399,33070	420	3,72372	-3,54	8,2	-48,69
	24	6	-151,19174	405	3,59073	-1,34	6	-25,19
S2-KCl	0,01	7,5	-329,26713	450	4,11885	-3,01	7,5	-43,90
	0,25	7,4	-318,34175	450	4,11885	-2,91	7,4	-43,01
	0,5	7,4	-318,34175	435	3,981555	-2,91	7,4	-43,01
	0,75	6,3	-198,16257	420	3,84426	-1,81	6,3	-31,45
	24	7	-274,64023	405	3,706965	-2,51	7	-39,23
S2-Control	0,01	7,5	-430,06237	450	5,0535	-4,83	7,5	-57,34
	0,25	7,4	-421,15765	450	5,0535	0,05	2,62	1,72
	0,5	7,4	-421,15765	435	4,88505	0,09	2,58	3,13
	0,75	6,3	-323,20573	420	4,7166	-0,07	2,74	-2,25
	24	7	-385,53877	405	4,54815	0,54	2,13	22,60

22-may-13	Time	$C_{aq}^{ads}(eq)$	$C_{s}^{ads}(eq)$	V_o	m_{soil}	$m_{s}^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d
pH 3	(h)	(mg/ml)	(mg/g)	(l)	(g)	(mg/l)	(mg/l)	(ml/g)
S1-Cit	0.01	0.0075	-0.40049	300	3.4635	-5.2989	7.5	-61.1969
	0.25	0.0138	-0.94618	300	3.4635	-11.5989	13.8	-72.8020
	0.5	0.016	-1.13674	285	3.2903	-13.7989	16	-74.7017
	0.75	0.0186	-1.36194	270	3.1172	-16.3989	18.6	-76.3673
	24	0.0438	-3.54471	255	2.9440	-41.5989	43.8	-82.2647
S1-SO	0.01	0.0075	-0.50848	300	3.1212	-5.7433	7.5	-73.6042
	0.25	0.0093	-0.68149	300	3.1212	-7.5433	9.3	-77.9615
	0.5	0.0107	-0.81605	285	2.9651	-8.9433	10.7	-80.3369
	0.75	0.0112	-0.86411	270	2.8091	-9.4433	11.2	-81.0414
	24	0.0227	-1.96946	255	2.6530	-20.9433	22.7	-88.6788
S1-HCl	0.01	0.0075	-0.43498	300	3.2532	-5.3611	7.5	-65.9180
	0.25	0.0091	-0.58253	300	3.2532	-6.9611	9.1	-70.5420
	0.5	0.0105	-0.71163	285	3.0905	-8.3611	10.5	-73.4320
	0.75	0.011	-0.75774	270	2.9279	-8.8611	11	-74.2858
	24	0.0244	-1.99345	255	2.7652	-22.2611	24.4	-84.1332
S1-KCl	0.01	0.0075	-0.49487	300	3.9021	-6.5078	7.5	-66.7105
	0.25	0.00035	0.05483	300	3.9021	0.6422	0.35	141.0718
	0.5	0.00028	0.06022	285	3.7070	0.7122	0.28	195.5601
	0.75	0.00023	0.06406	270	3.5119	0.7622	0.23	254.7866
	24	0.00098	0.00640	255	3.3168	0.7122	0.28	195.5601
S1-Kontrol	0.01	0.0075	-0.41036	300	4.1550	-6.0056	7.5	-57.8152
	0.25	0.00035	0.10588	300	4.1550	-0.1156	1.61	-5.1822
	0.5	0.00028	0.11094	285	3.9473	0.0044	1.49	0.2154
	0.75	0.00023	0.11455	270	3.7395	-0.0256	1.52	-1.2139
	24	0.00098	0.06040	255	3.5318	0.1044	1.39	5.4253

22. Mai 13	Time	$C_{aq}^{ads}(eq)$	$C_{s}^{ads}(eq)$	V_o	m_{soil}	$m_{s}^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d
pH 3	(h)	(mg/l)	(mg/g)	(l)	(g)	(mg/l)	(mg/l)	(ml/g)
S2-Cit	0,01	7,5	-468,5788	450	3,6945	-4,79	7,5	-77,74
	0,25	5,5	-224,9734	450	3,6945	-2,79	5,5	-61,71
	0,5	6,1	-298,0551	435	3,5714	-3,39	6,1	-67,62
	0,75	6,5	-346,7761	420	3,4482	-3,79	6,5	-70,96
	24	16,1	-1516,0819	405	3,3251	-13,39	16,1	-101,28
S2-SO	0,01	7,5	-488,7189	450	4,1499	-5,23	7,5	-75,57
	0,25	13,5	-1139,3370	450	4,1499	-11,23	13,5	-90,18
	0,5	6,4	-369,4390	435	4,0116	-4,13	6,4	-69,92
	0,75	6,7	-401,9699	420	3,8732	-4,43	6,7	-71,64
	24	16,1	-1421,2715	405	3,7349	-13,83	16,1	-93,13
S2-HCl	0,01	7,5	-456,1340	450	4,1702	-5,04	7,5	-72,52
	0,25	5,6	-251,1054	450	4,1702	-3,14	5,6	-60,51
	0,5	7	-402,1791	435	4,0311	-4,54	7	-69,99
	0,75	6,8	-380,5972	420	3,8921	-4,34	6,8	-68,87
	24	17,6	-1546,0229	405	3,7531	-15,14	17,6	-92,83
S2-KCl	0,01	7,5	-850,8176	450	3,4898	-6,57	7,5	-112,96
	0,25	0,91	-1,0432	450	3,4898	0,02	0,91	2,83
	0,5	0,93	-3,6222	435	3,3734	0,00	0,93	0,00
	0,75	0,87	4,1147	420	3,2571	0,06	0,87	8,89
	24	0,19	91,8001	405	3,1408	0,74	0,19	502,22
S2-Kontrol	0,01	7,5	-687,1636	450	4,2030	-6,50	7,5	-92,72
	0,25	0,91	18,4038	450	4,2030	-0,12	1,12	-11,03
	0,5	0,93	16,2625	435	4,0629	0,02	0,98	2,69
	0,75	0,87	22,6865	420	3,9228	0,12	0,88	15,17
	24	0,19	95,4916	405	3,7827	0,24	0,76	34,47

26. Jun 13	Time	$C_{aq}^{ads}(eq)$	$C_{s}^{ads}(eq)$	V_o	m_{soil}	$m_{s}^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d
pH 3	(h)	(mg/l)	(mg/g)	(l)	(g)	(mg/l)	(mg/l)	(ml/g)
S1-Cit	0,01	7,5	-259,5832751	300	3,4368	-3,65	7,5	-42,47
	0,25	4,56	-2,949196927	300	3,4368	-0,71	4,56	-13,57
	0,5	4,58	-4,695006983	285	3,26496	-0,73	4,58	-13,89
	0,75	4,52	0,542423184	270	3,09312	-0,67	4,52	-12,92
	24	4,45	6,65275838	255	2,92128	-0,60	4,45	-11,75
S1-SO	0,01	7,5	-450,5985889	300	2,4237	-4,09	7,5	-67,56
	0,25	4,54	-84,21661097	300	2,4237	-1,13	4,54	-30,90
	0,5	4,71	-105,2588192	285	2,302515	-1,30	4,71	-34,25
	0,75	4,44	-71,83884144	270	2,18133	-1,03	4,44	-28,81
	24	7,29	-424,6052729	255	2,060145	-3,88	7,29	-65,94
S1-HCl	0,01	7,5	-384,4941707	300	2,3931	-3,71	7,5	-62,03
	0,25	5,02	-73,60035101	300	2,3931	-1,23	5,02	-30,74
	0,5	4,82	-48,52826877	285	2,273445	-1,03	4,82	-26,82
	0,75	4,86	-53,54268522	270	2,15379	-1,07	4,86	-27,63
	24	6,18	-219,018428	255	2,034135	-2,39	6,18	-48,50
S1-KCl	0,01	7,5	-524,0790453	300	2,7402	-4,86	7,5	-70,91
	0,25	2,04	73,68754106	300	2,7402	0,60	2,04	32,32
	0,5	1,84	95,58375301	285	2,60319	0,80	1,84	47,73
	0,75	1,58	124,0488286	270	2,46618	1,06	1,58	73,60
	24	0,57	234,6246989	255	2,32917	2,07	0,57	398,02
S1-Kontrol	0,01	7,5	-458,9330622	300	3,0978	-4,83	7,5	-62,31
	0,25	2,04	69,8292853	300	3,0978	-0,18	2,85	-5,97
	0,5	1,84	89,19786946	285	2,94291	-0,47	3,14	-14,37
	0,75	1,58	114,3770289	270	2,78802	-0,25	2,92	-8,15
	24	0,57	212,1883788	255	2,63313	0,52	2,15	23,61

Lüneburg								
30. Aug 13	Time	$C_{aq}^{ads}(eq)$	$C_{s}^{ads}(eq)$	V_o	m_{soil}	$m_{s}^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d
pH 3	(h)	(mg/ml)	(mg/g)	(ml)	(g)	(mg/l)	(mg/l)	(ml/g)
BS-Control	0,01	0,0075	257,9010	375	3,68	-4,97	7,5	-67,51
	0,25	0,0122	257,4184	375	3,68	-9,71	12,24	-80,80
	0,5	0,0098	257,6719	360	3,54	-7,22	9,75	-75,43
	0,75	0,0083	257,8247	345	3,39	-5,72	8,25	-70,63
	24	0,0022	258,4397	330	3,24	0,32	2,21	14,61
BS-Cit	0,01	0,0075	450,5556	300	2,69	-3,97	7,5	-59,07
	0,25	0,0694	443,6501	300	2,69	-65,85	69,38	-105,92
	0,5	0,0705	443,5251	285	2,55	-66,97	70,5	-106,01
	0,75	0,0753	442,9873	270	2,42	-71,79	75,32	-106,36
	24	0,1038	439,8079	255	2,29	-100,28	103,81	-107,80
BS-KCl	0,01	0,0075	251,2537	300	3,17	-4,89	7,5	-61,81
	0,25	0,0304	249,0824	300	3,17	-27,81	30,42	-86,62
	0,5	0,0285	249,2671	285	3,01	-25,86	28,47	-86,06
	0,75	0,0222	249,8649	270	2,85	-19,55	22,16	-83,59
	24	0,0015	251,8259	255	2,69	1,15	1,46	74,40
RS-Kontrol	0,01	0,0075	281,616	600	5,82	-4,84	7,5	-66,52
	0,25	0,0130	281,053	600	5,82	-10,30	12,96	-81,93
	0,5	0,0147	280,877	585	5,68	-12,01	14,67	-84,39
	0,75	0,0153	280,808	570	5,53	-12,68	15,34	-85,21
	24	0,0006	282,324	555	5,38	2,03	0,63	332,12
RS-Cit	0,010	0,008	1157,485	450	3,59	-0,50	7,50	-8,42
	0,250	0,063	1150,476	450	3,59	-56,43	63,43	-111,51
	0,500	0,061	1150,740	435	3,47	-54,32	61,32	-111,03
	0,750	0,066	1150,192	420	3,35	-58,69	65,69	-111,98
	24,000	0,088	1147,364	405	3,23	-81,26	88,26	-115,39
RS-KCl	0,01	0,01	464,53	600	5,59	-3,77	7,50	-53,97
	0,25	0,04	461,50	600	5,59	-31,99	35,72	-96,06
	0,50	0,04	461,48	585	5,45	-32,23	35,96	-96,14
	0,75	0,03	461,72	570	5,31	-29,98	33,71	-95,40
	24,00	0,00	464,96	555	5,17	0,27	3,46	8,25

APPENDIX 4: : RELATIONSHIP BETWEEN THE SOIL/SOLUTION RATIO, SORPTION PERCENTAGE (A%) AND DISTRIBUTION COEFFICIENT (Kd)

Sampling date	Reactor	Time	V_o	m_{soil}	P cc initial	$m_s^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d	A	Soil/Solution
		(h)	(l)	(g)	(mg/l)	(mg/l)	(mg/l)	(ml/g)	(%)	(g/ml)
22 May 2013	S1-KCl	0,01	300	3,9021	0,9922	-6,5078	7,5000	-66,7105	-6,5588	0,0130
		0,25	300	3,9021	0,9922	0,6422	0,3500	141,0718	0,6473	0,0130
		0,5	285	3,7070	0,9922	0,7122	0,2800	195,5601	0,7178	0,0130
		0,75	270	3,5119	0,9922	0,7622	0,2300	254,7866	0,7682	0,0130
		24	255	3,3168	0,9922	0,7122	0,2800	195,5601	0,7178	0,0130
	S1-Control	0,01	300	4,1550	1,4944	-6,0056	7,5000	-57,8152	-4,0186	0,0139
		0,25	300	4,1550	1,4944	-0,1156	1,6100	-5,1822	-0,0773	0,0139
		0,5	285	3,9473	1,4944	0,0044	1,4900	0,2154	0,0030	0,0139
		0,75	270	3,7395	1,4944	-0,0256	1,5200	-1,2139	-0,0171	0,0139
		24	255	3,5318	1,4944	0,1044	1,3900	5,4253	0,0699	0,0139
	S2-KCl	0,01	450	3,4898	0,9300	-6,5700	7,5000	-112,9594	-7,0645	0,0078
		0,25	450	3,4898	0,9300	0,0200	0,9100	2,8340	0,0215	0,0078
		0,5	435	3,3734	0,9300	0,0000	0,9300	0,0000	0,0000	0,0078
		0,75	420	3,2571	0,9300	0,0600	0,8700	8,8930	0,0645	0,0078
		24	405	3,1408	0,9300	0,7400	0,1900	502,2227	0,7957	0,0078
	S2-Control	0,01	450	4,2030	1,0047	-6,4953	7,5000	-92,7242	-6,4652	0,0093
		0,25	450	4,2030	1,0047	-0,1153	1,1200	-11,0253	-0,1148	0,0093
		0,5	435	4,0629	1,0047	0,0247	0,9800	2,6949	0,0246	0,0093
		0,75	420	3,9228	1,0047	0,1247	0,8800	15,1677	0,1241	0,0093
		24	405	3,7827	1,0047	0,2447	0,7600	34,4679	0,2435	0,0093

Sampling date	Reactor	Time	V_o	m_{soil}	P cc initial	$m_s^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d	A	Soil/Solution
		(h)	(l)	(g)	(mg/l)	(mg/l)	(mg/l)	(ml/g)	(%)	(g/ml)
26. Jun. 13	S1-KCl	0,01	300	2,7402	2,6422	-4,8578	7,5000	-70,9113	-1,8385	0,0091
		0,25	300	2,7402	2,6422	0,6022	2,0400	32,3196	0,2279	0,0091
		0,5	285	2,6032	2,6422	0,8022	1,8400	47,7327	0,3036	0,0091
		0,75	270	2,4662	2,6422	1,0622	1,5800	73,6033	0,4020	0,0091
		24	255	2,3292	2,6422	2,0722	0,5700	398,0159	0,7843	0,0091
	S1-Control	0,01	300	3,0978	2,6742	-4,8258	7,5000	-62,3123	-1,8046	0,0103
		0,25	300	3,0978	2,6742	-0,1758	2,8500	-5,9729	-0,0657	0,0103
		0,5	285	2,9429	2,6742	-0,4658	3,1400	-14,3654	-0,1742	0,0103
		0,75	270	2,7880	2,6742	-0,2458	2,9200	-8,1513	-0,0919	0,0103
		24	255	2,6331	2,6742	0,5242	2,1500	23,6127	0,1960	0,0103
	S2-KCl	0,01	450	3,1469	2,5347	-0,1453	2,6800	-7,7547	-0,0573	0,0070
		0,25	450	3,1469	2,5347	0,0047	2,5300	0,2638	0,0018	0,0070
		0,5	435	3,0420	2,5347	0,2247	2,3100	13,9080	0,0886	0,0070
		0,75	420	2,9371	2,5347	0,5647	1,9700	40,9885	0,2228	0,0070
		24	405	2,8322	2,5347	1,8947	0,6400	423,3400	0,7475	0,0070
	S2-Control	0,01	450	4,7084	2,5200	-0,3100	2,8300	-10,4693	-0,1230	0,0105
		0,25	450	4,7084	2,5200	-0,2600	2,7800	-8,9387	-0,1032	0,0105
		0,5	435	4,5514	2,5200	-0,1500	2,6700	-5,3694	-0,0595	0,0105
		0,75	420	4,3945	2,5200	-0,1700	2,6900	-6,0400	-0,0675	0,0105
		24	405	4,2375	2,5200	0,2700	2,2500	11,4690	0,1071	0,0105

Sampling date	Reactor	Time	V_o	m_{soil}	P cc initial	$m_s^{ads}(eq)$	$m_{aq}^{ads}(eq)$	K_d	A	Soil/Solution
		(h)	(l)	(g)	(mg/l)	(mg/l)	(mg/l)	(ml/g)	(%)	(g/ml)
Lüneburg WWTP	BS-Control	0,01	375	3,6829	2,5271	-4,9729	7,5000	-67,5143	-1,9679	0,0098
		0,25	375	3,6829	2,5271	-9,7129	12,2400	-80,8004	-3,8436	0,0098
		0,5	360	3,5356	2,5271	-7,2229	9,7500	-75,4316	-2,8582	0,0098
		0,75	345	3,3882	2,5271	-5,7229	8,2500	-70,6332	-2,2647	0,0098
		24	330	3,2409	2,5271	0,3171	2,2100	14,6084	0,1255	0,0098
	BS-KCl	0,01	300	3,1668	2,6067	-4,8933	7,5000	-61,8079	-1,8772	0,0106
		0,25	300	3,1668	2,6067	-27,8133	30,4200	-86,6153	-10,6701	0,0106
		0,5	285	3,0085	2,6067	-25,8633	28,4700	-86,0593	-9,9220	0,0106
		0,75	270	2,8501	2,6067	-19,5533	22,1600	-83,5895	-7,5013	0,0106
		24	255	2,6918	2,6067	1,1467	1,4600	74,4021	0,4399	0,0106
	RS-Control	0,01	600	5,8206	2,6598	-4,8402	7,5000	-66,5248	-1,8197	0,0097
		0,25	600	5,8206	2,6598	-10,3002	12,9600	-81,9263	-3,8725	0,0097
		0,5	585	5,6751	2,6598	-12,0102	14,6700	-84,3923	-4,5154	0,0097
		0,75	570	5,5296	2,6598	-12,6802	15,3400	-85,2086	-4,7673	0,0097
		24	555	5,3841	2,6598	2,0298	0,6300	332,1245	0,7631	0,0097
	RS-KCl	0,01	600	5,5944	3,7261	-3,7739	7,5000	-53,9663	-1,0128	0,0093
		0,25	600	5,5944	3,7261	-31,9939	35,7200	-96,0623	-8,5863	0,0093
		0,5	585	5,4545	3,7261	-32,2339	35,9600	-96,1370	-8,6508	0,0093
		0,75	570	5,3147	3,7261	-29,9839	33,7100	-95,3952	-8,0469	0,0093
		24	555	5,1748	3,7261	0,2661	3,4600	8,2494	0,0714	0,0093