PHOSPHORUS RELEASE DURING TREATMENT OF SLUDGE DERIVED FROM A BENCH-SCALE EBPR PLANT

by

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To my parents Dimitri and Maria Belia for the genes and the upbringing they gave me and for making this possible

and to Duncan for being there

Declaration

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ABSTRACT

This thesis describes the development of enhanced biological phosphorus removal (EBPR) in a lab-scale sequencing batch reactor (SBR) and the release of phosphorus during the storage and thickening of sludge produced in this reactor. In the first phase of the experimental work a fast start-up method for EBPR development was established by the addition of a pure culture of *Acinetobacter lwoffi* to a conventional activated sludge. Investigations revealed that the performance EBPR depended on the combination of influent COD and phosphorus values and that in the investigated range, EBPR functioned independently of the sludge retention time. Low dissolved oxygen levels had no effect on the phosphorus removal properties of the sludge. The second phase of the experimental work involved the investigation of the phosphorus released during sludge handling. It was found that phosphorus resolubilisation during sludge treatment took place in three distinct phases which included an initial period of extremely low phosphorus release. Alterations of the reactor influent and operational parameters and the sludge characteristics, affected the amount of phosphorus released during anaerobic storage and gravity thickening. It was found that for short retention times in the sludge processing units (1-48 hours), decreasing the influent phosphorus concentration, increasing the oxidised nitrogen content of the excess sludge and wasting the excess sludge from the aeration tank decreased the amount of phosphorus resolubilised. For longer retention times (2-7 days), it was found that increasing the influent COD, having a lower total phosphorus sludge content, higher sludge "stabilisation" rates and quiescent conditions of storage, decreased the amount of phosphorus released.

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CHAPTER 1. INTRODUCTION

1.1. PROBLEM DEFINITION

1.1.1. Nutrients and the Problem of Eutrophication

Eutrophication can be defined as the natural ageing of water bodies. The slow input of nutrients into lakes, reservoirs and rivers, causes the increase of algae and plant life and subsequently the depletion of dissolved oxygen. The process is accelerated by the discharge of increased concentrations of nutrients derived from man related activities. Both phosphorus and nitrogen are often the limiting factors for biological growth in natural waters. However, in practice, growth prevention only needs a lowering of the phosphorus availability as cyanobacteria are capable of fixing molecular nitrogen, thus eliminating the requirements for ammonia or nitrate for their growth (Kortstee *et al.*, 1994). Therefore, the limiting nutrient in most lakes, rivers and reservoirs is phosphorus and even small amounts of this nutrient can initiate eutrophication with undesirable consequences such as the death of fish, algal blooms, toxins in potable water supplies, etc. The European Union guideline on effluent phosphorus, as stated in the Urban Wastewater Directive (CEC 1991), stands at 1 mg/l (100,000 pe).

The sources of phosphorus include agricultural run-off (from phosphorus rich fertilised land), excreta from livestock, municipal and industrial effluents (human waste contains orthophosphate and organic phosphorus compounds and both

domestic and industrial waste contain condensed phosphates originating from detergents), atmospheric deposition and diffusion of urban drainage. The above can be classified as point and non-point sources. Point sources, i.e. wastewater effluents, are the ones that can be most effectively controlled and their contribution to the eutrophication of water bodies limited. The elimination of phosphorus from domestic wastewater has proven to be an important step in the control of eutrophication.

1.1.2. Enhanced Biological Phosphorus Removal (EBPR) and the Problem of Handling EBPR Sludges

The efficient removal of phosphorus can be currently achieved by both chemical and biological methods, but biological methods are becoming increasingly attractive due to their low operational cost, low sludge production and the fertiliser value of the produced sludge.

Enhanced biological phosphorus removal (EBPR) is a combination of microbial action, precipitation and adsorption, depending on the process and operating conditions and relies on the fact that a group of microorganisms present in activated sludge can uptake phosphorus in excess of their growth requirements. The fundamental metabolic process that takes place during EBPR includes the release of phosphorus under anaerobic conditions, with the use of low molecular organics, followed by uptake and storage of phosphorus under aerobic conditions.

The biological phosphorus removal process can, in combination with a chemical precipitation polishing stage, meet any effluent standards set. The problems that the process poses to operators are mainly influent related but sludge handling is also a considerable concern.

Nutrient removal plants operating with the EBPR process, produce phosphorus laden sludges. Care must be taken to ensure minimal release of phosphorus from the solid to the liquid phase during sludge handling and disposal.

Phosphorus release during sludge treatment has been minimally investigated and the existing data includes sparse quantitative results on the effect of plant influent or

operational parameters. It is the aim of this study to investigate the phosphorus release potential of EBPR sludges during the handling of excess sludge and to determine whether any of the influent or operational parameters of a treatment plant affect the amount of phosphorus resolubilised during sludge treatment.

1.2. OVERVIEW OF THE STUDY

1.2.1. Objectives of the Study

For the achievement of the main aim of the study, as stated above, the experimental work was divided into two phases and a number of objectives were set.

The first phase of the experimental work dealt with the development of EBPR and had two objectives. The first was to establish a fast start-up method for the development of enhanced biological phosphorus removal in a lab-scale sequencing batch reactor (SBR) and following this, to operate the SBR in varying modes, with the intention to correlate the phosphorus removed and that released in the anaerobic zone with the parameters altered.

The second phase of the experimental work dealt with the problem of phosphorus resolubilisation during sludge treatment and had three main objectives. The first objective was to establish the different phases describing phosphorus release during sludge handling. The second was to determine any difference in the phosphorus release observed between the sludge handling methods employed. The final objective was to determine the effect of the reactor influent and operational parameters and the sludge characteristics on the amount of phosphorus released during sludge handling.

In this study the sludge handling methods investigated were quiescent anaerobic storage and gravity thickening. Anaerobic storage was selected as it routinely takes place at some stage in a treatment plant and gravity thickening as it is a popular and widely used dewatering method. Additional experiments employing fully mixed aerobic storage and fully mixed anaerobic storage were also performed. Other sludge

treatment processes such as anaerobic sludge digestion or dissolved air flotation, were not investigated because of the difficulty of simulating them in lab-scale conditions and because of the short time scale of this project.

1.2.2. Organisation of the Chapters

Following this brief introductory chapter, a review of the literature is presented in chapter 2. The purpose of the review is first to outline the principles of biological phosphorus removal and second to focus the reader on the specific aspects of the process that influence the release of phosphorus during the handling of phosphorus laden biological sludges.

Chapter 3 details the set-up and operation of the laboratory reactors used for the development of EBPR and for the sludge treatment experiments. The analytical and microbiological techniques used are also described.

The results obtained in this study have been included in chapters 4 and 5. Chapter 4 describes the development of enhanced biological phosphorus removal in the lab sequencing batch reactor during start-up and the operation of the reactor for the development of the excess sludge used in the thickening and storage experiments that followed.

Chapter 5 discusses the process of phosphorus resolubilisation during the treatment of excess sludge. As already mentioned, the sludge handling methods investigated were quiescent anaerobic storage and gravity thickening. For the two methods, the amount of phosphorus resolubilised with increasing retention time was compared. The phosphorus released, for each of the sludge handling methods, from sludges that had been derived from runs with different operational parameters was also investigated.

Finally, a summary of the results obtained in this study is presented in the concluding chapter.

CHAPTER 2. LITERATURE REVIEW

2.1. INTRODUCTION

The purpose of this chapter is first to outline the principles of biological phosphorus removal and second to focus the reader on the specific aspects of the process that influence the release of phosphorus during the handling of phosphorus laden biological sludges.

It is not intended that an extensive literature review of biological phosphorus removal is presented here. For comprehensive reviews of the process the reader is referred to Fuhs and Chen (1975), Yeoman *et al.* (1988a) and Toerien *et al.* (1990). Also for the existing full scale process configurations the book Design and Retrofit of Wastewater Treatment Plants for Biological Nutrient Removal (1992), is suggested.

Comprehending the mechanisms that induce phosphorus release in the sludge stream requires an overall understanding of biological phosphorus removal and particularly the function of the anaerobic zone. Therefore, after a short historic perspective of the development of enhanced biological phosphorus removal (EBPR), the parameters which are thought to affect phosphorus release will be reviewed.

As literature on the specific subject of phosphorus release during the handling of EBPR sludges is scarce, a large part of this chapter deals with the biological phosphorus removal process itself. The author considers this to be crucial for the understanding of the philosophy behind the design of the experiments to follow and the interpretation of the results obtained.

2.2. HISTORIC PERSPECTIVE OF ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

The first detailed laboratory investigation into enhanced biological phosphorus removal (EBPR), has been attributed to Srinath *et al.* (1959). The authors noted that in the activated sludge systems investigated, more phosphorus was removed than could be accounted for by normal bacterial growth. They also observed that the magnitude of the removal appeared to be linked to the intensity of aeration. In 1965, Levin and Shapiro reported on an extensive investigation into phosphorus uptake and release. They proposed a biochemical mechanism explaining the phenomenon on the basis of biological uptake. They speculated that the metabolic pathways employed were normal of aerobic organisms. They were the first to associate volutin granules as a potential intracellular storage place for the unaccounted for phosphorus. They also observed that for aerated sewage samples carbon addition resulted in higher phosphorus uptake and that aerated sludge took up phosphorus while unaerated sludge released.

Further evidence on the biological nature of the phenomenon was provided by other early researchers who employed biochemical inhibitors and induced phosphorus release (Sekikawa *et al.*, 1967). Around this time the detrimental effect of nitrate in the anaerobic zone was also noted (Barnard, 1974).

So far, the understanding of the process of EBPR extended little beyond the recognition of (i) the necessity of an anaerobic/aerobic sequence in the system and (ii) the adverse influence of nitrate recycled to the anaerobic zone. The reasons for the above were not recognised but experimental data suggested that the presence of an anaerobic zone stimulated EBPR and that nitrate recycled to the anaerobic phase significantly reduced it (Barnard, 1974, 1975a,b, 1976; Nicholls, 1975; Davelaar *et al.*, 1976).

Having developed system configurations in which it was possible to minimise nitrate recycle into the anaerobic phase, e.g. with nitrification-denitrification systems

(Barnard, 1974), researchers proceeded to develop nitrification denitrification kinetic models and applied the simplified steady state denitrification theory to nitrification-denitrification enhanced biological phosphorus removal (NDEBPR) processes (Dold *et al.*, 1980; Ekama *et al.*, 1983).

Investigations into full scale plants, identified two key parameters which appeared to influence EBPR systems: the anaerobic sludge mass fraction and the concentration of readily biodegradable soluble substrate (RBCOD) in the anaerobic zone (Rabinowitz and Oldham, 1986; Siebritz *et al.*, 1980).

It was observed that by increasing the anaerobic sludge mass fraction an increase in EBPR is observed but at a decreasing rate, and that an increase in the RBCOD concentration led to an increase in EBPR.

These parameters also clarified the detrimental effect of nitrate in the anaerobic zone. It was concluded that if nitrate is present in the anaerobic phase, the soluble fraction of the substrate was utilised preferably with nitrate as the electron acceptor causing a reduction in RBCOD concentration and therefore a reduction in EBPR. These concepts were used at this stage for the development of design procedures which gave empirical estimations of the above two parameters and the mass of sludge (active, endogenous and inert) wasted per day (Siebritz *et al.*, 1983; Ekama *et al.*, 1983).

Around this stage, parallel research had identified specific organism groups that stored inorganic polyphosphate in intracellular storage granules. The approach to EBPR was thus shifted and from considering the active sludge mass as a whole, specific organism groups mediating EBPR were studied, termed polyphosphate organisms (recommended term: phosphorus accumulating organisms, IAWPRC Task Group, 1991).

The first to suggest that specific bacteria were responsible for phosphorus removal in wastewater plants were Fuhs and Chen who in 1975 isolated and identified them as *Acinetobacter* spp. The presence and importance of these bacteria was firmly established by Buchan (1981, 1983), Deinema *et al.* (1980), Brodisch and Joyner (1983), and many others since. A variety of other microorganisms has been implicated

since then and in addition to this the quantitative importance of *Acinetobacter* spp. has been questioned. The subject is still under investigation.

The next advancement came with the establishment of biochemical models (Marais *et al.*, 1983; Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Mino *et al.*, 1987), which laid down the main metabolic pathways involved. Over the past decade a consensus has been reached regarding many of the key features of the behaviour of the organisms mediating EBPR. However some issues regarding specific metabolic aspects of the process are still to be resolved and research is continuing.

In the last five years, research has focused on resolving the remaining inconsistencies in the biochemical pathways involved (Smolders *et al.*, 1995a; Matsuo *et al.*, 1992; Jenkins and Tandoi, 1991), on identifying more of the organisms responsible (Auling *et al.*, 1991; Knight *et al.*, 1995a) and incorporating the latest research finds into a general activated sludge kinetic model (Gujer *et al.*, 1995; Henze *et al.*, 1995).

2.3. OVERVIEW OF THE MAIN PARAMETERS INVOLVED IN EBPR

2.3.1. The Anaerobic Zone

2.3.1.1. The role of the anaerobic zone

The primary prerequisite for obtaining biological phosphorus removal is the re-circulation of the sludge through first an anaerobic and then an aerobic zone. Barnard (1975b, 1976) was the first who explicitly stated that for excess phosphorus uptake the mixed liquor needs at some point in the process to be subject to anaerobic conditions (absence of both dissolved oxygen and nitrate). The reason for incorporating an anaerobic zone into the EBPR process has been the subject of intense speculation.

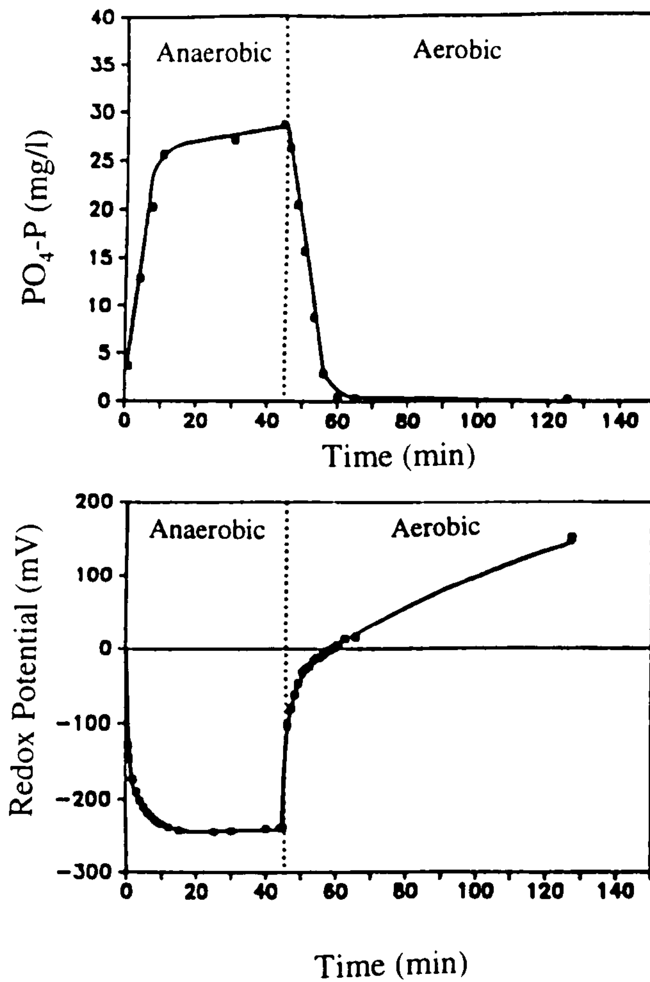


Fig. 2.1. Typical anaerobic-aerobic ORP and phosphorus profiles, during EBPR (from Tracy and Flammino, 1987).

It has been thought that it operates as a stress zone for the microbial population, resulting in enzyme reactions similar to those during nutrient imbalances which lead to polyphosphate formation (Nicholls and Osborn, 1979).

Fuhs and Chen (1975), suggested that the anaerobic zone serves as a fermentation period for acetogenic bacteria which convert complex compounds into low molecular fatty acids used by phosphorus accumulating bacteria.

Although it still remains to be proved, it is at the moment generally accepted, that the anaerobic zone functions as follows. Facultative aerobic organisms convert part of the influent COD to low molecular fatty acids. Polyphosphate accumulating bacteria take them up and store them as poly-β-hydroxyalkanoates, mainly poly-β-hydroxybutyrate (PHB). The energy for this transport is supplied by the hydrolysis of the intracellular glycogen and polyphosphate which is thus released to the bulk liquid. There is a strong correlation between phosphorus release and fatty acid uptake, although published ratios vary from 0.25 to 0.75 P-mol/C-mol (Jenkins and Tandoi, 1991).

Due to this anaerobic uptake of fatty acids, polyphosphate accumulating bacteria acquire an advantage over the rest of the strict aerobes as they have access to substrate under anaerobic conditions (Marais *et al.*, 1983; Comeau *et al.*, 1986; Gerber *et al.*, 1987; Wentzel *et al.*, 1985, 1986; Smolders *et al.*, 1994).

Over the years research has pointed out certain parameters that are thought to effect the anaerobic release of phosphorus. The most important ones follow.

2.3.1.2. Oxygen redox potential

The oxygen redox potential (ORP) has been cited as being an important parameter regulating phosphorus release in the anaerobic stage. Evidence to support this has been given by Marais *et al.* (1983), who noted that phosphorus was not released immediately after the dissolved oxygen concentration reached zero, indicating that redox potential is the parameter that needs to be low enough for release to be initiated. Rapid release appears to be triggered off once the redox

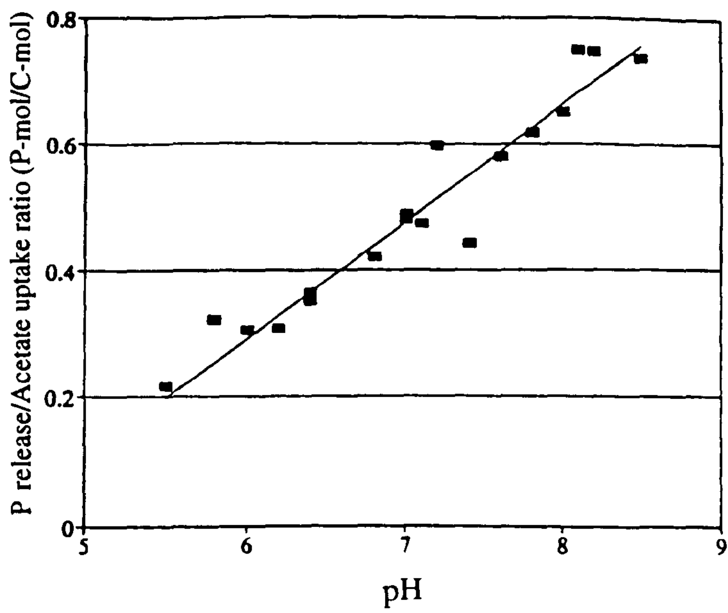


Fig. 2.2. Phosphorus release as a function of the pH (from Smolders *et al.*, 1994).

Rensink *et al.* (1981), correlated the presence of lower fatty acids with the redox state in the anaerobic reactor. The lower the redox potential the more the growth of facultative anaerobes will be promoted in the system and the greater the production of lower fatty acids and therefore the higher the phosphorus removal.

Conflicting results were published by Randal *et al.* (1970) and Schön *et al.* (1993), who concluded that there is no evidence to support the hypothesis that redox potential has a direct influence on inducing phosphorus release.

2.3.1.3. The role of the anaerobic sludge mass fraction

The anaerobic mass fraction was implicated in the phosphorus removal process with the work of Siebritz *et al.* (1980, 1983). It is defined as the ratio of the mass of sludge in the anaerobic reactor to the total mass of sludge in the system. The authors identified it as one of the main factors affecting the release and consequent uptake of phosphorus in EBPR systems.

Extensive testing of the concepts has verified this approach validating the hypothesis that the greater the fraction of total mass in the anaerobic reactor, the greater the net phosphorus removal (Marais *et al.*, 1983).

2.3.1.4. pH

Recently the effect of the pH in the anaerobic zone has been shown to affect phosphorus release (figure 2.2.). At low pH, the energy required for acetate uptake is less than at high pH. The implications are that at low pH less of the intracellularly stored phosphorus is needed, therefore the rest could be used for other purposes such as an increase in biomass yield. This could mean a higher fraction of polyphosphate accumulating organisms in the process (Smolders *et al.*, 1994).

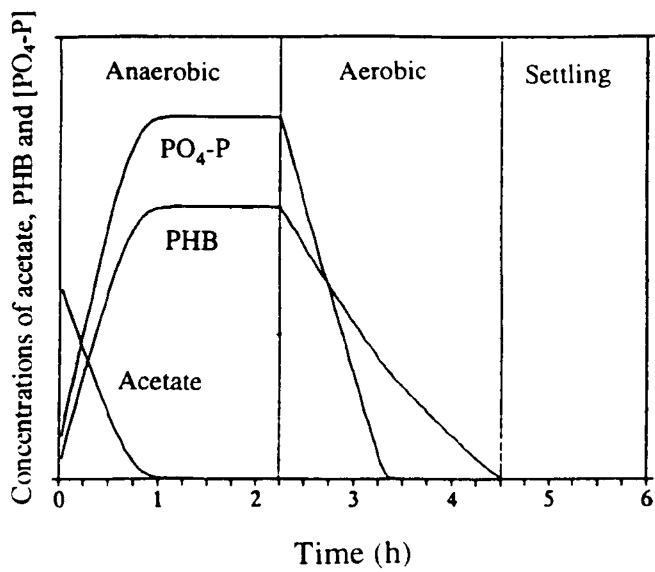


Fig. 2.3. Phosphorus, acetate and PHB concentrations during a full cycle in a sequencing batch process (from Smolders *et al.*, 1994).

2.3.2. The Aerobic Zone

The metabolic pathways involved in the aerobic phase are much less studied even though the actual removal of phosphorus takes place in this stage. The anaerobically stored PHB is broken down and is used for cell growth and for polyphosphate and glycogen synthesis (figure 2.3.). This results in the uptake of phosphorus from the liquid.

2.3.3. Effect of Nitrate

2.3.3.1. Effect of nitrate in the anaerobic zone

The detrimental effect of nitrate in the anaerobic zone is well documented (Barnard, 1976; Manning and Irvine, 1985; Hascoet *et al.*, 1985b; Van Groenestijn and Deinema, 1985). Several explanations have been offered for the observed decrease in phosphorus release when oxidised nitrogen is present.

With denitrification there is the increased potential of phosphorus precipitation (Arvin and Kristensen, 1983; Hascoet and Florentz, 1985a). It has also been speculated that the enzymes involved in the release of phosphorus could, with the presence of nitrate, be inhibited (Lötter, 1984; Lötter and Van der Merwe, 1987). Another implication is that the readily available substrates will be consumed for denitrification rather than phosphorus storage (Comeau *et al.*, 1986; Mostert *et al.*, 1988). A fourth reason given in the literature is the possible increase in the redox potential above levels necessary for phosphorus release (Peirano *et al.*, 1983). Finally the presence of denitrifying bacteria which also accumulate large quantities of phosphorus has been cited (Shin *et al.*, 1992a; Auling *et al.*, 1991; Koch and Oldham, 1985; Vlekke *et al.*, 1988).

There is not enough experimental evidence to point conclusively to any one of the above reasons. Phosphorus precipitation alone cannot explain the reduction of phosphorus in the presence of nitrate (Appeldoorn *et al.*, 1992) and the variable effects observed in full scale plants also support the proposal that it is probably not

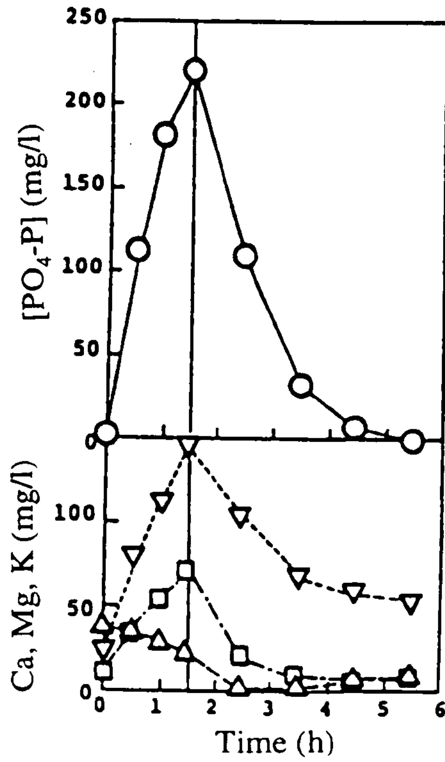


Fig. 2.4. Uptake and release of cations and phosphorus under anaerobic and aerobic conditions. Phosphorus (O), Calcium (Δ), Magnesium (□) and Potassium (∇) (from Miya *et al.*, 1987).

the nitrate induced increase in the ORP that has an inhibitory effect on phosphorus release (Schön *et al.*, 1993). It seems that the behaviour of the sludge during anaerobic conditions in the presence of nitrate is affected by a combination of the above mentioned parameters.

Other researchers have tried to quantify the effect. Hascoet *et al.* (1985b) for example, concluded that occasional addition of nitrate during the anaerobic phase has a minor effect on the phosphorus removal process whereas continuous addition deteriorates the process. Osborn and Nicholls (1978) cited as an acceptable concentration less than 2 mg/l and Raper (1983), suggested a concentration below 5 mg/l.

2.3.3.2. Anoxic release and uptake of phosphorus

Hascoet *et al.* (1985b), observed phosphorus release in the anoxic phase when adequate COD was present. According to these researchers the anoxic release may lie in the following factors: (a) the nitrate oxygen is masked by the presence of high concentrations of organic matter and (b) addition of substrate causes a drop in the ORP which triggers release.

The presence of adequate concentrations of VFAs has been quoted to assist phosphorus release when nitrates are present by other researchers too (Gerber *et al.*, 1986; Nicholls *et al.*, 1985).

Phosphorus uptake has also been shown to take place by the consumption of oxidised nitrogen instead of dissolved oxygen (Comeau *et al.*, 1986; Hascoet *et al.*, 1985b; Shin and Jun, 1992b). This observation suggests that a sub-population of the phosphorus accumulating bacteria is capable of nitrate reduction and/or denitrification (Comeau *et al.*, 1986; Lötter, 1985; Van Groenestijn and Deinema, 1985).

TABLE 2.1. MOLAR RATIOS OF CATIONS CO-TRANSPORTED WITH PHOSPHORUS

Reference	Cation/P ratio (mole/mole)				Direction of transport
	K^+/P	Mg^{2+}/P	Ca^{2+}/P	Sum/P[1]	
Miyamoto-Mills *et al.* (1983)	0.27	0.26	0.00	0.79	Release
Arvin & Kristensen (1985)	0.23	0.32	0.05	0.97	Release
Comeau *et al.* (1986)	0.34	0.24	0.06	0.94	Release/uptake
Comeau *et al.* (1986)	0.20	0.28	0.09	0.94	Release
Comeau *et al.* (1986)	0.24	0.27	0.12	1.01	Uptake
Fukase *et al.* (1984)	0.29	0.34	-	-	Release/uptake
Jardin and Pöpel (1994)	0.26	0.30	-	-	Release/uptake
Rickard and McClintock (1992)	0.21	0.30	-	-	Release/uptake
Wentzel *et al.* (1988)	0.30	0.26	-	-	Release/uptake

1. Sum = $K^+ + 2 \times (Mg^{2+} + Ca^{2+})$

2.3.4. The Role of Cations

Phosphorus uptake and release is accompanied by the uptake and release of mono and divalent metal ions (figure 2.4.) (Arvin and Kristensen, 1985; Comeau *et al.*, 1986).

Polyphosphate granules contain Ca^{2+}, Mg^{2+} and K^+ (Van Groenestijn and Deinema, 1985), which are used for the stabilisation of the electrochemical gradient across cell membranes and for the stabilisation of the charges of the polyanionic polyphosphates (Kulaev, 1979; Buchan, 1981, 1983). Mg^{2+} and K^+ are thought to be the most important ones, whereas Ca^{2+} is not thought to be equally significant for successful EBPR (Kortstee *et al.*, 1994; Rickard and McClintock, 1992).

From the literature, table 2.1. was assembled showing the observed molar ratios of cation and phosphorus co-transport (adapted from Comeau *et al.*, 1986). From table 2.1., it appears that on average one charge of transported phosphorus is neutralised by one of the cations Comeau *et al.* (1986). Similar results were given by Mino *et al.* (1987), who quoted an equivalent molar ratio of 2. Ratios in the range of 3.4 to 0.7 have been observed (Jenkins and Tandoi, 1991).

2.3.5. Biologically Mediated Chemical Precipitation

During the anaerobic phase in EBPR processes, excessive amounts of phosphorus are released into the liquid phase. It has been suggested that the increased phosphorus concentration may lead to chemical precipitation thus reducing the measured phosphorus released by the microbial population (Arvin 1983; Arvin and Kristensen, 1983; Beccari *et al.*, 1985; Miya *et al.*, 1987). Biologically mediated chemical precipitation may occur in two ways. Firstly, the elevated phosphorus concentrations created by anaerobic conditions can initiate and accelerate calcium phosphate precipitation. Secondly, biological denitrification can lead to phosphate precipitation by calcium, magnesium or iron, due to elevated pH inside the flocs (Arvin, 1983).

Levin and Shapiro (1965) first tried to determine the extend of co-precipitation with phosphorus uptake especially at high aeration rates, which were thought to strip CO_2 and raise pH thus possibly causing calcium precipitation. After conducting batch experiments at different pH levels, they concluded that chemical precipitation was not involved in biological phosphorus uptake. Menar and Jenkins (1969), suggested that 20 to 30% of the overall removal was due to metabolic reactions and the rest was due to chemical precipitation. Other researchers suggested that chemical precipitation takes place when the influent characteristics are suitable (350 mg/l calcium carbonate, 24 mg/l Mg, pH 7.5 - 8.5) and tried to determine the precipitation rates (Ferguson *et al.*, 1973). Bundgaard *et al.* (1983) and Lan *et al.* (1983), demonstrated the removal of phosphorus in activated sludge systems to be primarily due to chemical precipitation. Marais *et al.* (1983), commenting on these results, pointed out that there is evidence of a species of calcium phosphate precipitant with a low solubility product that forms at pH 7 to 8 but its rate of formation is extremely slow in the presence of organic material. Their conclusion that the mechanism is not physicochemical but mainly biological, has been verified by other investigators like, Barnard (1976), Buchan (1981) and Rensink *et al.* (1981).

In order to evaluate the mechanisms in operation in EBPR systems it is essential to fractionate the phosphorus compounds in the sludge. Thus mass balances of phosphorus, calcium, magnesium, iron and aluminium should be included in every study of the process (Appeldoorn *et al.*, 1992).

2.3.6. The Role of Readily Biodegradable COD (RBCOD)

Municipal influents contain two biodegradable COD fractions, a readily biodegradable soluble (RBCOD) and a slowly biodegradable particulate (SBCOD) fraction. RBCOD is the portion of the influent wastewater consisting of small, simple molecules that can pass directly through the cell wall for synthesis and oxidative metabolism by the organism. SBCOD is the portion of the influent that requires adsorption and extracellular breakdown before entering the cell (Dold *et al.*, 1980).

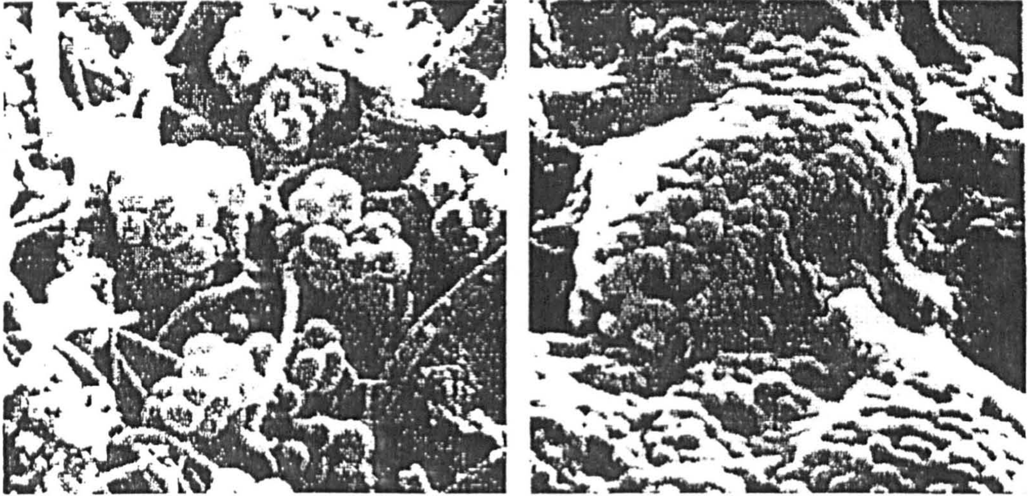
The magnitude of phosphorus removal is linked directly to the magnitude of RBCOD in the influent. In contrast SBCOD appears to have little influence on EBPR (Marais *et al.*, 1983). Indeed, according to Siebritz *et al.* (1980), it is the presence of readily biodegradable COD in the anaerobic reactor that induces phosphorus release and excess removal.

Low influent COD has been shown to adversely affect EBPR (Pitman *et al.*, 1983; Fukase *et al.*, 1985). When the RBCOD or volatile fatty acid (VFA) content of the sludge is high (greater then 100 mg/l) biological phosphorus removal does not present great problems. When that fraction of the influent drops below 50 mg/l the design of the plant must incorporate facilities to increase the RBCOD content of the feed (Pitman *et al.*, 1992). This can be done by the production of VFAs either in the mainstream process i.e. the anaerobic zone, or in side stream units like primary sedimentation tanks, thickeners or acid digesters (Nicholls *et al.*, 1985; Pitman *et al.*, 1983; Skalsky and Daigger, 1995; Rabinowitz and Oldham, 1986).

2.3.7. Organisms Involved in EBPR

2.3.7.1. The significance of *Acinetobacter* spp.

The first connection of EBPR with a change in microbial populations was made by Wells in 1967, when he stated that the sludge from plants that exhibited biological phosphorus removal was different to those that did not. The first to link biological phosphorus removal with specific types of organisms though, were Fuhs and Chen (1975). Microscopic investigations of sludge used in their batch experiments indicated that phosphorus accumulating organisms belonged to one bacterial strain. They identified it as being of the *Acinetobacter* genus. They postulated that the appearance of this bacterium was related to the anaerobic-aerobic sequence. Other researchers followed and *Acinetobacter* was identified as the genus responsible for EBPR (Fuhs and Chen, 1975; Buchan, 1981, 1983; Brodisch and Joyner, 1983; Hascoet *et al.*, 1985b).



(a) (b)

Fig. 2.5. SEM of phosphorus accumulating sludge floc, showing the diversity of *Acinetobacter* spp. sludge clusters. (a) small poorly organised clusters (from Beacham *et al.*, 1990. Magnification: x3500) and (b) larger tight clusters surrounded by capsular material (from Hascoet *et al.*, 1985b. Magnification x4800).

Bacteria belonging to this species are non-motile, gram-negative and strictly aerobic. They are short plump rods 1 to 1.5 μm by 1.5 to 2.5 μm in the logarithmic phase, approaching coccus shape in the stationary phase. They are predominantly found in pairs or short chains (Buchan, 1983). Lötter (1985), noticed that *Acinetobacter* spp. appeared to be covered by a thin layer of extracellular material which caused them to clump together (figure 2.5.). They are normal inhabitants of the human skin and may cause opportunistic and nosocomial infections (Wagner *et al.*, 1994). They are also found in soil, water and sewage. They accumulate phosphorus as polyphosphate in metachromatic granules which can occupy up to 60% of the cell volume (Buchan, 1983). *Acinetobacter* spp. require oxygen as electron acceptor (Juni, 1978), but more recently it has been shown that some strains can utilise nitrate as external electron acceptor (Lötter, 1985; Van Groenestijn and Deinema, 1985).

Comparison of sludges with different uptake capabilities indicated that intracellular polyphosphate accumulation is related to the tendency of the *Acinetobacter* cells to increase in size and aggregate into clusters. It also appears that the cells needed to undergo morphological and physiological changes when they developed the ability to remove phosphorus. The general pattern observed was for chains of small cells to develop into chains of larger cells which ultimately disintegrated to form clusters of very large cells containing massive (almost covering the total cell volume) quantities of polyphosphate (see figure 2.5.) (Buchan, 1983).

A very important point of the process is the fact that the polyphosphate accumulating organisms will be unable to utilise glucose or other high molecular weight carbon sources, directly, for the production of PHB under anaerobic conditions. They rely on other non-polyphosphate accumulating organisms to convert the complex fraction of the RBCOD to short chain fatty acids (SCFA) under anaerobic conditions.

More recently though the significance of *Acinetobacter* spp. in EBPR has been disputed. One of the reasons for doubt over the extent of their involvement, is the discrepancy found between the numbers of *Acinetobacter* spp. isolated from activated sludge by conventional plate count methods and estimations inferred from direct examinations by chemotaxonomic and immunochemical techniques. Conventional

methods have shown that *Acinetobacter* spp. represent a major part of the bacteria present in EBPR systems (Buchan, 1983; Deinema *et al.*, 1985; Fuhs and Chen, 1975). Chemotaxonomic and immunochemical data have indicated that they may constitute only 10% of the total biomass (Auling *et al.*, 1991; Cloete and Steyn, 1988; Hiraishi *et al.*, 1989). These results indicate that bacteria other than *Acinetobacter* spp. must be involved in EBPR (Wagner *et al.*, 1994). Other recent *in situ* identification methods have also shown that the community compositions determined by cultivation were dramatically different from the *in situ* compositions. The nutrient rich medium strongly favoured the growth of bacteria belonging to the class *Proteobacteria* and mostly *Acinetobacter* spp. Because of the strong selectivity of the presumably "non selective media" used in most studies, it was concluded that the importance of *Acinetobacter* spp. in EBPR had been overestimated (Wagner *et al.*, 1994).

Auling *et al.* (1991), using *in situ* identification methods of *Acinetobacter* spp. found a diverse spectrum of polyphosphate accumulating bacteria present in the sludges examined. By using electron microscopy, unicellular, multicellular (filamentous), gram-negative and gram-positive bacteria with polyphosphate granules were observed. 50% were allocated to the different genera and the other half were identified as *Acinetobacters* spp. The isolates clearly allocated to the genus *Acinetobacter* displayed the highest intracellular phosphorus storage in batch cultures with acetate or lactate as the substrate. The authors found evidence that the loading regime of the plant were the sample sludge was derived affected the population. In plants with a high organic loading and no nitrification-denitrification (F/M: 0.25 and 0.6 - 0.8 kgBOD/kg solids.d), members of the genus *Acinetobacter* represented only a minority of the population. In plants with low organic loading (F/M: 0.04 - 0.18 kgBOD/kg solids.d) considerably higher amounts (up to 50%) were found signifying that *Acinetobacter* spp. were enriched by this process mode. In these plants *Acinetobacter* constituted a major if not dominant fraction of the biomass.

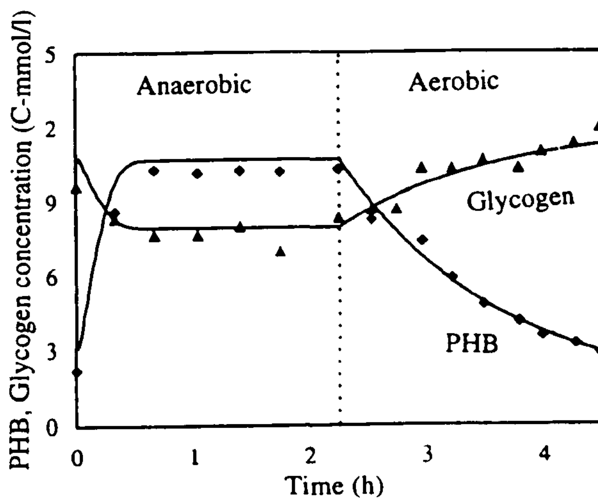


Fig. 2.6. PHB (♦) and glycogen (▲) concentration during anaerobic and aerobic conditions (from Smolders *et al.*, 1995a).

2.3.7.2. The involvement of other bacterial species

Polyphosphates reserves have been detected in many bacterial species isolated from activated sludge. Among them are: *Acinetobacter, Aeromonas, Azotobacter, Escheria coli, Nitrobacter, Nitrosomonas, Pseudomonas* (Kulaev, 1979; Lötter and Murphy, 1985). Certain species of actinomycetes, algae, fungi and protozoa can also accumulate phosphorus as shown by Kulaev (1979). It is only recently though, with the development of new isolation and identification techniques, that it has been established that some of these bacteria can accumulate polyphosphate at even higher quantities than *Acinetobacter* spp.

Among these bacteria are: *Pseudomonas* spp. (Brodisch and Joyner, 1983; Lötter, 1985; Suresh *et al.*, 1985), *M. Parvicella*, a filamentous, gram-positive bacterium (Wagner *et al.*, 1994), *Bacillus cereus* (Florentz and Hartemann, 1984) and an *Arthrobacter* isolate from soil which was found to accumulate 20% phosphorus per dry cell mass (Ohsumi *et al.*, 1980).

2.4. REVIEW OF METABOLIC PROCESSES INVOLVED IN EBPR

2.4.1. Carbon Metabolism

Bacterial carbon storage in EBPR occurs in the form of glycogen and poly-β-hydroxyalkanoates with poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV) as the most important ones (Dawes, 1992). The accumulation of PHB and/or glycogen has been observed in EBPR sludges under anaerobic conditions (Fuhs and Chen, 1975; Fukase *et al.*, 1984; Comeau *et al.*, 1987).

During the anaerobic period the amount of cellular glycogen decreases (Mino *et al.*, 1987) and PHB is stored. In the aerobic period glycogen is formed from PHB (figure 2.6.). In addition to glycogen formation, PHB also supports growth, phosphorus uptake and polyphosphate formation.

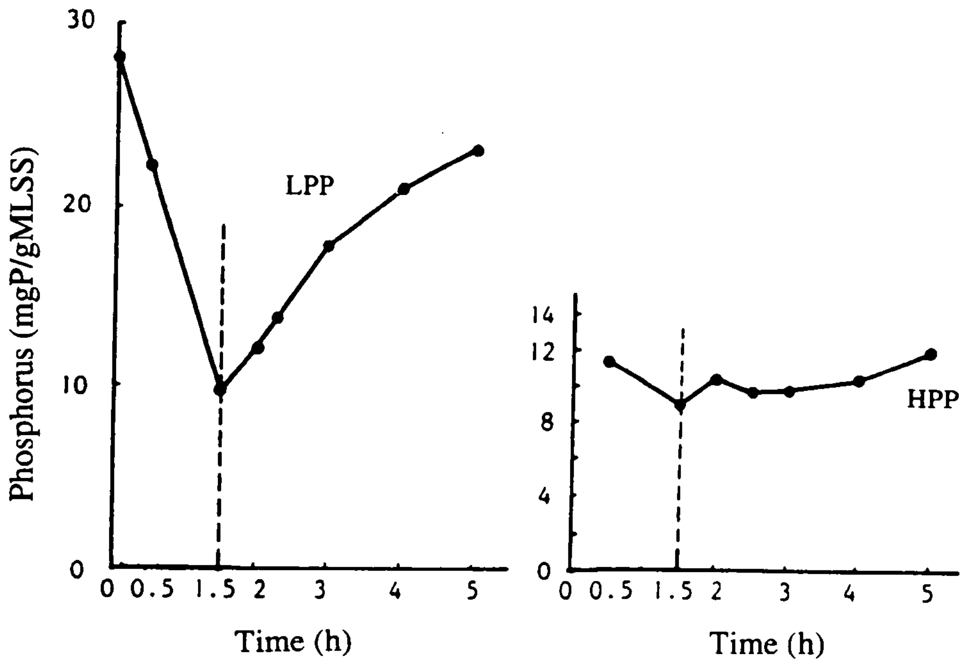


Fig. 2.7. Changes in low-molecular (LPP) and high-molecular (HPP) polyphosphate during anaerobic and aerobic conditions (adapted from Mino *et al.*, 1985).

2.4.2. Polyphosphate Metabolism in EBPR

2.4.2.1. Polyphosphate fractions and location in bacterial cells

Polyphosphates (with the common formula $M_{n+2}P_nO_{3n+1}$) are polymers of orthophosphate with phosphoanhydride linkages equivalent to the bonds found in ATP and ADP (Harold, 1966; Kulaev, 1979). They are stored intracellularly in volutin granules and are used by bacteria as phosphorus and energy reserves.

In sludges exhibiting EBPR, both a low molecular weight polyphosphate (LPP) fraction and a high molecular weight polyphosphate (HPP) fraction has been identified (Mino *et al.*, 1985). The LPP has been associated with the phosphorus turnover observed in the anaerobic zone, while the HPP does not change substantially during anaerobic conditions (figure 2.7.).

It is thought that the HPP fraction serves only as a phosphorus reserve while the LPP fraction serves as an energy pool for substrate transfer, like anaerobic carbon uptake. As seen in figure 2.7., during the anaerobic phase only the LPP is hydrolysed, but during the aerobic phase the extracellular orthophosphate is incorporated in both the LPP and HPP fractions (Mino *et al.*, 1985).

Low molecular weight polyphosphates (acid soluble) appear to be in a free state inside the cell, whereas high molecular weight species (acid insoluble) are thought to be complexed with other polymers in the cell, in particular proteins, polypeptides, nucleic acids, phospholipids and polysaccharides (Harold, 1966; Kulaev, 1979).

Experiments on pure cultures of *Acinetobacter lwoffi* showed the presence of two polyphosphate pools, one on the cell surface and one in the cytoplasm. The surface pool was found to be metabolically more active and it was preferentially degraded in low nutrient or anaerobic conditions (Halvorson *et al.*, 1987; Suresh *et al.*, 1985). On the other hand, Streichan and Schön (1991), found that in all *Acinetobacter* strains investigated polyphosphate occurred inside the cell. Unlike *Acinetobacter*, the *Moraxella* strain investigated by the same researchers, could incorporate polyphosphate in the periplasm. In the same bacterial strain the researchers also found a surface bound chemically precipitated phosphorus fraction.

Hill *et al.* (1989), also found that part of the polyphosphate is located outside the cytoplasmic membrane and is complexed with metal cations. This polyphosphate, along with the degrading enzyme polyphosphatase is bound to the cell wall and/or the cytoplasmic membrane.

Continuation of the work of the previous researchers was done by Jing *et al.* (1992). They based their work on the hypothesis that micro-organisms use phosphorus in a variety of ways and therefore the polyphosphate will be stored in different cellular locations, in different polymer sizes and with different mobilities. Their work indicated that the location of the stored polyphosphate changes according to the type of substrate used. Glucose based feed solutions resulted in phosphorus being stored as HPP, part of which was located outside the cytoplasmic membrane and was cell bound. No motile polyphosphate was found inside the cytoplasm. On the other hand, starch fed bacteria stored phosphorus primarily as LPP inside the membrane. A portion of the polyphosphate located inside the cells was attached (immobilised) to cell material (probably the inside surface of the cytoplasmic membrane), while a portion of the polyphosphate was mobile (not cell bound) and resided in the cytoplasm.

Bark *et al.* (1992), observed in pure cultures of *Acinetobacter* a considerable amount of phosphorus adsorbed at the exocellular polymer (ECP) on the outside of the cell. They suggested that phosphorus adsorption must be considered in addition to phosphorus metabolism. All strains investigated showed ECP production resulting in bioflocculation. Under anaerobic conditions fewer biopolymers are released and therefore anaerobic sludges contain less ECP with higher concentration of protein and Ca, Mg, K, Fe and phosphorus (Morgan *et al.*, 1990). It seems probable as Arvin (1979) pointed out that calcium phosphate is precipitated in ECP, however the presence of calcium may also be explained by equilibrium between calcium or other metals and the anionic surface of the ECP.

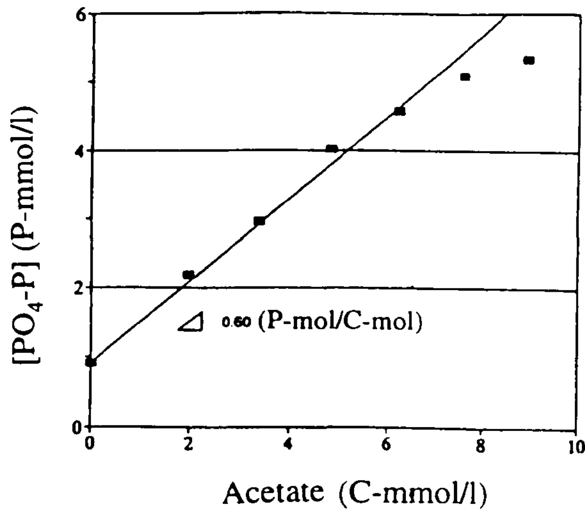


Fig. 2.8. Phosphorus release per mol-C acetate consumed during anaerobic conditions. Batch experiments with increasing acetate concentration at pH 7.4 (from Smolders *et al.*, 1994).

TABLE 2.2. GENERAL OBSERVATIONS DURING ANAEROBIC AND AEROBIC CONDITIONS IN EBPR SYSTEMS.

Anaerobic Conditions	Aerobic Conditions
short chain fatty acids (SCFA) decrease	-
intracellular glycogen decreases	intracellular glycogen increases
intracellular PHB increases	intracellular PHB decreases
intracellular polyphosphate decreases	intracellular poly-P increases
phosphorus in the bulk liquid increases	phosphorus in the bulk liquid decreases
cation concentrations increase	cation concentrations decrease
pH does not change	pH increases

Table compiled from Wentzel *et al.* (1991) and Mino *et al.* (1987).

strongly dependent on the available acetate (figure 2.8.). During the subsequent aerobic phase polyphosphate is re-synthesised through the utilisation of the previously stored PHB. The polyphosphate synthesis rate is dependent on the external phosphorus concentration and the amount of intracellular PHB and polyphosphate (Smolders *et al.*, 1995a).

2.4.3. Biochemical Models for EBPR

Two biochemical models have been proposed so far. One is the Comeau/Wenzel model and the other is the Mino model. The first only takes PHB and polyphosphate storage into account, whereas the second includes an additional storage polymer, glycogen. Both models are called to describe the metabolic pathways lying behind the observations summarised in table 2.2.

The model proposed by Comeau (1986) and Wentzel *et al.* (1986), is based on the metabolic pathways specific to *Acinetobacter* spp., and uses acetate as the sole substrate. The above model does not address the anaerobic decrease and aerobic increase in intracellular and extracellular carbohydrate (Mino *et al.*, 1987). The model proposed by Mino *et al.* (1987), assumes that a single organism causes the observed changes in both the carbohydrate and PHB. Recently though, another group of bacteria, termed "G-bacteria" (Cech and Hartman, 1990) has been shown to be able to accumulate glycogen in anaerobic-aerobic systems. Not much of their microbiology is yet understood but they can take up organic substrates under anaerobic conditions without releasing phosphorus. They have been implicated with EBPR deterioration although their actual effect on the process is still under investigation.

In the figures that follow (figures 2.9. and 2.10.) the anaerobic and aerobic metabolic pathways for the biochemical models described above are presented.

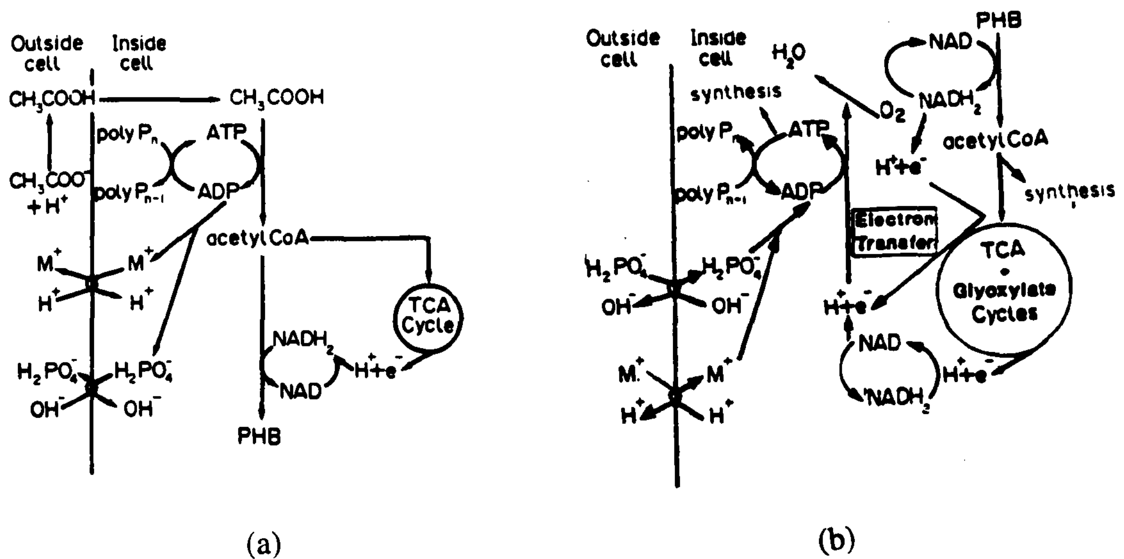


Fig. 2.9. Metabolic model proposed by Wentzel *et al.* (1986). (a) anaerobic conditions and (b) aerobic conditions.

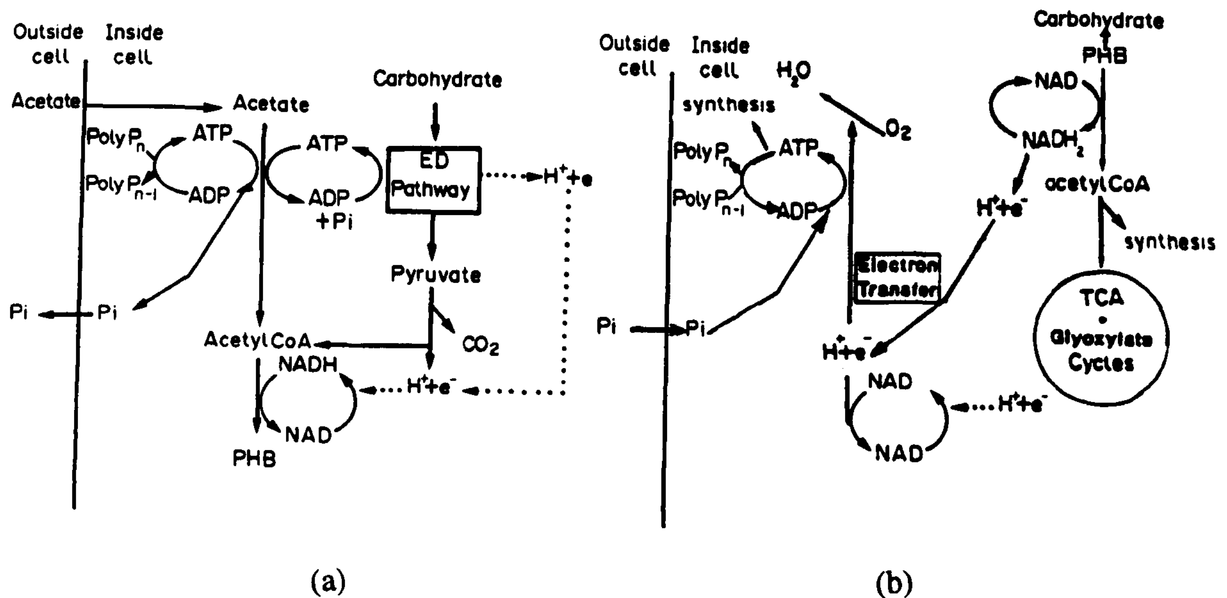


Fig. 2.10. Metabolic model proposed by Mino *et al.* (1987).

(a) anaerobic conditions and (b) aerobic conditions.

Nomenclature: ATP = adenosine triphosphate, ADP = adenosine diphosphate, polyP = polyphosphate, M = cation, acetylCoA = acetyl coenzyme A, NAD and NADH = nicotinamide-adenine dinucleotide, TCA Cycle = tricarboxylic acid cycle, Pi = inorganic phosphorus, ED pathway = Entner-Doudoroff pathway.

2.5. DESIGN AND OPERATIONAL PARAMETERS INFLUENCING THE PROCESS

2.5.1. Temperature

The effect of temperature on phosphorus removal has been the subject of much research but conflicting evidence has been presented so far. The majority of research suggests that the optimum operating temperature is between 20 and 30 °C (Fuhs and Chen, 1975; Van Groenestijn and Deinema, 1985; Mamais and Jenkins, 1992). It also happens that the most studied range of temperatures has been that of 20 to 30°C. More specifically, Shapiro *et al.* (1967), studied phosphorus release between 10 and 30°C and found that it decreased by 2.1 - 2.6 times for every 10°C drop in the temperature. The same was found for phosphorus uptake (Hashimoto and Furukawa, 1984) and McClintock *et al.* (1993), confirmed that lowering the temperature decreases the phosphorus removal capacity of the sludge. This temperature range also coincides with the optimum growth temperature of *Acinetobacter* spp. known to be the predominant bacterial species in EBPR plants (Juni, 1978).

Conflicting evidence comes from Sell *et al.* (1981), who stated that phosphorus accumulating bacteria are psychrophilic and that EBPR performs better at colder (5°C) climates. Krichten *et al.* (1985), found that phosphorus removal is higher at 5°C than 15°C. Barnard *et al.* (1985), reported that phosphorus removal efficiency was higher by 40% at 5°C than at 15°C and Kang *et al.* (1985) also found phosphorus removal to be effective around 10°C.

Lately, Converti *et al.* (1995), have stated that the overall removal yield of a sludge seemed to be independent of temperature. The time necessary to achieve it on the other hand, is strongly increased by a temperature decrease.

2.5.2. Oxygen Concentration

Levin and Shapiro (1965) investigated the effect of the extent of aeration on phosphorus uptake. In batch experiments at different aeration rates they noted that inadequate aeration adversely affected phosphorus uptake but aeration beyond a certain rate did not lead to indefinite uptake improvement. They also noted that pure oxygen would give higher phosphorus removal than a mixture of air and oxygen.

The oxygen requirements for effective phosphorus removal have been stated as being between 2 and 6 mg/l (Wells, 1969; Milbury *et al.*, 1971; Kerdachi and Roberts, 1983; Raper, 1983; Malnou *et al.*, 1984). Over-reaction (>5mg/l) was detrimental to phosphorus removal causing aerobic digestion of solids, excessive nitrification and phosphorus re-release (Wells, 1969).

2.5.3. Mean Cell Residence Time (MCRT)

It has been found that within normal operating temperatures (10-30°C), EBPR functions independently of MCRT when above 2.9 days (Mamais and Jenkins, 1992). McClintock *et al.* (1993) found that phosphorus removal was greatest at a MCRT of 5 days (range studied 2-15 d), at 20°C, which was the lowest MCRT that provided complete nitrification. Similar results were obtained at 5 and 15°C. Okada *et al.* (1991), observed that at an operating temperature of 20°C phosphorus accumulating bacteria could not accumulate in the reactor if it operated at MCRT less than 25 days.

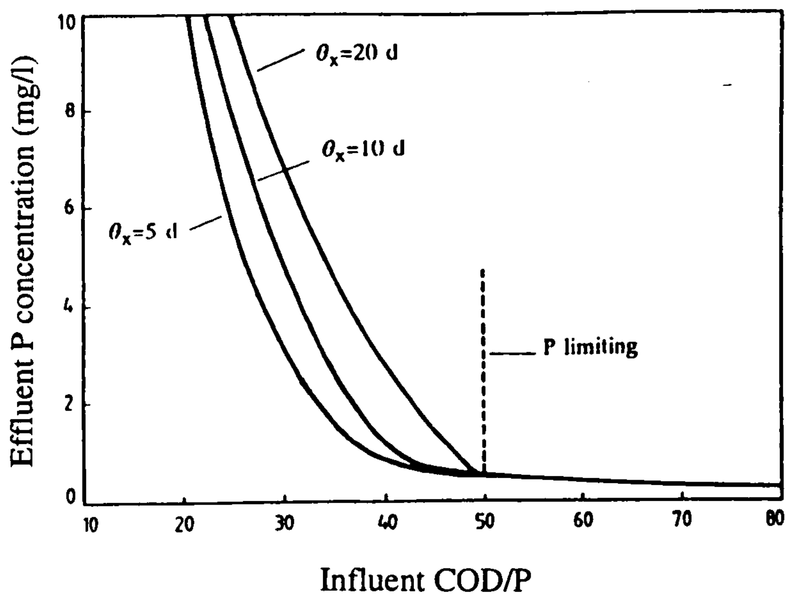


Fig. 2.11. Effect of influent COD/P ratio on effluent phosphorus concentration (from: Design and Retrofit of Wastewater Treatment Plants for Biological Nutrient Removal, 1992).

TABLE 2.3. OPERATING COD/P RATIOS IN FULL SCALE PLANTS.

Treatment Facility	Operating COD/P
Baviaansport, Pretoria (Wet *et al.*, 1992)	43:1
Northern Works, Johannesburg (Wet *et al.*, 1992)	40:1
Goundkoppies, Johannesburg (Wet *et al.*, 1992)	72:1
Olifantsvlei, Johannesburg (Wet *et al.*, 1992)	67:1
Mutare, Zimbabwe (Wet *et al.*, 1992)	91:1
Bulawayo, Zimbabwe (Wet *et al.*, 1992)	60:1
Palmetto, Florida USA (Wet *et al.*, 1992)	36:1
Kelnowa, B.C. Canada (Wet *et al.*, 1992)	41:1
Patapsco, Md, USA (Wet *et al.*, 1992)	36:1
Brasilia (Wet *et al.*, 1992)	81:1
Wolfsburg, Germany (Kayser, 1992)	53:1

2.5.4. Influent Characteristics

2.5.4.1. Influent COD/P ratios

The influent COD/P ratio is a critical factor for design, because it determines whether the system is operated with a phosphorus or a carbon limitation. It has also been shown to affect the settling characteristics of activated sludge (Bates and Torabian, 1981). In general higher COD/P ratios seem to favour EBPR with a COD/P ratio of 50 being the limit below which phosphorus becomes limiting and EBPR cannot function (figure 2.11.). Table 2.3. shows operating COD/TP ratios in plants around the world treating domestic sewage.

2.5.4.2. Influent F/M ratios

Somiya *et al.* (1988), investigated the effect of F/M ratio on phosphorus release and uptake. They concluded that anaerobic release increases as the initial F/M ratio increases until it reaches a value of 0.04 mgTOC/mgMLSS.d. For F/M values greater than 0.04 mgTOC/mgMLSS.d, no marked difference is observed in both the phosphorus release pattern and the amount of phosphorus released per unit MLSS. The ratio between the concentration of organic substrate removed and phosphorus released does not appear to be affected by the influent F/M ratio.

2.6. PHOSPHORUS RELEASE DURING SLUDGE TREATMENT

2.6.1. The Extent of the Problem

Because of the mechanism of EBPR, sludges derived from biological phosphorus removal plants, have a higher content of biologically bound phosphorus. Typical phosphorus concentrations in waste activated sludge exhibiting EBPR are 6-

10% by weight (Barnard, 1975a and others), versus 2-3% for conventional waste activated sludge. One of the major problems of biological phosphorus removal systems, is the potential resolubilisation of phosphorus during anaerobic handling of excess sludge. The problem arises when process waters are recycled back to the head of the works, increasing the influent phosphorus concentration. The capacity of the system may in this way be exceeded, resulting in an increased phosphorus concentration in the effluent. Thus it is often recommended that sludges derived from such systems be kept aerobic and if possible processed as quickly as possible.

The mechanism of phosphorus leakage during sludge handling is not known, but cell lysis is the reason most often cited. Since the stored phosphorus has been associated with the active sludge mass fraction, conditions of endogenous respiration would result in biomass reduction and phosphorus release (Marais *et al.*, 1983). It may also be possible, that release is caused by intracellular carbohydrate consumption in a similar way to the one observed in the anaerobic stage (Arun *et al.*, 1988).

The most important parameter shown to induce phosphorus solubilisation during sludge treatment is the anaerobic retention time. The longer the sludge is retained, for example, in primary sedimentation tanks and thickeners, the greater the degree of phosphorus solubilisation (Pitman *et al.*, 1991; Pöpel and Jardin, 1993).

The solids capture in dissolved air flotation (DAF) units and dewatering equipment has also been found to be of importance. The poorer the solids capture the greater the suspended solids content of the liquid phase, the greater the extent of potential phosphorus release (Pitman *et al.*, 1991).

Mixing primary and excess activated sludge can result in a substantial release as shown by the literature. When phosphorus rich solids come into contact with untreated sewage, as occurs on recycle to the inlet of the primary sedimentation tanks (PST), a large degree of phosphorus release occurs in response to the assimilation of soluble substrates in the sewage by the phosphorus accumulating biomass (Pitman *et al.*, 1991).

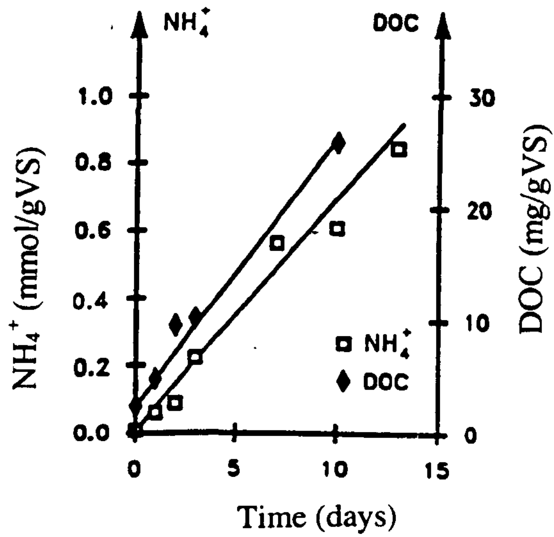


Fig. 2.12. Development of bulk water concentration of NH_4^+ (mmol/gVS) and DOC (mgC/gVS) during anaerobic storage (from Rasmussen *et al.*, 1994).

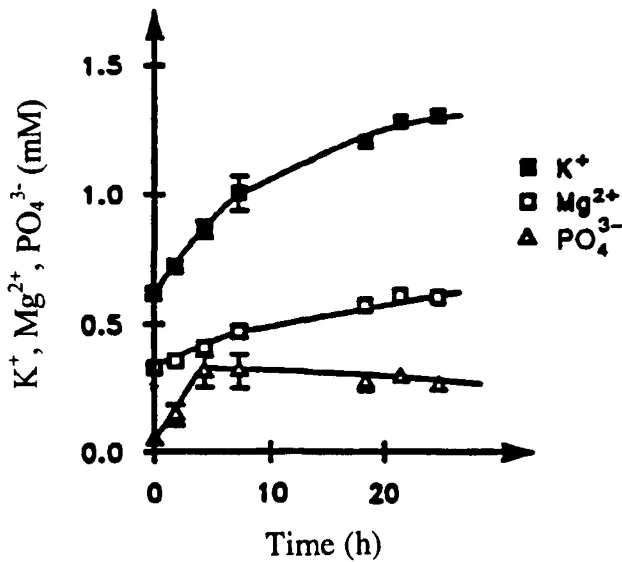


Fig. 2.13. Concentrations of K^+, Mg^{2+} and PO_4^{3-}during the first day of anaerobic storage. Bars show standard deviation, n=2 (from Rasmussen *et al.*, 1994).

2.6.2. Phosphorus Release During Anaerobic Sludge Storage

Anaerobic storage has been shown to effect several sludge properties. Long term storage effects the sludge structure and microbial composition due to the hydrolysis of the extracellular polymers and degradation of the sludge matrix (Ryssov-Nielsen, 1975). In a comprehensive investigation of EBPR sludge by Rasmussen *et al.* (1994) storage was shown to decrease the dewaterability of the sludge and increase its turbidity and conductivity.

Dissolved Organic Carbon (DOC) and NH_4^+ profiles showing a linear increase of both parameters, with rates of 2.38 mgC/gVS·day and 81 mmol/gVS·day respectively, pointed to a constant mineralisation of the organic matter (figure 2.12.) (Rasmussen *et al.*, 1994).

The same authors noted that as no oxygen or nitrate were present during their storage experiments, hydrolysis, fermentation and respiration by reduction of Fe(III) and sulphate could be important processes in the mineralisation of the biomass. They also hypothesised that microbial reduction of Fe(III) may have taken place because they detected soluble Fe(II) after a few hours of storage.

Release of phosphorus, potassium and magnesium ions appeared to be linear with time. In experiments done by Rasmussen *et al.* (1994), the increase in phosphorus in the first hours was closely correlated to the amount of organic matter. After the initial release the phosphorus concentration remained almost constant. K^+ and Mg^{2+} on the other hand continued to increase after 4 hours but with a slower rate (figure 2.13.).

The authors found the presence of polyphosphate accumulating bacteria in their sludge samples and attributed the release of K^+, Mg^{2+} and PO_4^{3-} to these bacteria. They postulated that the continued release of $K^+$and Mg^{2+} after the release of PO_4^{3-} had stopped was due to the obligate aerobic bacterial population leaking out high concentrations of internal K^+ under anaerobic conditions.

In this study the researchers concluded that the minimum anaerobic storage time before phosphorus release commences, for phosphorus laden sludges was only a few

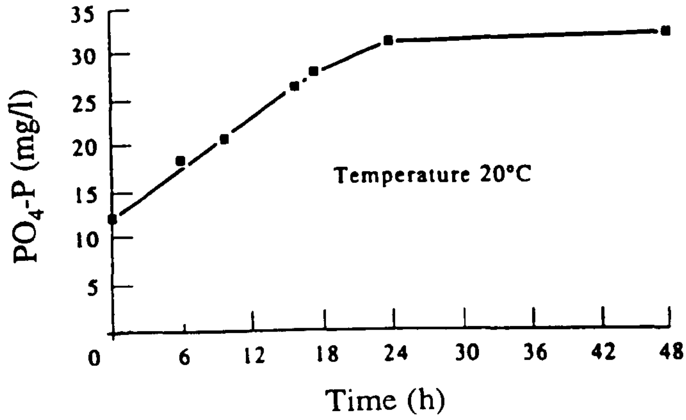


Fig. 2.14. Phosphorus release under anaerobic conditions (from Kroiss and Negm, 1994).

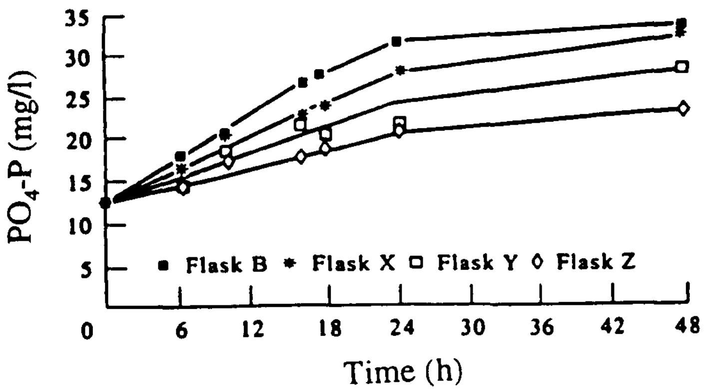


Fig. 2.15. Effect of initial nitrate concentration on phosphorus release. Flask B=4.5, X=29.5, Y=54.5 and Z=104.5 all in mg/l NO_3-N (from Kroiss and Negm, 1994).

hours, whereas the dewaterability showed a continued deterioration with time (Rasmussen *et al.*, 1994).

2.6.3. Phosphorus Release During Sludge Gravity Thickening

During gravity thickening phosphorus release can be induced by the anaerobic conditions and/or by the contact of excess waste activated sludge (WAS) solids with large amounts of volatile fatty acids (VFA). The latter can take place when WAS is co-thickened with primary sludge (Pöpel and Jardin, 1993). The thickener supernatant will then contribute to increased phosphorus concentrations in the following two ways. First through the recycle of soluble phosphorus and second through the recycle of phosphorus rich suspended solids. These solids, through the return of the process waters to the head of the works, will settle in the PST with the potential of inducing high phosphorus release due to the presence of organic acids in the primary sludge (Pöpel and Jardin, 1993).

Kroiss and Negm (1994), investigated the release of phosphorus from EBPR sludges during anaerobic and anoxic completely mixed thickening experiments. They observed that phosphorus release occurred in two phases; an initial rapid release phase, followed by a stationary phase (figure 2.14.). They attributed the release observed in the first phase (up to 15 hours) to phosphorus taken up through the EBPR phenomenon, to cell lysis as well as phosphorus precipitation. The second phase (15-48 hours) they attributed to hydrolysis processes and again phosphorus precipitation.

The same researchers investigated the effect nitrate on phosphorus release. They found that the phosphorus released to the liquid phase decreased as initial nitrate concentration increased (figure 2.15.). They postulated that this was due to the presence of organic substrates generated through sludge hydrolysis. Denitrification occurring outside the flocs would consume part of the available carbon, whereas the rest would penetrate the interior were anaerobic conditions prevail and be used for phosphorus release.

Simultaneous thickening of WAS and primary sludge has been shown to lead to high phosphorus release rates. Concentrations of as high as 60 to 100 mg/l during gravity thickening of mixed sludges have been noted (Murakami *et al.*, 1987). Tanaka *et al.* (1987), investigated the release of phosphorus during co-thickening of primary and waste activated sludge (WAS). The quantity of phosphorus released from the mixed sludge was about ten times larger than from the excess activated sludge. Sludge fractionations revealed that 30% of the biologically stored polyphosphate would be returned to the head of the works.

2.6.4. Phosphorus Release During Other Sludge Treatment Processes

2.6.4.1. Phosphorus release during sludge conditioning and dewatering

When treating sludges which have the potential of releasing phosphorus under anaerobic conditions, dewatering with plate and belt filter presses is preferred, because of the low anaerobic retention time needed. High phosphorus concentrations in the filtrate can be expected if the sludge treated had previously released phosphorus during anaerobic digestion (Murakami *et al.*, 1987).

The phosphorus feedback caused by incomplete separation during sludge dewatering is considered to be very low. When using centrifuges for sludge dewatering the phosphorus feedback is significantly higher because of the relatively low solids recovery rates (Pöpel and Jardin, 1993).

For sludges with high phosphorus contents (6-7%) a phosphorus feedback of up to 40% of the incoming phosphorus can be expected (Jardin and Pöpel, 1994).

2.6.4.2. Phosphorus release during anaerobic digestion

During anaerobic digestion there is a high probability of phosphorus release due to the anaerobic conditions and the high concentration of organic acids.

The release of phosphorus is accompanied by a release of potassium and magnesium (Pöpel and Jardin, 1993). The resolubilisation of Mg^{2+} along with phosphorus results in the precipitation of significant amounts of phosphorus as struvite ($MgNH_4PO_46H_2O$). Calcium in solution in the sludge entering the anaerobic digester can also precipitate some of the soluble phosphorus. Sen and Randall (1988) stated that 60 to 80% of the phosphorus content can be expected to solubilise during anaerobic digestion. Because of precipitation though, only 30% of the phosphorus removed across the plant can be expected to return to its inlet.

The amount of the maximum releasable phosphorus depends mainly on the phosphorus content of WAS. The major part of phosphorus released during digestion can be attributed to the phosphorus fraction accumulated as polyphosphate during EBPR. Miya *et al.* (1987), found that most of the polyphosphate fraction was released within 7 days of digestion but Jardin and Pöpel, (1994) noted that only a part of the released phosphorus remains in soluble form as phosphorus elimination processes take place in the anaerobic digester; primarily chemical fixation. Phosphorus fixation in anaerobic digesters occurs primarily in the form of hydroxyapatite, magnesium-ammonium-phosphate and struvite (Jardin and Pöpel, 1994).

2.7. MEASURES TO PREVENT PHOSPHORUS RELEASE DURING SLUDGE HANDLING

For the prevention of the recycling to the head of the works the released phosphorus the following strategies can be implemented:

- Minimisation of return flows
- Minimisation of phosphorus release to the liquid phase through efficient primary sludge thickening and through wasting from the bioreactor rather than the FST.
- Avoidance of co-thickening of primary and WAS
- Thickening with DAF rather than gravity
- Centrifuging instead of using belt filter presses.

Apart from the above, sludge aeration and/or chemical precipitation with metal salts can always reduce high phosphorus levels from process waters. This can be achieved by conditioning of either the raw or digested sludge or the process water (Fujimoto *et al.*, 1991; Tanaka *et al.*, 1987). Experiments on aeration of primary, WAS and anaerobically digested sludge have been carried out by Pitman *et al.* (1991) and Tanaka *et al.* (1987). Although interesting results were obtained, full scale application evaluation is yet to be made.

2.8. OVERVIEW

Enhanced biological phosphorus removal relies on the activity of a group of microorganisms, the Phosphate Accumulating Organisms (PAOs), who accumulate phosphorus in excess of their growth requirements. The primary prerequisite for obtaining biological phosphorus removal is the re-circulation of the sludge through first an anaerobic and then an aerobic zone. Taking the PAOs through the anaerobic-aerobic sequence allows them to accumulate internal energy stores, such as organic polymers (polyalkanoetes and glycogen) and polyphosphate.

A key factor for the success of the process is the availability of simple organics, mainly fatty acids, in the anaerobic zone. On the other hand, dissolved oxygen and nitrate in the same zone may limit the efficiency of the process.

The organisms responsible for EBPR development have been found to successfully operate in a temperature range of 5 to 30 °C. Within the range of 10 to 30°C, EBPR functions independently of MCRT when above 3 days. Excessive oxygen (> 5 mg/l) in the aeration zone has been found to be detrimental to the process as has a low (< 50) influent COD/P ratio.

Biological phosphorus removal can meet most effluent standards, especially in combination with a chemical precipitation back-up system. Problems arise when the influent wastewater has a low content of fatty acids, when nitrates are re-circulated to

the anaerobic tank, during extended storm water input, or high solids concentrations in the effluent.

Sludge handling of EBPR sludges is another major problem of biological phosphorus removal systems, as there exists an increased potential of phosphorus resolubilisation during anaerobic treatment. Research in this area is scarce and the mechanism of phosphorus leakage during sludge handling is not known. Cell lysis has been most often cited as the reason of phosphorus resolubilisation, with anaerobic retention time, solids capture and contact of the excess EBPR sludge with primary being the parameters influencing the extent of the release.

Sludge storage and gravity thickening experiments have concluded that release is initiated within the first few hours of anaerobic processing and the presence of nitrate in the sludge acts as a reducing factor to the expected phosphorus release. On the other hand anaerobic digestion does not lead to significant release of phosphorus.

Although EBPR is a well established process for removing phosphorus from wastewater, research has still to decide a number of issues.

Many of the microorganisms responsible for EBPR are still unknown and controversy exists about the contribution of a group of bacteria belonging to the *Acinetobacter* genus, who were initially closely associated with the process.

Although the metabolism of the PAOs has been largely established the details of the biochemical pathways involved have yet to be decided.

Research has not thoroughly investigated the effect on the process of influent and operational parameters such as influent phosphorus, carbon, dissolved oxygen concentration and sludge retention time.

Finally, one of the major problems that EBPR poses to plant operators, phosphorus resolubilisation during sludge handling, has been largely left uninvestigated as designers opt for chemical phosphorus precipitation of the process waters.

Researchers so far have given only very general explanations of the phenomenon of phosphorus resolubilisation during anaerobic treatment and the cell lysis theory can not explain the release observed during the first few hours of sludge

handling, as EBPR sludges are subject to anaerobic conditions daily and have therefore been acclimatised to this type of stress.

There exists a gap in the knowledge regarding the different phases describing phosphorus release during sludge handling, the effect of the sludge handling method employed or the effect that plant operational and influent parameters have on the phosphorus released during treatment. It is in this area that the research presented in this thesis intends to contribute.

CHAPTER 3. MATERIALS AND METHODS

3.1. INTRODUCTION

In the following chapter the experimental design of the laboratory reactors used for the development of EBPR and also for the storage and thickening experiments is presented. All analytical and microbiological techniques used are described as are the grades and names of the suppliers of the employed chemicals.

An short introduction to the operation of Sequencing Batch Reactors has been provided since the theory could not be included in the literature review chapter.

3.2. SEQUENCING BATCH REACTORS

3.2.1. Description of the Process

The experimental system used, consisted of Sequencing Batch Reactors (SBR). The SBR is a fill and draw, time oriented, unsteady-state, activated sludge system. For a comprehensive review on SBRs the reader is referred to Irvine and Ketchum (1989).

Each SBR cycle of operation has five discrete periods: FILL, REACT, SETTLE, DRAW and IDLE (figure 3.1.).

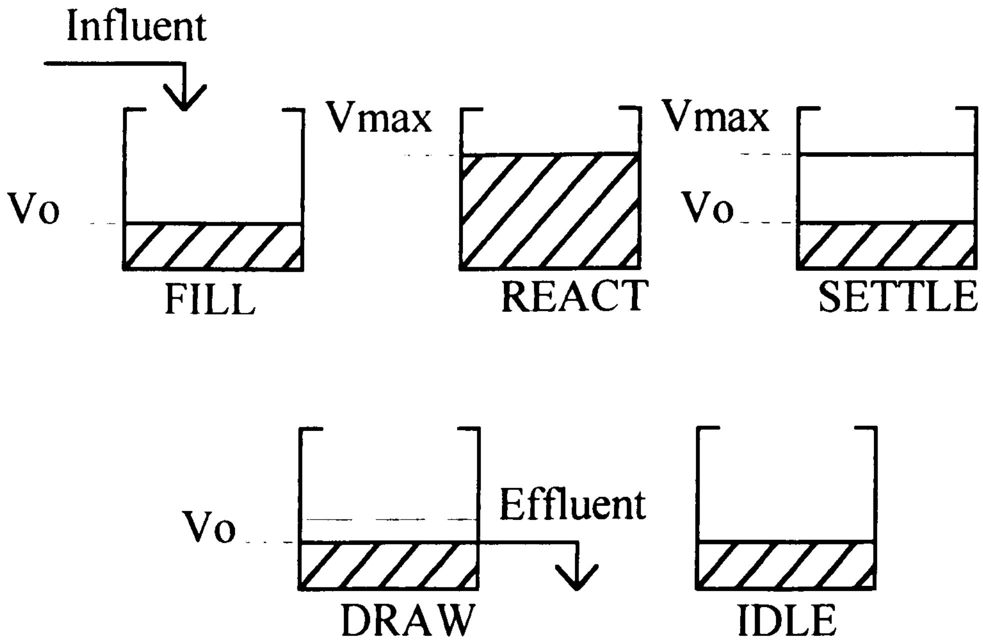


Fig. 3.1. Schematic representation of the SBR operating stages.

During FILL, the influent wastewater is added to the biomass which remained in the tank from the previous cycle. The liquid volume increases from the initial level (V_0) to the maximum (V_{max}).

During REACT, stirring and/or aeration takes place and the reactions initiated during FILL are completed. Although the liquid level is shown to remain at maximum (figure 3.1.), sludge wasting can take place during this period.

During SETTLE, all energy input is stopped and the biomass is allowed to flocculate and settle. In the SBR, solids separation (SETTLE) takes place under quiescent conditions. Because all the biomass remains in the same container, no sludge recycle lines are needed. The time of SETTLE should be long enough to ensure that the sludge blanket remains below the withdrawal line. Longer times pose problems of sludge bulking due to gas evolution.

During DRAW, part of the supernatant is removed. Usually the volume removed is equal to the volume added during FILL. After DRAW the tank is ready to receive additional wastewater. The time between the end of DRAW and the beginning of FILL is termed IDLE. Sludge wasting can take place during this stage too.

The relation between sludge age, mass loading, hydraulic retention time and tank sizing are not clear for SBRs because of the dramatic effect of the aeration and mixing strategies on the overall performance (Irvine and Ketchum, 1989). Nevertheless they are convenient terms and they are being used in this study (for definitions see section 3.3.3).

3.2.2. Advantages of SBR Systems

The experimental set-up for the project described in this thesis had several requirements. The lab scale reactor had to be able to accommodate the varying operational conditions (anaerobic/aerobic) necessary for EBPR. As one of the objectives of the study was to investigate the performance of EBPR with varying parameters, the reactor had to be flexible and simple to operate. Finally, it had to be safe from overflows, blockages and sludge bulking problems when left unattended.

The SBR could meet all the requirements stated above and was therefore selected as the reactor configuration to be employed in this study. The most important advantage of the SBR process is the fact that it is time oriented, which gives it flexibility of operation. As all reactions take place in one tank, it is simple to operate and has a reduced amount of connecting tubing and thus blockage problems. Growth of filamentous bacteria can be easily controlled by varying the operating strategies of fill and therefore filamentous sludge bulking is not a problem.

Although sequencing batch reactor technology is not as widely employed in full scale plants as continuous processes, the results of full-scale applications have demonstrated that it can be both a successful and attractive alternative for both conventional treatment (removal of organics and suspended matter) and more complicated processes such as combined nitrification - denitrification - EBPR (Irvine *et al.*, 1983; Ketchum *et al.*, 1987).

Apart from the ones detailed above, SBR operation has a number of other advantages, especially applicable to full-scale treatment, which are summarised below (Irvine and Ketchum, 1989).

- It can easily tolerate peak flows and shock loads of biochemical oxygen demand (BOD) without degradation of effluent quality due to the fact that it acts as an equalisation basin and a buffer during fill.
- The periodic effluent discharge allows the effluent to be held in the reactor until it meets effluent standards.
- No return sludge pumping is required since the mixed liquid always remains in the same tank.
- Solid - liquid separation occurs under nearly ideal quiescent conditions.
- It has been proven to be able to achieve nitrification-denitrification and phosphorus removal without the addition of chemicals, because of the easily achievable variations in operating strategies.

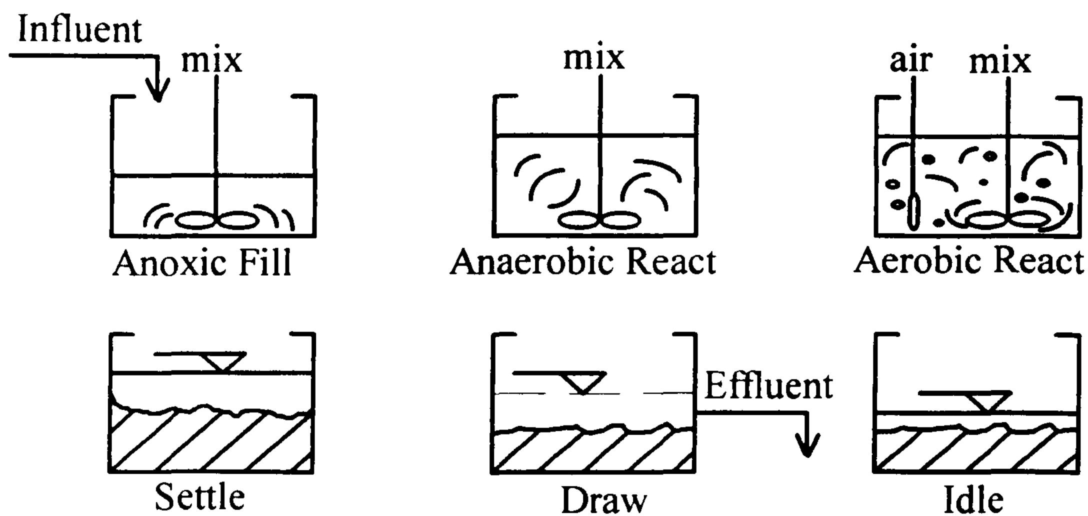


Fig. 3.2. SBR operation for EBPR.

3.2.3. Biological Phosphorus Removal in SBRs

In EBPR, a number of groups of organisms are involved. Denitrifying organisms, facultative organisms manufacturing fermentation products, phosphorus accumulating organisms and aerobic autotrophs and heterotrophs. The SBR operating strategy must ensure that the conditions necessary for the enrichment of all organisms involved are met.

Figure 3.2. illustrates the basic SBR operation for the achievement of EBPR. Fill is accomplished without aeration but with mixing, since for EBPR development the biomass needs to be initially exposed to anaerobic conditions (absence of NO_x-N and D.O.). Mixing ensures that the heterotrophic population comes in contact with the high substrate concentration quickly, consuming all available oxygen. After D.O. depletion phosphorus accumulating and denitrifying bacteria compete for substrate under anoxic conditions. When NO_x-N is eliminated, fill will continue under anaerobic conditions (Manning and Irvine, 1985; Ketchum *et al.*, 1987).

After the end of fill the reactor continues running under anaerobic conditions. The incoming organics are used by facultative aerobic organisms to produce VFAs. Phosphorus accumulating organisms take up this readily biodegradable products and release phosphorus which remains in solution. The aerobic react period follows.

In an SBR react cycle the oxygen uptake rate (OUR) and the F/M ratio are constantly changing. When aeration is initiated F/M can range from 0.6 to 1.0 and the OUR can exceed 125 mg/l hr. At the end of the aeration period the F/M should be near zero. The wide swings in the F/M ratio put selective pressures on the biomass. During the feast period (high F/M, plenty of air), the D.O. remains near zero because the demand exceeds the maximum aeration capacity of the system. This period inhibits the growth of slow growing filaments and encourages the growth of floc forming zooglean organisms. The period when the F/M ratio and OUR are low is a famine period where all available food has been utilised. The famine period inhibits the growth of fast growing filaments (Norcross, 1992).

The previously described strategy (figure 3.2.), ensures the removal of organics and phosphorus. During the aerobic period the ammoniacal nitrogen will be converted to nitrates, providing the operating temperature and aerobic retention time are sufficient. If the nitrates are to be converted to nitrogen gas through denitrification, the addition of a post anoxic stage is needed. After dissolved oxygen depletion the mixture is converted to anoxic and in the presence of dissolved or intracellularly stored carbon denitrification will commence. This anoxic stage in combined nitrification-denitrification EBPR (NDEBPR) systems should not be prolonged as secondary phosphorus release may take place, thus affecting the overall phosphorus removal. Denitrification may also continue during settle, draw and idle with possible detrimental consequences on sludge settling characteristics. If nitrates are still present at the beginning of the next cycle, denitrification will take place during the first part of fill, as fresh carbon supply is added and should be completed before the beginning of the next anaerobic phase.

3.3. SEQUENCING BATCH REACTORS - EXPERIMENTAL SET UP

The SBRs used for the development of EBPR in the laboratory, were of two different types; type A and type B, run in parallel. The different characteristics of the two types are presented in detail bellow.

3.3.1. Reactor Design

3.3.1.1. Reactor A

Reactor A consisted of a purposely built plexi-glass cylinder with ports for effluent and sludge extraction. It had a capacity of 10 litres and a working volume of 5 litres. Air was administered through a glass diffuser, connected to an air pump and controlled with a vertical flow rate meter. A mechanical stirrer was used for mixing,

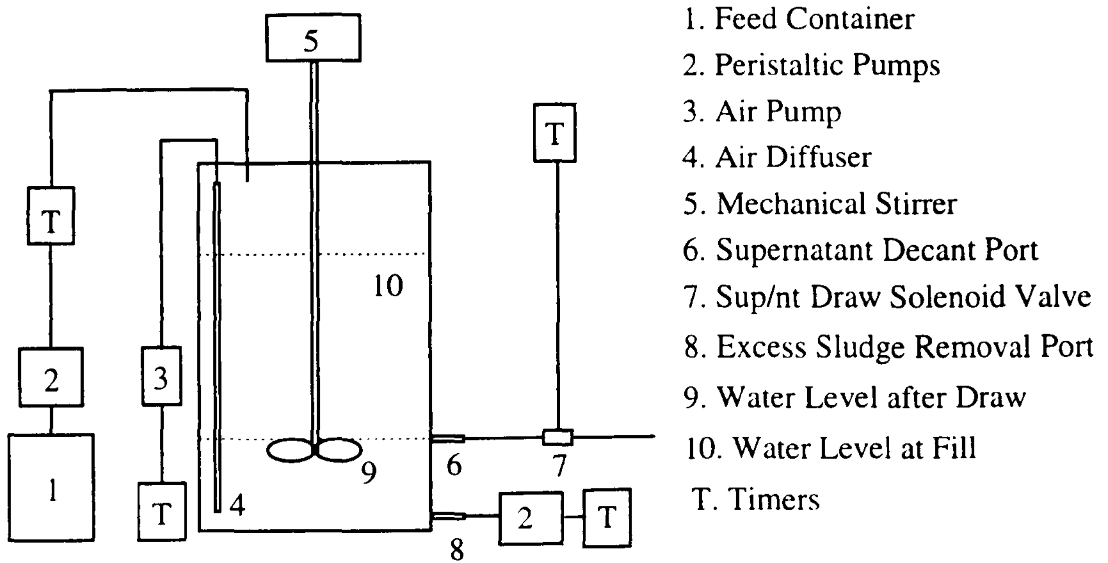


Fig. 3.3. Reactor A.

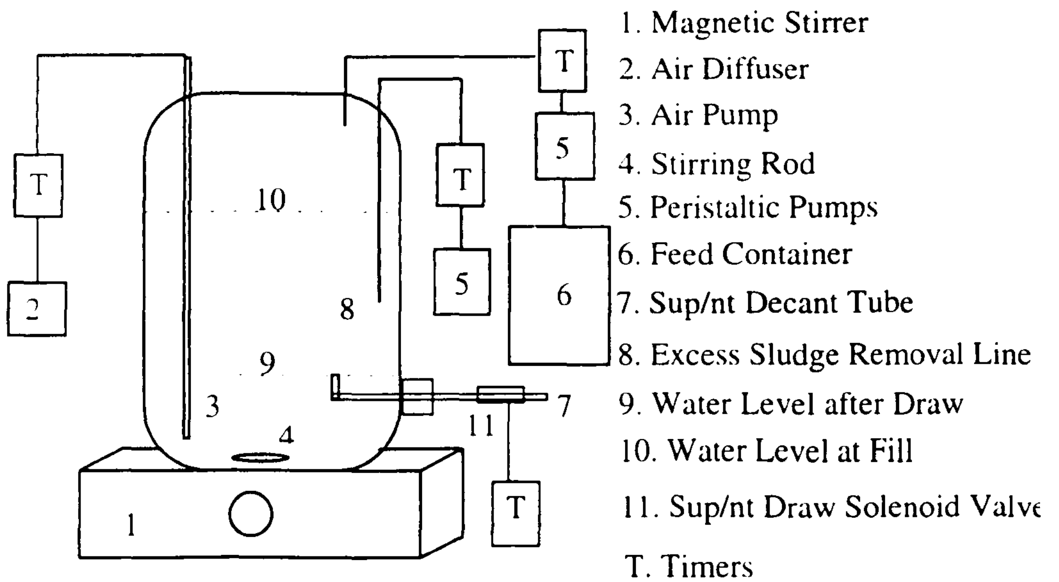


Fig. 3.4. Reactor B.

running at 73 rpm, a velocity proven to provide adequate mixing without surface turbulence, which would affect anoxic and anaerobic conditions. Further provision against the introduction of oxygen during the anaerobic conditions was the addition of a fitted cover on the top of the reactor. For effluent discharge a solenoid valve was used, connected to a timer. Feed was administered with a peristaltic pump. Sludge was wasted with the use of a peristaltic pump and the excess sludge was collected in a settling container. All equipment were run on timers. The reactor is shown in a schematic diagram in figure 3.3.

3.3.1.2. Reactor B

For reactor B, a 5 litre glass aspirator was used. Air was administered through a glass diffuser, connected to an air pump. The air flow was regulated by an air flow meter attached to the plastic tube connecting the pump and the diffuser. For mixing, a magnetic stirrer was used and feed was administered with a peristaltic pump. Effluent was decanted with the use of a solenoid valve. Sludge was also wasted by a peristaltic pump and all equipment were run on timers. The reactor is shown in figure 3.4.

3.3.2. Comparison of Reactors A and B

Reactor A was the first to be constructed and was purposely built. The need of a second reactor running in parallel led to the conversion of a glass aspirator to a chemostat. Although of a simpler design, reactor two proved to be equally successful with an identical performance to reactor A. This avoided the necessity of building a second, more expensive reactor, similar to A.

The only significant difference between the two reactors was the method of stirring. The use of a magnetic stirrer for reactor B, proved initially difficult to regulate. After a number of trials the correct combination of stirrer speed setting and size of magnet was achieved which resulted in consistent stirring to a comparable speed to the one used for reactor A.

TABLE 3.1. DIMENSIONS AND OPERATING VOLUMES OF REACTORS USED.

	Reactor Type	
	A	B
Material	Clear plexi-glass	Clear glass
Internal Diameter (cm)	17.1	16.5
Area (cm^2)	229.7	213.8
Operating Volume V_f (l)	5	5
Draw Volume V_d (l)	2.25	2.82
Effluent Volume V_e (l)	2.75	2.18

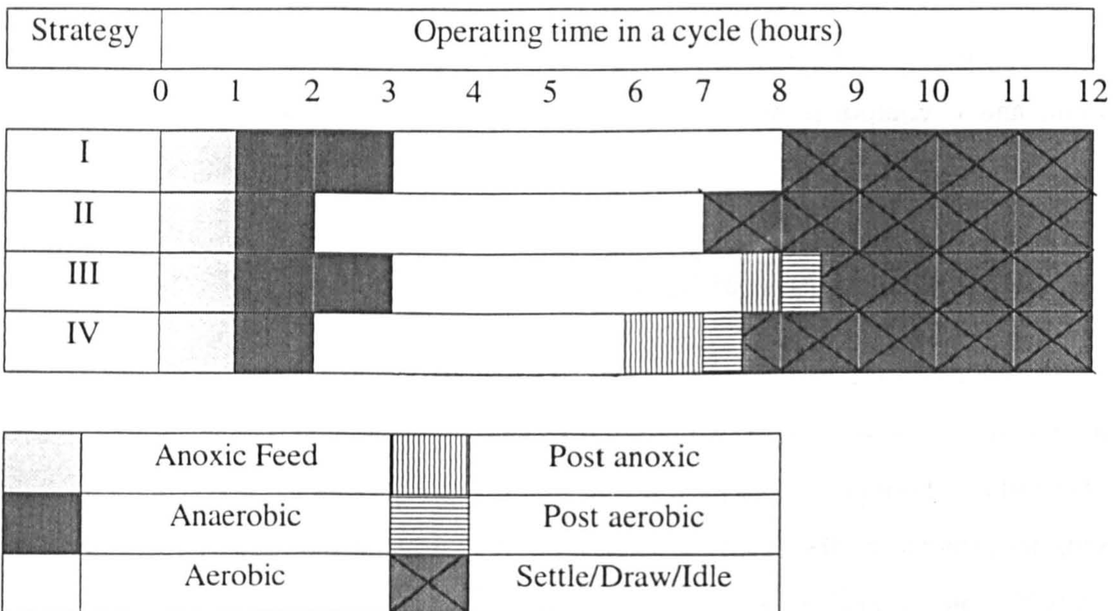


Fig. 3.5. Operating conditions during different experimental runs.

The dimensions and operating volumes of the two types of reactors are shown in table 3.1.

3.3.3. Operating Parameters

3.3.3.1. Fill strategy

A fill strategy carried out with no aeration, results in the best nitrogen and phosphorus removal performance and also in the best settling characteristics of the sludge (Okada and Sudo, 1986). Therefore, anoxic feed was selected, that is, feed addition over a specified period of time with mixing only. The time of fill was selected to be 1 hour. Longer feed periods do not favour floc forming micro-organisms and shorter ones prevent denitrification of nitrates carried over from the previous cycle (Okada and Sudo, 1986).

3.3.3.2. Fill volume to draw volume ratio

The employed ratio between the volume after fill (V_f) and the volume after draw (V_d), varies in the literature. The one most commonly chosen though, is the $V_f/V_d = 3$; that is the remaining volume in the reactor is 1/3 of the total operational volume.

For reactor A, a ratio of 2.2 was chosen and for reactor B a ratio of 1.8. The choice of a lower ratio resulted in a reduction of the feed volumes, making the simultaneous operation of the two reactors more manageable.

3.3.3.3. Operating strategies

The reactors were operated with two 12 hour cycles per day. The length of the anaerobic and aerobic stages and the influent and operational parameters of the SBRs differed according to the experimental protocol. The experimental runs included in this study can be found in Appendix I.

The length and configuration of the phases was named as the operating strategy and was given a capital latin number (figure 3.5.). The feed time was always 1 hour and the anaerobic period varied between 1 and 2 hours. The aerobic time was 4-5 hours. When denitrification was desired an anoxic stage was added after the aerobic one. The length of this stage was chosen between 0.5 - 1 hours (operating strategies III, IV in figure 3.5.). Shorter anoxic periods are desirable for combined nitrification-denitrification EBPR systems, so as to avoid the secondary release of phosphorus observed when the oxidised nitrogen is completely denitrified. A post-aerobic stage was usually included after the anoxic with a length of 0.5 hour.

3.3.3.4. Sludge retention time

The sludge retention time (SRT), was varied between 13 to 40 days as it was one of the parameters under investigation. As the phosphate removing organisms grow only under aerobic conditions, the process was actually designed on the basis of the aerobic or effective SRT (SRT_E) which was calculated according to equation (1). The equivalent operating SRT_E was 5 - 16 days.

$$SRT_E = f \cdot SRT = f \cdot \frac{V_f \cdot MLVSS}{EVSS \cdot Q_e + MLVSS \cdot Q_w} \quad (1)$$

Where: SRT_E = effective sludge retention time (d)

f = aerated fraction of the whole cycle = T_a / T_t

T_a = aeration time in cycle (h)

T_t = total cycle time (h)

V_f = reactor volume at fill (l)

MLVSS = aerobic mixed liquor volatile suspended solids (mg/l)

EVSS = effluent volatile solids (mg/l)

Q_e = effluent flow rate (l/d)

Q_w = waste sludge flow rate (l/d)

The amount of sludge to be wasted was calculated from the aerobic volatile suspended solids concentration and the selected cell residence time. Wasting took place at the end of the aeration period.

3.3.3.5. Organic loading (L)

Although the definition of the organic mass loading is obscure because of the SBRs combination of aerated and unaerated periods, it is a convenient and widely used term and it was therefore used in this study. An appropriate expression for SBR operating with aerated and unaerated sequences is the one shown in equation (2) and this was used in this study.

$$L = \frac{Q \cdot S_o}{f \cdot V_f \cdot MLVSS} \qquad (2)$$

Where: L = organic loading (mgCOD/gVSS.d)

Q = influent flow rate (l/d)

S_o = influent COD (mg/l)

f = aerated fraction of the whole cycle

V_f = reactor volume at fill (l)

MLVSS = aerobic mixed liquor volatile suspended solids (g/l)

3.3.3.6. Available phosphorus

To describe the amount of available phosphorus for the aerobic fraction of the biomass the term P/M was used. It was calculated according to equation (3).

$$P/M = \frac{V_i \cdot P_i}{V_f \cdot MLVSS} \qquad (3)$$

Where: P/M = available phosphorus (mgP/gVSS)

V_i = influent volume (l)

P_i = influent [PO_4-P] (mg/l)

V_f = reactor volume at fill (l)

MLVSS = aerobic mixed liquor volatile suspended solids (g/l)

3.3.3.7. Calculations of mass of phosphorus removed and anaerobically released

In chapter 4 four terms are used for the description of the performance of the EBPR process.

The % influent phosphorus removed describes the phosphorus removal efficiency of the reactor.

The term [PO_4-P] accumulated (in mgP/gVSS), describes the mass of phosphorus per unit MLVSS residing in the reactor at the end of the aerobic stage.

The amount of influent phosphorus removed per unit aerobic MLVSS, for one operating cycle, was calculated according to equation (4).

$$P_{rem} = (P_i - P_{eff})/MLVSS \quad (4)$$

Where: P_{rem} = phosphorus removed per unit MLVSS (mgP/gVSS)

P_i = mass of influent [PO_4-P] per cycle (mg)

P_{eff} = effluent [PO_4-P] per cycle (mg)

MLVSS = mass of aerobic volatile suspended solids (g)

The mass of dissolved orthophosphate released during anaerobic conditions per unit MLVSS, for one operating cycle, was calculated according to equation (5).

$$P_{rel} = [\max P_{an} - (P_{idleprev} + P_i)]/MLVSS \quad (5)$$

Where: P_{rel} = net phosphorus released per unit MLVSS (mgP/gVSS)

$maxP_{an}$ = mass of maximum observed [PO_4-P] released under anaerobic conditions (mg)

$P_{idleprev}$ = mass of [PO_4-P] remaining in the sup/nt after the end of the previous cycle (mg)

P_i = influent [PO_4-P] per cycle (mg)

MLVSS = mass of aerobic volatile suspended solids (g)

3.3.3.8. Synthetic feed composition

The reactors were fed with synthetic wastewater prepared daily. The synthetic feed consisted of two parts. The main nutrient solution and a trace element solution. The main nutrient solution, which included carbon, nitrogen, phosphorus, magnesium

TABLE 3.2. MAIN FEED COMPOSITION.

Chemical	Amount (g/l)	Feed element (mg/l)
CH_3COONa	0.52 - 1[1]	290 - 750 as COD
NH_4Cl	0.15 - 0.25[1]	35 - 52 as [NH_4-N]
KH_2PO_4	0.03 - 0.14[1]	4 - 30 as [PO_4-P]
$MgSO_4$ $7H_2O$	0.12	16 as Mg
$CaCl_2$	0.04	15 as Ca

1. Varied according to experimental run

TABLE 3.3. TRACE ELEMENTS USED IN FEED SOLUTION.

Trace Elements	Amount (mg)[1]
$FeCl_3$ $6H_2O$	500
$MnCl_2$ $4H_2O$	200
$ZnCl_2$	200
KBr	2.5
KI	2.5
$CuSO_4$ $5H_2O$	0.05
$CoCl_2$	0.05
H_3BO_3	10.0
$Na_3C_6H_5O_7$ $2H_2O$	1000

1. In 1l of water

and calcium, was weighed every day and dissolved into tap water. A solution containing trace elements was prepared on a monthly basis and kept in the refrigerator. The composition of the main nutrients is presented in table 3.2. It must be mentioned here that both carbon and phosphorus concentrations were altered during the different runs (for details see chapter 4).

The trace elements were prepared at the beginning of each month and were kept in the refrigerator. Their composition is presented in table 3.3. and it was selected according to Manning and Irvine (1985). The amount shown was diluted in 1 litre of water and of it 1 ml per litre was added to the main feed.

Feed pH varied between 7.5 and 7.7 except for the occasions were it was kept for longer than 24 hours, in which case the pH increased to about 8. After pH monitoring in the first stages of the reactors' operation, it was decided that the reactor showed adequate buffering capacity, making the adjustment of feed pH unnecessary.

3.4. STORAGE AND THICKENING LAB-SCALE REACTORS

3.4.1. Bench Scale Storage

3.4.1.1. Quiescent bench scale storage

For the quiescent batch storage experiments 1 litre of excess sludge was withrawn from the SBR and placed in a 1 litre, glass volumetric cylinder. The storage experiments were performed with the addition of well mixed excess sludge, which was then left to settle. Sampling took place at variable time intervals according to the experiments from both the supernatant and the settled sludge. In the quiescent storage experiments the contents of the cylinder were not mixed.

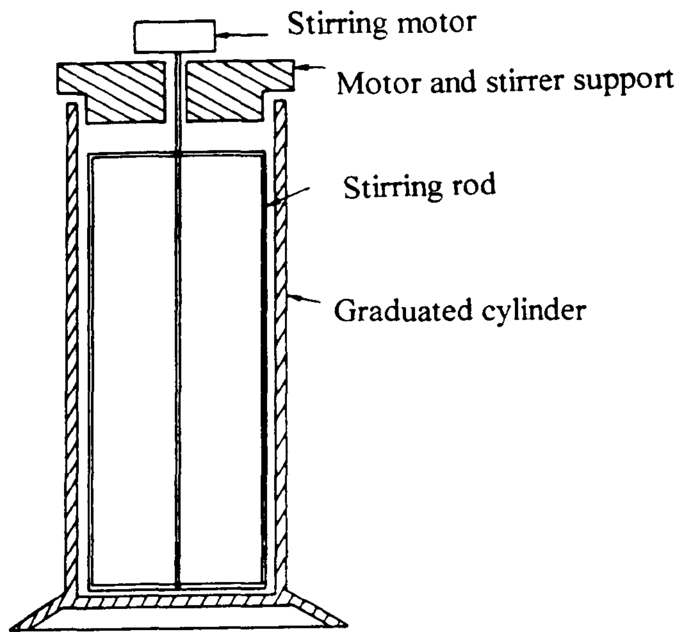


Fig. 3.6. Bench scale thickening apparatus (stirred sludge volume index apparatus, APHA, 1989).

3.4.1.2. Fully stirred anaerobic and aerobic bench scale storage

An identical procedure to 3.4.1.1. was used. The main difference between the quiescent and fully stirred experiments was that in the latter the sludge was continuously stirred. In the anaerobic experiments the cylinder was sealed and its contents were stirred with a magnetic stirrer. In the aerobic experiments, in addition to magnetic stirring, air was administered through a glass diffuser.

3.4.2. Bench Scale Thickening

The batch thickening experiments were performed in a 1.2 litre column with a stirring mechanism rotating at approximately 1.3 cm/sec (figure 3.6.). The apparatus was chosen because its slow stirring closely approximates the thickening characteristics obtained in full scale thickeners (Dick and Ewing, 1967; Vesilind, 1980). As with the storage experiments, the sampling positions for both the supernatant and the sludge, were kept constant and sampling took place at various time intervals during the progress of the experiment.

3.4.3. Sampling

For the determination of the phosphorus released during the sludge storage and thickening experiments, a 15 ml sample of the supernatant was removed. The sampling position was in the centre, 4 cm above the sludge blanket - supernatant interface and was kept constant. This was considered appropriate as it has been shown that in full scale thickeners the dissolved phosphate concentration near the bottom and centre of the basin is greater. As the ploughs move through the sludge they cause some turbulence. The sludge settles again but the dissolved orthophosphate does not and as a result an increase in concentration is observed immediately above the sludge blanket (Shapiro *et al.*, 1967).

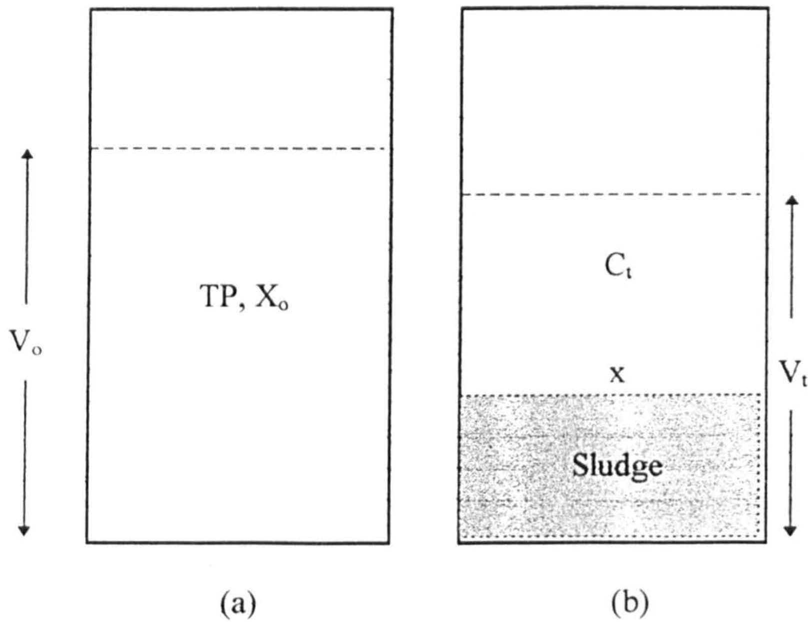


Fig. 3.7. Sampling position and volume and concentration definitions during a typical thickening/storage experiment at (a) start of the experiment and (b) at time t after the start of the experiment. The supernatant sampling position is marked by x.

V_o: initial volume of completely mixed sludge

TP: total phosphorus concentration as measured by sludge digestion at t=0

X_o: VSS concentration at t=0

C_t: measured dissolved [PO_4-P] concentration at time t

V_t: combined volume of supernatant and sludge at time t

Sludge samples for the determination of phosphorus fractions in the sludge and the solids concentration were collected from the centre of the container, 2 cm above the bottom. The volume removed was 10 ml.

3.4.4. Mass Calculations

In this study, for calculating the amount of phosphorus released during sludge treatment, it was assumed that the measured concentration was uniform throughout the thickener or storage container. The sampling position was chosen to assure that the inaccuracy introduced by this assumption was an over-estimation of the phosphorus released rather than an under-estimation.

Figure 3.7., shows the sampling position (marked by x) and the volume and concentration definitions during a typical thickening/storage experiment at the start of the experiment and at time t after the start of the experiment.

For the calculation of the mass of phosphorus released per unit biomass equation (6) was used.

$$[PO_4 - P]\text{released} = \frac{C_t \cdot V_t}{X_o \cdot V_o} \qquad (6)$$

Where: $[PO_4\text{-}P]$ released = phosphorus released per unit of initial volatile solids (mgP/gVSS)

C_t = measured dissolved $[PO_4\text{-}P]$ concentration at time t (mg/l)

V_t = combined volume of supernatant and sludge at time t (l)

V_o = initial volume of completely mixed sludge (l)

X_o = VSS concentration at t=0 (g/l)

For the calculation of the mass of phosphorus released as a percentage of the initial total phosphorus sludge content equation (7) was used.

$$\%\text{TPreleased} = \frac{C_t \cdot V_t}{TP \cdot V_o} \cdot 100 \qquad (7)$$

Where: %TP released = phosphorus released as a percentage of the initial total phosphorus sludge content

C_t = measured dissolved [PO_4-P] concentration at time t (mg/l)

V_t = combined volume of supernatant and sludge at time t (l)

TP = total phosphorus concentration as measured by sludge digestion at t=0 (mg/l)

V_o = initial volume of completely mixed sludge (l)

3.5. SAMPLE STORAGE

Samples for all measurements were collected in glass bottles and analysed on the same day. When that was not possible, they were acidified by addition of 0.8 ml conc. H_2SO_4/l of sample and stored overnight at 4°C. No samples were analysed later than 24 hours after their collection.

3.6. REAGENTS AND SUPPLIERS

All chemical reagents used for the preparation of feed solutions were general purpose reagents and the suppliers were BDH Chemicals.

All chemical reagents used for analytical determinations were of analytical grade. The suppliers were BDH Chemicals apart for the Methyl orange solution which was supplied by Merck Ltd.

3.7. ANALYTICAL TECHNIQUES

3.7.1. Determination of Physical and Aggregate Properties

3.7.1.1. Solids determination

All solids were determined according to Standard Methods for the Examination of Water and Wastewater (1989). For the determination of total solids and total suspended solids, method numbers 2540 B, page 2-72 and 2540 D, page 2-75 were used respectively. Fixed and volatile solids were measured according to method 2540 E, page 2-77. All samples were filtered through Whatman GF/C filter papers.

3.7.1.2. Sludge volume index determination

Measurements of settled sludge volume, sludge volume index and zone settling rate were done according to Standard Methods for the Examination of Water and Wastewater (1989). The methods followed were 2710 C, page 2-83, 2710 D, page 2-84 and 2710 E, page 2-84, respectively.

3.7.2. Determination of Metals

3.7.2.1. Total metals

The total concentration of three metals, calcium, magnesium and potassium, was determined by digestion with the nitric acid digestion method and with the nitric acid - sulphuric acid digestion method (numbers 3030 E, page 3-8 and 3030 G, page 3-9, respectively).

3.7.2.2. Pretreatment of samples for the determination of dissolved and suspended metals

Dissolved and suspended concentrations of three metals, calcium, magnesium and potassium, were determined. Dissolved and suspended metals were determined after preliminary filtration according to Standard Method 3030 B, page 3-5.

3.7.2.3. Determination of metals by atomic absorption spectrometry

For the determination of dissolved and suspended concentrations of calcium and magnesium, atomic absorption spectrometry was used (Standard Method numbers 3500-Ca B, page 3-85 and 3500-Mg B, page 3-112, respectively). The instrument used was a PYE UNICAM SP9 atomic absorption spectrophotometer. Both calcium and magnesium were determined with the same hollow cathode lamp, type 3QNY/Ca-Mg (Cathodeon Ltd.).

Calcium was determined at a wave length of 422.7 nm. The band pass was 0.2 nm and the flame air/acetylene, stoichiometric. The lamp current was set at 4 mA. The detection limit of the method is 0.08 mg/l.

Magnesium was determined at a wave length of 285.2 nm. The band pass was 0.4 nm and the flame air/acetylene, stoichiometric. The lamp current was set at 4 mA. The detection limit of the method is 0.003 mg/l.

3.7.2.4. Determination of metals by flame emission spectrometry

Potassium was determined by flame emission spectrometry (Standard Method 3500-K D, page 3-125). The wave length used was 766.5 nm, using a fuel lean air/acetylene flame. The same spectrophotometer as in 3.6.2.2. was used. The detection limit of the method is 0.011 mg/l.

3.7.2.5. Determination of intracellular and extracellular metals

For the fractionation of the cation content of the cells the method described by Bonting *et al.* (1993) was followed. To a well mixed 1ml sample of sludge 0.4ml of

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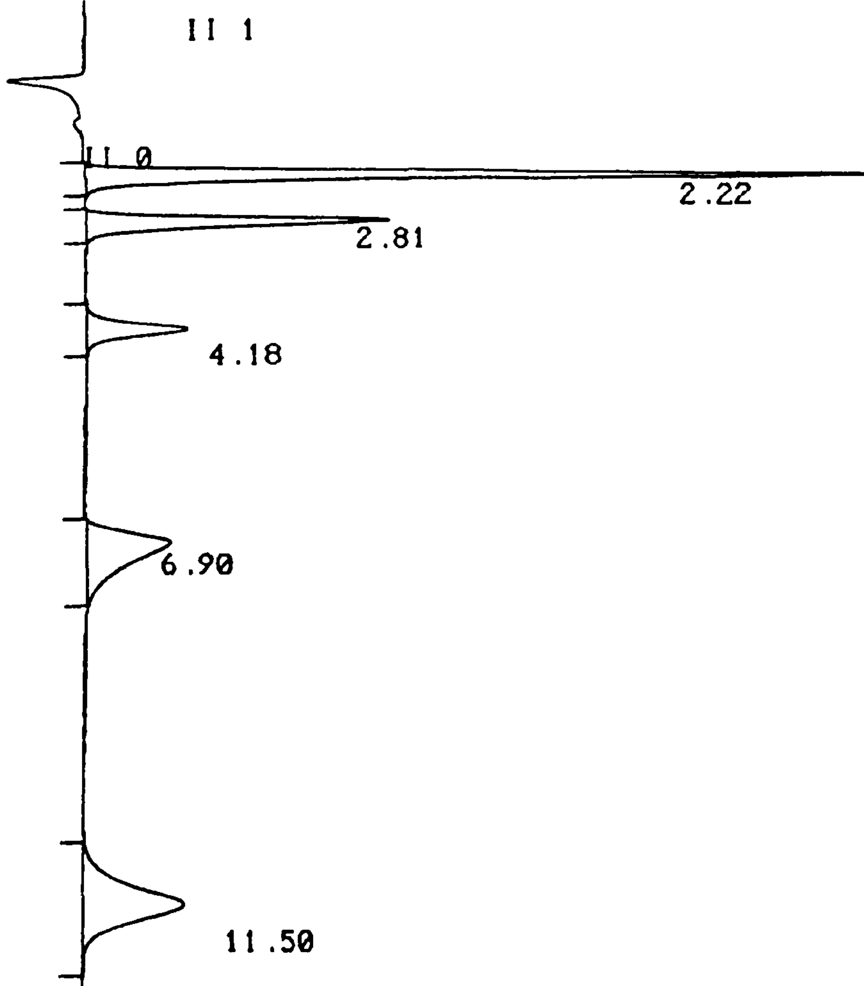

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31-08-94 18:43:00 CH= "A" PS=

FILE 1. METHOD 5. RUN 422 INDEX 2 BIN

ANALYST: LINA

NAME	MG/L	RT	AREA	BC	RF
CHLORIDE	4.805	2.22	6984513	01	1453592.716
NITRITE	4.709	2.81	3477813	01	738545.976
PHOSPHATE	4.6	4.18	1628595	01	354042.392
NITRATE	4.743	6.9	3104509	01	654545.436
SULPHATE	4.874	11.5	4264532	01	874955.272

Figure 3.8. Typical chromatogram for the determination of anions with ion chromatography.

silicon oil (d=1.023) was added. The samle was then centrifuged. The supernatant and silicon oil were discarded, the pellet was resuspended in water and the total cation content was determined as described in 3.7.2.1. This fraction constituted the intracellular portion of cations. The amount of extracellular cations was calculated as the total cation content of the initial sludge fraction minus the intracellular fraction.

3.7.3. Determination of Inorganic Non-metallic Constituents

3.7.3.1. Determination of anions by ion chromatography

Chloride, nitrite, nitrate, phosphate and sulphate were determined by ion chromatography (Standard Method 4110 B, page 4-2). The chromatograph used was a DIONEX 2000i/SP. The separation column used was an IonPac AS4 (P/N 35311). This was preceded by a guard column, IonPac AG4A, 4mm (P/N 37042). The mobile phase consisted of the eluant (1.7 mM $NaHCO_3$ and 1.8 mM Na_2CO_3). The regenerant consisted of a 25 mN solution of sulphuric acid. The eluant flow rate was 2 ml/min and the regenerant flow rate was 3 ml/min. The sample loop volume used was 50μL. The chromatograph had a background conductivity of 15-19 μS. The working output range was 30 μS.

Samples were filtered prior to injection through Whatman 0.2 μm (25 mm in diameter), cellulose nitrate membrane filters. All dilutions as well as the eluant and regenerant were prepared with de-ionised water (NANOpure II, 16.7 megohm·cm).

The data were processed by an datajet integrator made by Spectra-Physics Analytical. A typical chromatogram with retention times and peak areas for the five anions measured is shown in figure 3.8. The detection limit of the process is dependent on the daily signal to noise ratio, but in this study the lower concentration regarded as above the detection limit was 0.5 mg/l for all anions.

3.7.3.2. Determination of ammonia

Ammoniacal nitrogen was determined with the Nesslerisation method (Standard Method 4500-NH_3 C, page 4-117). The spectrophotometer was a SP6-550 UV/VIS, made by PYE UNICAM. Ammonia was determined at 400 nm with a light path of 1 cm. The detection limit of the method is 20μg NH_3-N/l.

3.7.3.3. Determination of organic nitrogen

Total Kjeldal Nitrogen (TKN) was determined with the semi-micro-Kjeldal method (Standard Method 4500-NH_{org} C, page 4-147). The same spectrophotometer and conditions as in 3.6.3.2. apply. The detection limit of the method is 0.2 mg/l TKN.

3.7.3.4. Determination of phosphorus

Dissolved orthophosphate was determined by both ion chromatography and by the vanadomolybdophosphoric acid colorimetric method (Standard Method 4500-P C, page 4-173). For concentrations between 1.0-5.0 mgP/l, a wave length of 400 nm was used. For concentrations between 2.0-10.0 mgP/l, the wave length was 420 nm. The minimum detectable concentration is 200μgP/l.

Total phosphorus was determined by the persulphate digestion method (Standard Method 4500-P B.5, page 4-172) followed by orthophosphate determination as above.

3.7.3.5. Determination of dissolved oxygen (D.O.)

Dissolved oxygen (D.O.) was measured using a glass membrane electrode (Standard Method 4500-O F, page 4-158). The D.O. meter was a Kent EIL 7135 model.

Extract	Phosphorus Determination and Fraction
50ml mixed liquor	Total phosphorus
↓ centrifuged at 8.650 rpm for 5 min	
Filtered Centrate	Soluble orthophosphate
Centrate	Orthophosphate and colloidal organic phosphorus
Residue	Washed: 0.1M tris buffer at pH 7.5 (x1)
↓ centrifuged at 8.650 rpm for 5 min	
Centrate	Tris fraction - Exocellular phosphorus
Residue	Extr.: 0.5N PCA (5°C) for 30min (x2). Washed: 0.5N PCA (x1)
↓ centrifuged at 8.650 rpm for 5 min	
Centrate	Cold acid fraction - orthophosphate, LMW polyphosphate
Residue	Washed: 80% EtOH (5°C) (x2). Washed: 95%EtOH (5°C) (x2)
↓ centrifuged at 8.650 rpm for 5 min	
Centrate	EtOH fraction - Lipid phosphorus
Residue	Extr.: EtOH/Ether (3:1) (60°C) for 5 min (x2). Washed: 95% EtOH (5°C) (x1)
↓ centrifuged at 8.650 rpm for 5 min	
Centrate	EtOH/Ether fraction - Lipid phosphorus
Residue	Extr.: 1N PCA (20°C) overnight. Extr.: 0.5N PCA (90°C) for 10min (x2). Washed: 0.5N PCA (x1)
↓ centrifuge at 8.650 rpm for 5 min	
Centrate	Hot acid fraction - HMW polyphosphate and TNA
Residue	Extr.: 2% NaOH (90°C) for 10 min (x2)
↓ centrifuge at 8.650 rpm for 5 min	
Centrate	Alkaline fraction - Protein phosphorus
Residue	Total phosphorus

Figure 3.9. Phosphorus fractionation procedure according to the modified STS method (Mino *et al.*, 1984). LMW: low molecular weight, HMW: high molecular weight an TNA: total nucleic acids.

3.7.3.6. Determination of oxygen redox potential (ORP)

Oxygen redox potential (ORP) was measured with a combined hydrogen glass electrode. The O.R.P. meter was supplied by Hanna Instruments (model HI 8424) and the combined glass electrode was the HI 3230 model.

3.7.3.7. Determination of pH

pH was measured with the use of a glass electrode pH meter (Standard Method 4500-H^+ B, page 4-95). The pH stick was a FISONS PHK-121-800J and the glass electrode a FISONS PHK-124-030X.

3.7.3.8. Determination of COD

Chemical oxygen demand was determined with the closed reflux, titrimetric method (Standard Method 5220 C, page 5-14). The detection limit of the method is 50 mgO_2/l and the variation ± 11 mgO_2/l.

3.7.4. Phosphate Fractionation Method

For the fractionation of phosphorus compounds the modified STS method was used (Mino *et al.*, 1984; Jing *et al.*, 1992). The procedure is detailed in figure 3.9. and the fractions of phosphorus extracted at each step have been included in table 3.4.

Measurements were done in triplicate and the values averaged out. It was found that the recovery was between 90 and 105%. Due to the time necessary to perform the fractionation steps, only a few representative measurement were done for each experimental run. The accuracy of the method has been tested by ^{31}P-NMR spectroscopy (Mino *et al.*, 1984; Jing *et al.*, 1992).

TABLE 3.4. PHOSPHORUS FRACTION EXTRACTED AT EACH STEP OF THE MODIFIED STS METHOD

Fraction	Possible Phosphorus Content
Mixed liquor	All phosphorus compounds
Centrifuged/filtered effluent sup/nt	Orthophosphates
Centrifuged effluent sup/nt	Orthophosphates, colloidal organic phosphorus
Tris fraction	Exocellular phosphate
Cold acid fraction	Orthophosphates, low molecular polyphosphate
Ethanol fraction	Ethanol soluble phosphorus (lipids)
Ether/Ethanol fraction	Lipid phosphates
Hot acid fraction	Total nucleic acids, high molecular polyphosphate
Alkaline fraction	Proteins containing phosphorus compounds
Residue	Remaining unextractable phosphorus

3.8. MICROBIOLOGICAL METHODS

3.8.1. Pure Culture Development

3.8.1.1. Culture details

During the course of the experimental work the need for a pure culture of polyphosphate accumulating bacteria arose. The strain selected was *Acinetobacter lwoffi* (NCIMB 12456). The inoculum for the preparation of the laboratory culture were freeze-dried bacterial cells in dried horse serum and nutrient broth. The cultures were obtained from the National Collection of Industrial and Marine Bacteria Ltd, 23 st. Machar Drive, Aberdeen, AB2 1RY, Scotland, U.K.

3.8.1.2. Revival of the culture

The freeze-dried sample was received in a vacuum packed, glass ampoule. The ampoule was cracked and 0.5 ml of sterile Ringers solution was added to the dry contents of the ampoule. The resulting suspension was sub-cultured to 10 petri dishes filled with solid nutrient agar (pH 6.8). The subcultures were then incubated at 30 °C for 48 hours. After the incubation period the colonies were removed from the plates by a sterile loop and placed in sterile liquid media containing the same concentration of nutrients as shown in table 3.2. at pH 7.0. After 48 hours of aeration, the culture was added to the activated sludge reactor at a concentration of 2.3×10^8 cells per ml. This technique resulted in the bioaugmentation of the activated sludge SBR with *Acinetobacter lwoffi*.

3.8.2. Staining

3.8.2.1. Neissers' methylene blue

Although there are doubts as to whether the Neisser staining procedure can successfully differentiate between polyphosphate and poly-β-hydroxybutyrate granules (Streichan *et al.*, 1990), it is widely used and was also applied in this study. The method used was the modified Neisser method as presented by Cruickshank *et al.* (1975).

3.8.2.2. Sudan black

Sudan black was used for the staining of intracellular lipids, namely poly-β-hydroxybutyrate. The method used the modified Burdons' method as presented by Cruickshank *et al.* (1975).

3.8.2.3. Preparation of slides for staining

Activated sludge samples were removed from the SBR at different times during its daily operation. The samples were homogenised at a rapid mixed and then diluted with ringers solution to a final concentration of 10, 100 and 1000 times the original. A glass slide was smeared with the use of a sterile loop and then fixed either by flame or air drying. The staining procedure was then followed accordingly.

CHAPTER 4. THE DEVELOPMENT OF ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

4.1. INTRODUCTION

This chapter describes the first two phases of the experimental work.

The first phase, presented in section 4.2., consists of the development of enhanced biological phosphorus removal (EBPR) in the lab sequencing batch reactor (SBR) during start-up. A description of the three methods employed (enhanced culture development, pure culture bioaugmentation and EBPR sludge inoculation) for the development of EBPR is presented, along with the performance of the reactor for each method.

These results have been presented in the First IAWQ Specialised Conference on Sequencing Batch Reactor Technology, held in Munich, Germany, in March 1996 and will be published in the journal of Water Science and Technology (Belia and Smith, in print).

In the second phase of the experimental programme (section 4.3.), the operation of the reactor for the development of excess sludge to be used for the thickening and storage experiments, is presented. Since very little has been published on the effect of operational parameters on phosphorus release during sludge treatment, it was decided to investigate the operational parameters that have been shown to influence anaerobic phosphorus release during the first stage of the operating cycle of the SBR, the anaerobic stage. This, second experimental phase, explores the correlation between

the operational parameters employed and the extent of the anaerobic release observed, as well as the phosphorus removal capabilities of the sludge produced.

4.2. PHASE 1: ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL - DEVELOPMENT OF START-UP METHOD

4.2.1. Objective

The objective of the first phase of the experimental work was the establishment of a fast method for the development of enhanced biological phosphorus removal (EBPR) in the laboratory sequencing batch reactor. This was necessary as EBPR sludge for the sludge treatment experiments was otherwise unobtainable.

4.2.2. Methodology for the Determination of Optimum Start-up Method for EBPR Development

4.2.2.1. Reactor operation

Initially two methods were employed and compared for their effectiveness regarding the development of EBPR. The first aimed at the development of an enhanced culture of polyP organisms through the application of certain operating procedures, widely quoted in the literature (Wentzel *et al.*, 1988; Kortstee *et al.*, 1994 and many others). These procedures, consisted of the addition of conventional activated sludge to the reactor, operating the reactor in an anaerobic - aerobic sequence, providing adequate RBCOD in the influent (use of acetate as the carbon source) and minimising nitrate-nitrogen recycle in the anaerobic zone (long idle period for denitrification and appropriate TKN/COD ratios).

TABLE 4.1. OPERATIONAL PARAMETERS OF THE RUNS USED FOR THE DETERMINATION OF THE OPTIMUM START-UP METHOD.

RUN	**N/COD** (gN/gCOD)	**P/M** (mgP/gVSS)	**L** (gCOD/gVSS.d)	**STR_E** (d)
Control	0.06	3.7	0.76	9
Bioaugmentation	0.06	4.1	0.76	9
EBPR inoculum	0.10	5.5	0.64	8

The second method, designated as the bioaugmentation procedure, consisted of operating the reactor in an identical mode as in method one and adding, a pure culture of *A.lwoffi* at the start of the run to the conventional activated sludge. The bacterial culture was resuscitated from a freeze dried sample according to the method described in chapter 3 (Materials and Methods, section 3.8.1.) and was added at an average concentration of 2.3×10^{8} cells per ml.

After the successful application of the bioaugmentation procedure a third method was also employed, designated as the EBPR sludge inoculation method. The reactor was operated as in methods one and two and a volume of EBPR sludge (10% - 50% on the basis of VSS), retained from a bioaugmentation run, was added at the start of the run to the conventional activated sludge.

4.2.2.2. Operational parameters

The selection of the influent characteristics during this first experimental phase, was based on the values quoted in the literature as being optimum. The intention was to speed up the process by providing the optimum growth conditions, in the form of the influent COD/P ratio and organic loading (L), as well as the effective sludge retention time (STR_E), for the phosphorus removing microorganisms (Table 4.1.). For a detailed description of the choice of the operational parameters the reader is referred to chapter 3 (Materials and Methods). Influent values and operating solids concentrations are included in Appendix II, Table II.1.

4.2.3. Results of Phase 1

4.2.3.1. Enhanced culture development - control run

As already mentioned (section 4.2.2.1.), for the first method of EBPR development, the control SBR was seeded with activated sludge from a municipal sewage treatment plant with no phosphorus removing capabilities and an enhanced culture of polyphosphate accumulating bacteria was allowed to develop.

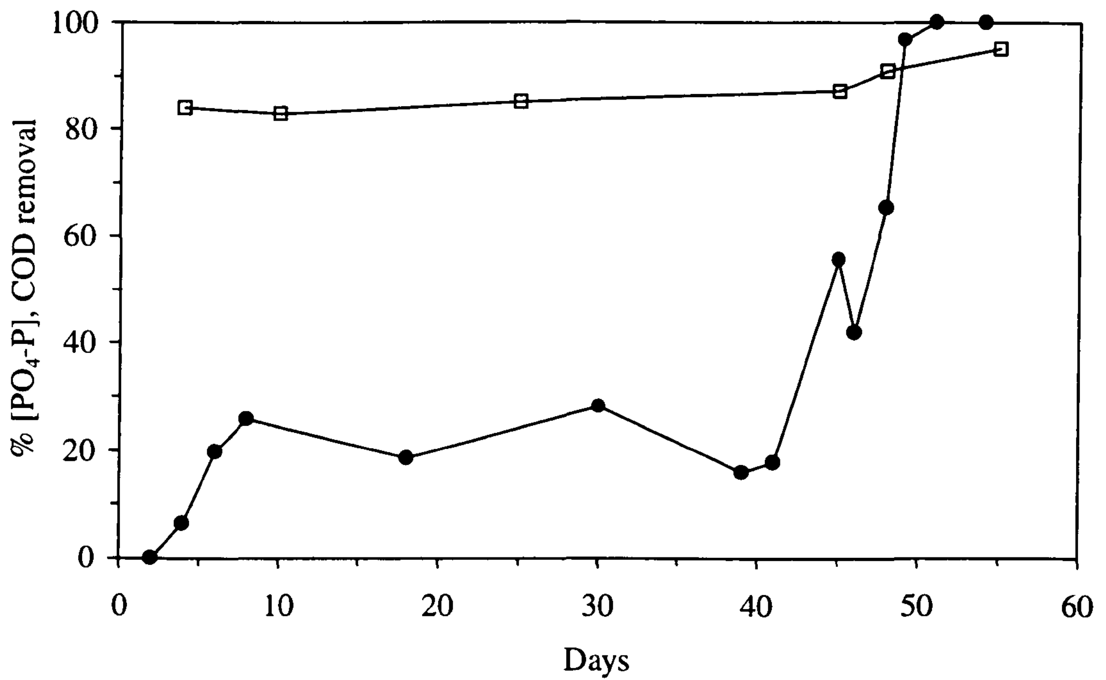


Fig. 4.1. EBPR development during a control run. (□) % influent COD removed and (●) % influent P removed.

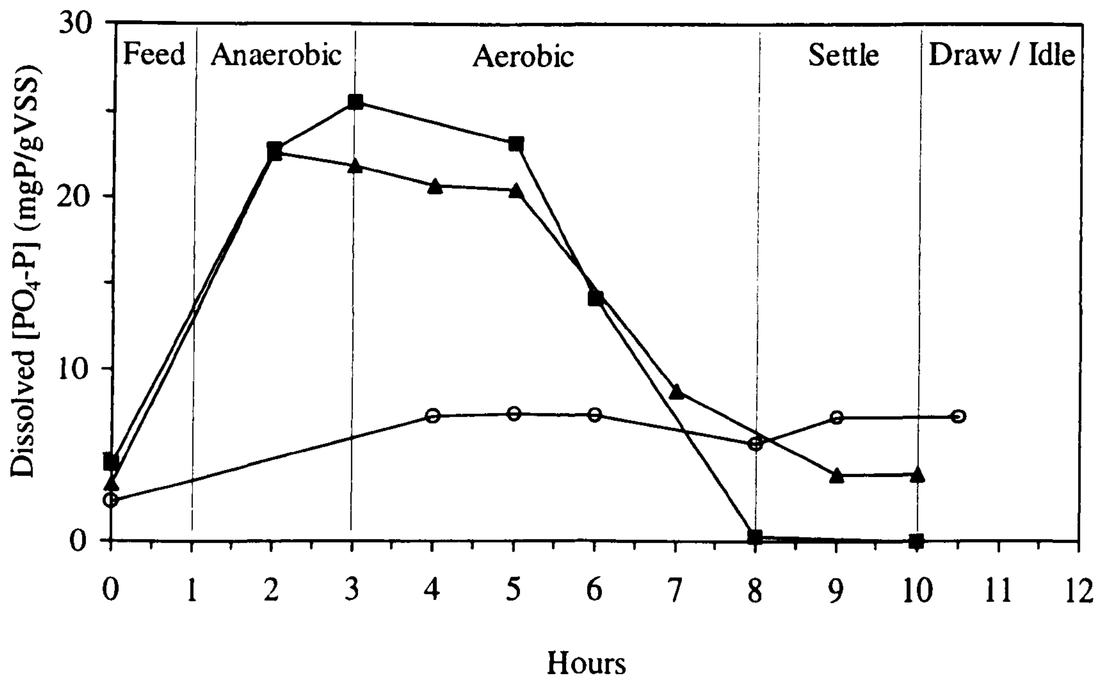


Fig. 4.2. Change of daily dissolved phosphorus profile with the development of EBPR during a full cycle of a control run. (O) no EBPR - 6% infl. P removal, (▲) low EBPR - 65% infl. P removal, (■) high EBPR - 100% infl. P removal.

An enhanced culture can be described as a culture of polyP organisms that grow and dominate in the activated sludge system because the applied substrate and environmental conditions favour them. Growth of the rest of the heterotrophic biomass that can not successfully compete is slowly reduced but does not seize. There is no positive exclusion of interactive effects between the different microorganism groups or of predation by higher organisms (Wentzel *et al.*, 1988). Finally, with this method, a strain of polyP organisms will be selected that may be different from the ones isolated and grown by pure culture studies. It may also not be the same bacterial strain for different start-up sludges

During the start up of lab scale reactors following the above procedure, EBPR has been shown to develop between 30 and 100 days (Manning and Irvine, 1985; Cech and Hartman, 1993; Appeldoorn *et al.*, 1992). The control reactor in this study, developed EBPR characteristics, as indicated by both the phosphorus removal rates (figure 4.1.) and the phosphorus profile (figure 4.2.) during the SBR cycle, over a period of 50 to 60 days.

The COD removal properties of the reactor were high (above 80%) from the beginning of the run (figure 4.1.).

The daily profile, at the end of the 50 day period, displayed the typical characteristics of EBPR; high anaerobic release and rapid aerobic uptake with undetectable phosphorus concentration in the effluent (figure 4.2.).

4.2.3.2. EBPR development through pure culture addition - bioaugmentation run

The second method that was implemented was the addition of a pure culture of resuscitated bacteria of the genus *Acinetobacter*. In this study the addition of a pure culture to a mixed culture for the purpose of improved reactor performance is designated the bioaugmentation method.

The use of bioaugmentation for the improvement of the operation of activated sludge plants has had mixed success and published results on its effectiveness have been judged inconclusive (Stephenson and Stephenson, 1992). When successfully applied though, bioaugmentation has been shown to assist with plant start up

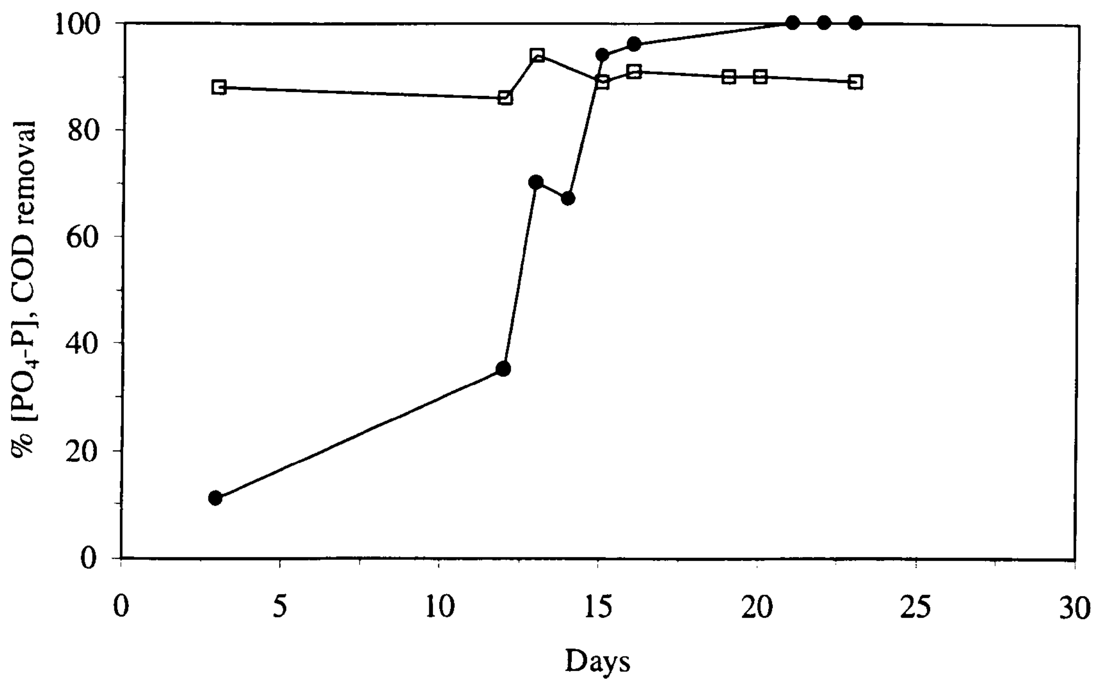


Fig. 4.3. The development of EBPR for bioaugmentation at t=0. (□) % influent COD removed and (●) % influent P removed.

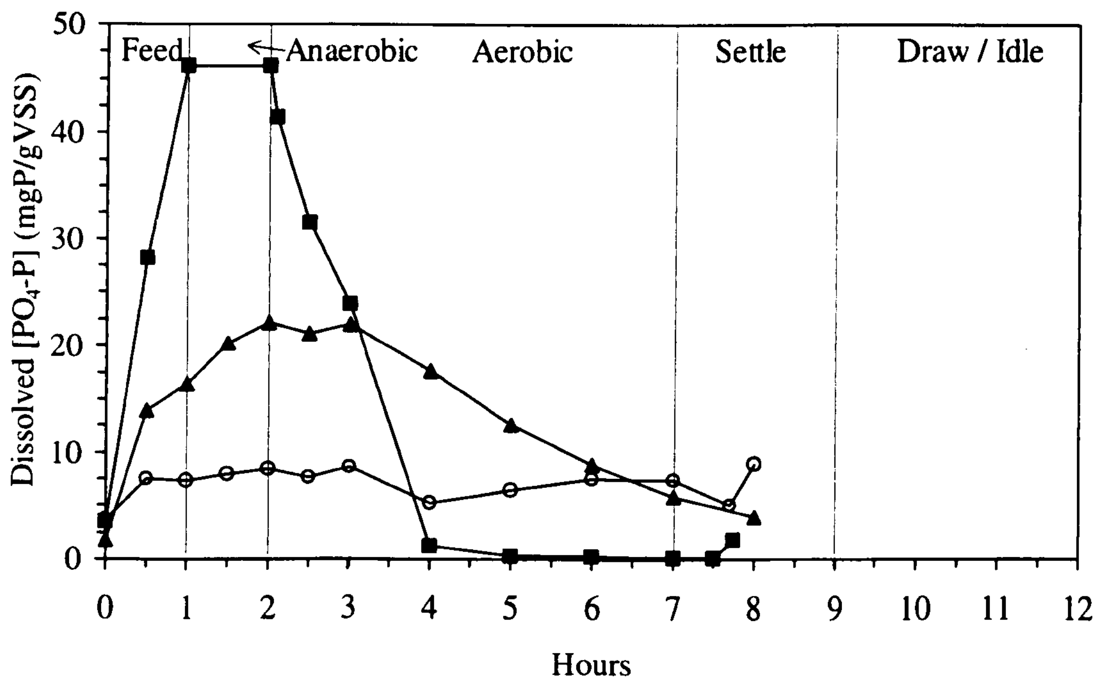


Fig. 4.4. Change of dissolved phosphorus profile with the development of EBPR during a full cycle. (○) no EBPR - 6% infl. P removal, (▲) low EBPR - 65% infl. P removal, (■) high EBPR - 100% infl. P removal.

(Stevens, 1989; Wilderer *et al.*, 1991), organic and hydraulic overloading (Stevens, 1989), removal of toxic compounds or other problematic wastewater components (Ying *et al.*, 1986) and sludge settleability (Chambers, 1981).

Pure culture addition for the development of EBPR has only rarely appeared in the literature. Fuhs and Chen (1975), quoting their unpublished results, noted that addition of a pure culture of *Acinetobacter lwoffi* to a laboratory activated sludge culture resulted in phosphate accumulation with no biomass acclimatisation. Yeoman *et al.* (1988b), also found that for operating temperatures between 18-25°C and a sludge age of 12 days the augmented reactor gave 70% removal as compared to 40% in the control.

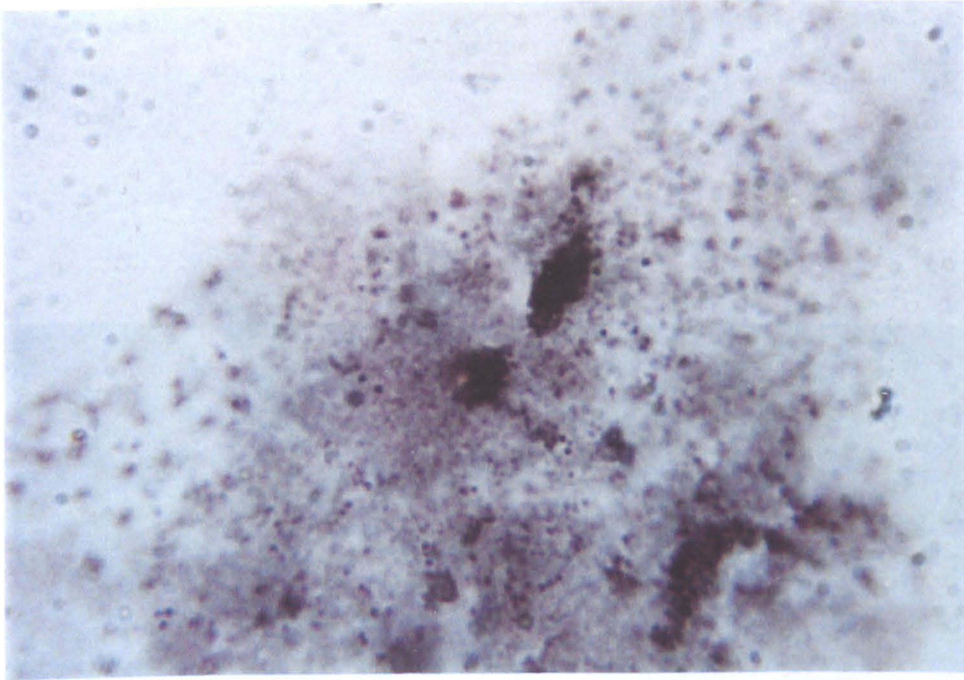
In this study, the reactor was started with conventional activated sludge to which the pure culture was added at run time t=0 and the effect of the bioaugmentation on the development of EBPR was investigated. The reactor required only 15 days to reach removal rates of 90% (figure 4.3).

As with the control run, the COD removal rate was above 80% of the influent from the beginning of the run (figure 4.3.) and the daily profile developed the typical characteristics of EBPR in a far shorter time than the control (figure 4.4.).

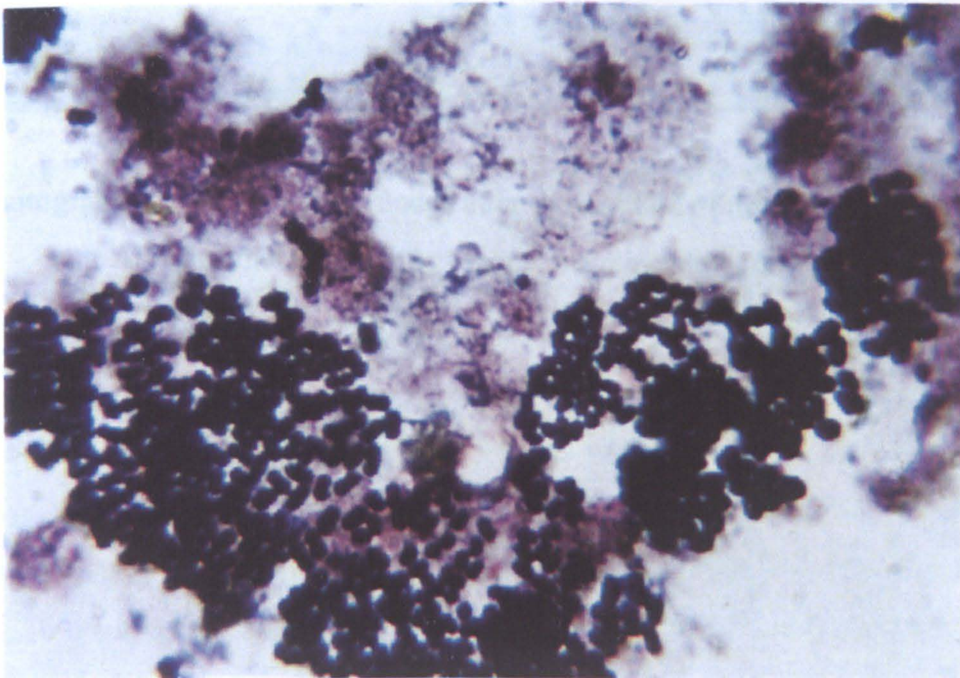
Microscopic investigation of the sludge before (photograph 4.1.) and after (photograph 4.2.) the addition of the pure culture revealed the change that took place in the biomass. Phosphorus removing bacteria, stained Neisser positive, dominated and their increasing rate of appearance was concomitant with the increasing phosphorus removal capabilities of the reactor. Gram staining revealed them to be gram negative rods and cocci (photograph 4.3.).

4.2.3.3. EBPR development through the addition of bioaugmented sludge - EBPR inoculum run

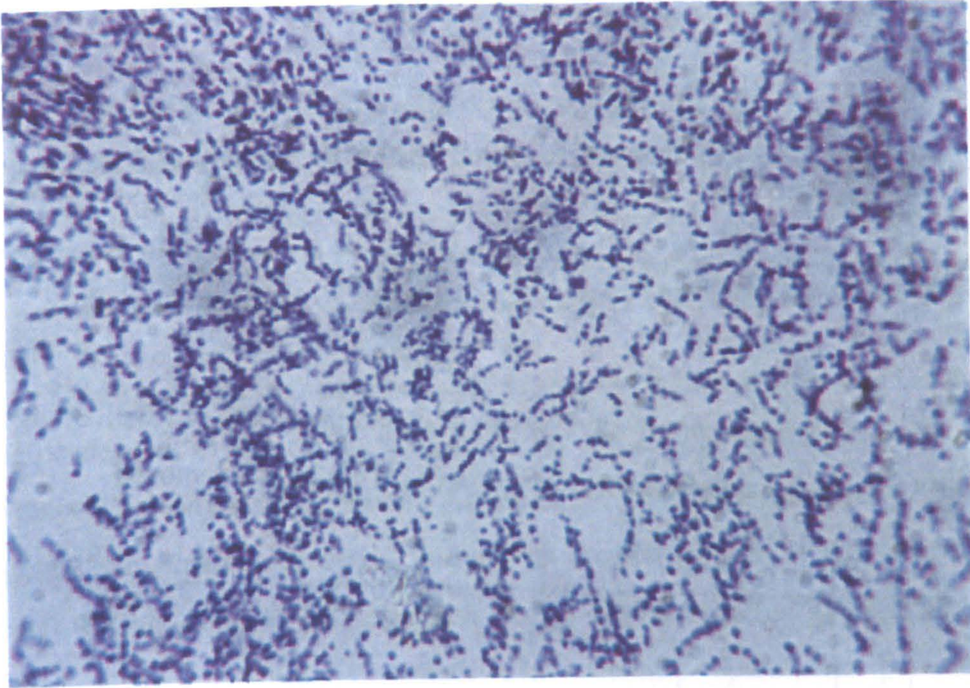
The third method of start-up consisted of adding to the SBR a mixture of conventional activated sludge and a percentage (10% - 50%) of EBPR sludge (percentages on the basis of VSS weight), retained from a previous bioaugmentation run. The higher the percentage of EBPR added, the shorter the start-up time required.



Photograph 4.1. Neisser stained sludge before the addition of the pure culture. (Oil immersion light microscopy, magnification 1000).



Photograph 4.2. Neisser stained sludge after the addition of the pure culture. (Oil immersion light microscopy, magnification 1000).



Photograph 4.3. Gram stained sludge after the addition of the pure culture. (Oil immersion light microscopy, magnification 1000).

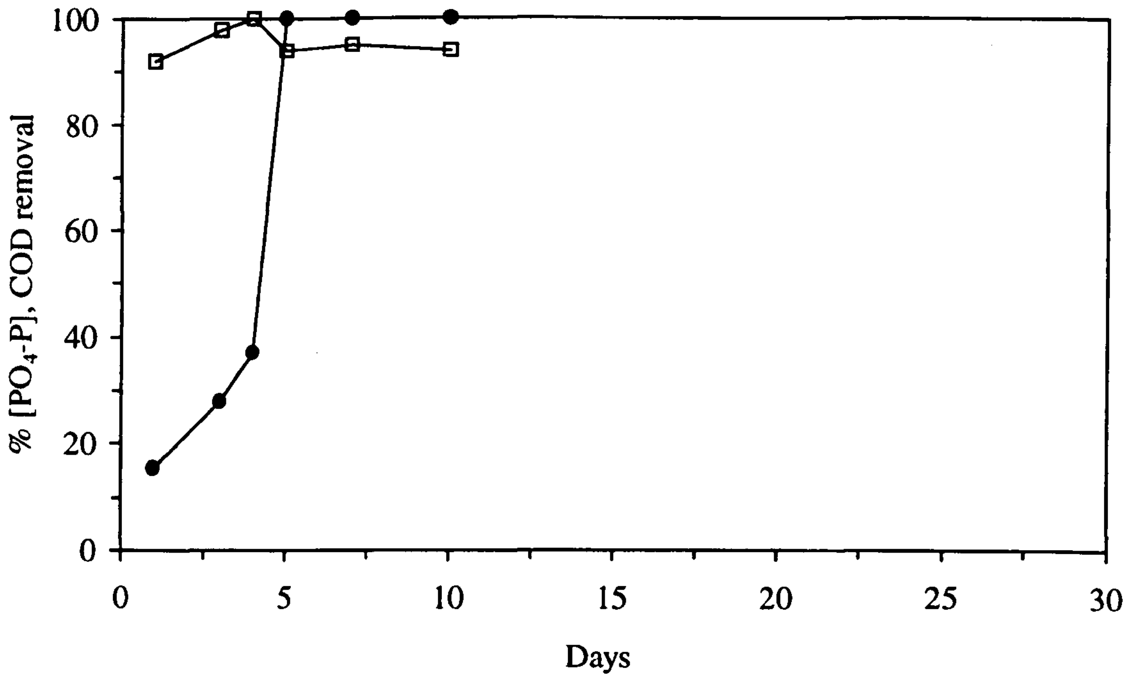


Fig. 4.5. The development of EBPR with inoculation of 10% of EBPR sludge. (□) % influent COD removed and (●) % influent P removed.

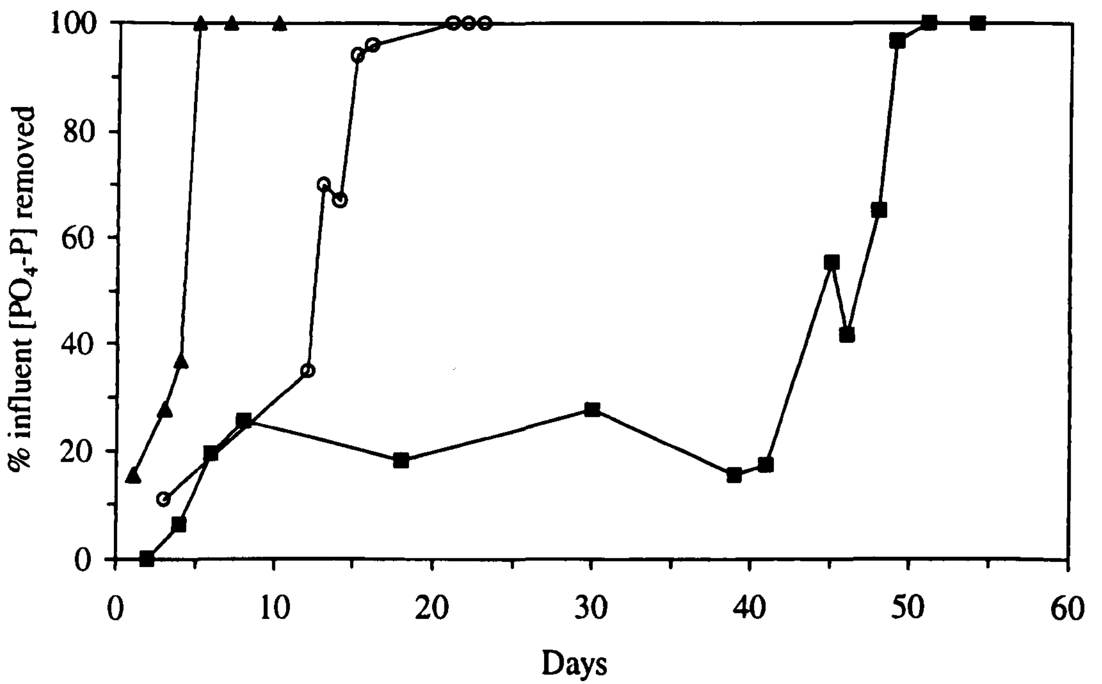


Fig. 4.6. Comparison of start up times for the three start-up procedures employed: (▲) EBPR sludge inoculum, (o) pure culture addition at t=0 and (■) control.

For percentages of around 50 and above, no start-up time was required, as the reactor characteristics, e.g. phosphorus removal rates and daily phosphorus profiles were unaffected.

Figure 4.5. shows the development of EBPR for a run start with the addition of 10% by VSS weight of EBPR sludge. As with previous runs COD removal was established immediately and was unaffected by the sludge addition.

4.2.4. Discussion of EBPR Start-up Results

4.2.4.1. Comparison of start-up times for the control and bioaugmentation runs

The first aspect of the reactor that needed to be investigated was the amount of time needed with three different methods for full EBPR (90% influent removal) to develop and steady state to prevail. Although the SBR is an inherently unsteady state system, it can be assumed that after a certain amount of time, steady state conditions prevail. Steady state in SBR systems and in EBPR systems in particular, can be defined as the condition in which with constant operational parameters, no accumulation of biomass or storage polymer increase takes place between two operational cycles. In the literature steady state has been defined as effluent phosphorus < 0.5 mg/l (Manning & Irvine, 1985), steady state regarding VSS concentration and constant phosphorus content in biomass (Appeldoorn *et al.*, 1992) and as steady state (VSS) with phosphorus removal rates > 90% of the influent (Cech & Hartman, 1993; Smolders *et al.*, 1994; Converti *et al.*, 1995). The latter has been adopted as the definition of steady state in this study. Therefore the time needed for the attainment of steady state is defined as the start-up time.

Figure 4.6. shows the start-up time necessary for the three different methods of EBPR development expressed on the basis of reactor phosphorus removal efficiency. The control, took 50 days to reach high levels of phosphorus removal. The bioaugmented reactor, on the other hand, needed only 14 days to reach similar efficiency levels - a substantial reduction in start-up time.

TABLE 4.2. START UP TIMES FOR EBPR DEVELOPMENT AS STATED IN THE LITERATURE IN SBR SYSTEMS

Reference	Inoculum used for the SBR	Start up time (d)
Manning & Irvine, 1985	conventional activated sludge	35-56[1]
Shin *et al.*, 1992	conventional activated sludge	125[1]
Appeldoorn *et al.*, 1992	P removing activated sludge (Renpho)	54[2]
Cech & Hartman, 1993	P removing activated sludge	100[3]
Smolders *et al.*, 1994	P removing activated sludge	50[3]
Converti *et al.*, 1995c	conventional activated sludge	90[3]
Converti *et al.*, 1995	acclimatised conventional activated sludge	20[3]
Smolders *et al.*, 1995c	conv. act. sludge + unidentified polyP biomass	7[3]
This study	conventional activated sludge	57[3]
This study	conventional activated sludge + pure culture	14[3]
This study	conventional activated sludge + 10% EBPR	5[3]

Start up time defined as: ([1]) Effluent phosphorus < 0.5 mg/l, ([2]) steady state, constant phosphorus content in biomass and ([3]) steady state, phosphorus removal rates > 90%.

The shortest time by far, was achieved by method number three, the inoculation of the conventional activated sludge with 10% bioaugmented sludge.

A comparison with the start up times published in the literature for the achievement of similar removal rates (Table 4.2.) clearly indicates the advantage of the addition of pure cultures of *Acinetobacter lwoffi*, as compared to other methods of EBPR development. These results indicate that polyP bacteria, at least of this particular strain, do not have slow growth rates (< 1 day^{-1}) as thought by Okada *et al.* (1991, 1992). These researchers suggested that a minimum SRT of 30 days was necessary for the accumulation of polyP bacteria of the *Acinetobacter* species and that a SRT below 25 days would result in washout of these organisms from the system. This study shows that effective EBPR can be achieved with an SRT_E of 9 days which is equivalent to a SRT of 20 days.

The same picture emerges if the phosphorus accumulation capability of the sludges is plotted against the days of the run (figure 4.7.). If the maximum sludge phosphorus requirement for maintenance and growth is approximately 1.0 - 1.5 mgP/gVSS, than as figure 4.7. shows, the control reactor was operating for the first 45 days on minimum requirement levels, with no excess phosphorus accumulation. For the bioaugmented reactor the beginning of EBPR characteristics as described in section 4.2.3.2. and figure 4.3., coincided with a phosphorus accumulation value of 3 mgP/gVSS, which was attained within the first 10 days of operation. The bioaugmented sludge reached its maximum accumulated phosphorus value (4.2 mgP/gVSS) after 20 days compared to the 50 days needed by the control reactor.

The shorter start up times achieved with the addition of the pure culture or the addition of 10% of EBPR sludge, point to the fact that EBPR development relies on an increase in the population of polyP organisms. During the control runs, the initial ratio of heterotrophic biomass to polyP biomass is high. The small numbers of polyP bacteria cannot consume all the influent acetate, added at the start of the cycle. Acetate passes on to the aerobic phase allowing non polyP bacteria to grow. Bioaugmentation, provides a sufficient number of bacteria able to consume acetate during anaerobic conditions immediately. Thus, polyP predomination and heterotrophic wash out occur sooner than in the control runs.

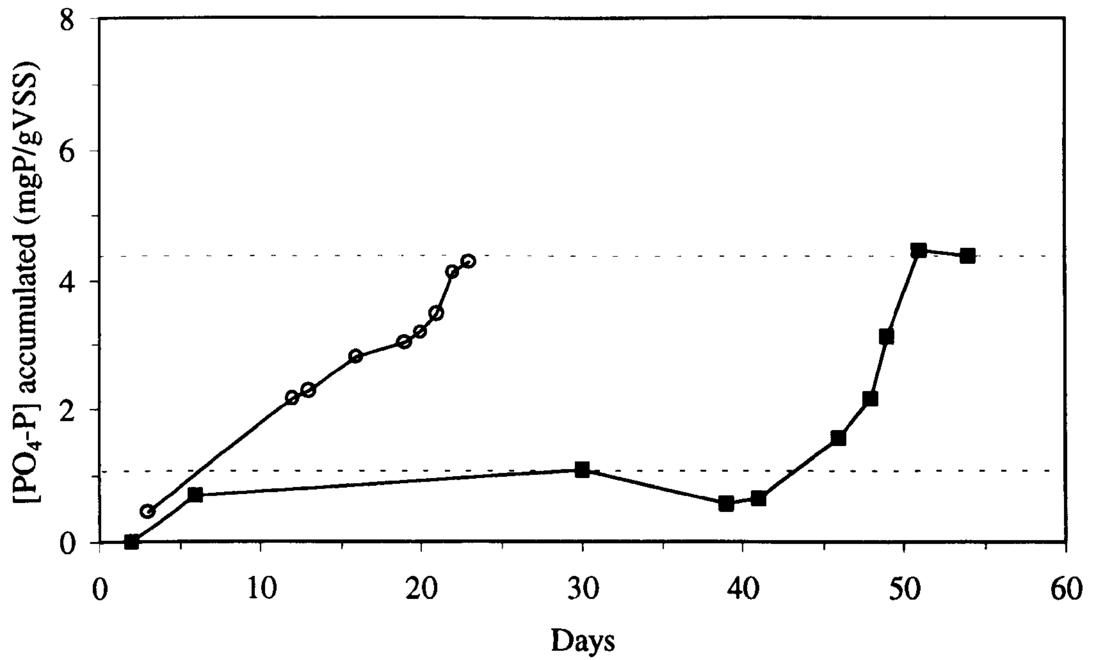


Fig. 4.7. Comparison of P removal per unit VSS for two of the start-up procedures employed: (o) pure culture addition at t=0 and (■) control.

TABLE 4.3. OPERATIONAL PARAMETERS USED FOR THE DETERMINATION OF THE EFFECT OF THE L VALUE DURING START-UP.

Bioaugmentation at t=0			
Run	P/M (mgP/gVSS)	L (gCOD/gVSS.d)	STR_E (d)
B 1	4.1	0.76	9
B 4	4.3	0.44	10
B 5	1.5	0.44	9
B 2	1.5	0.73	9

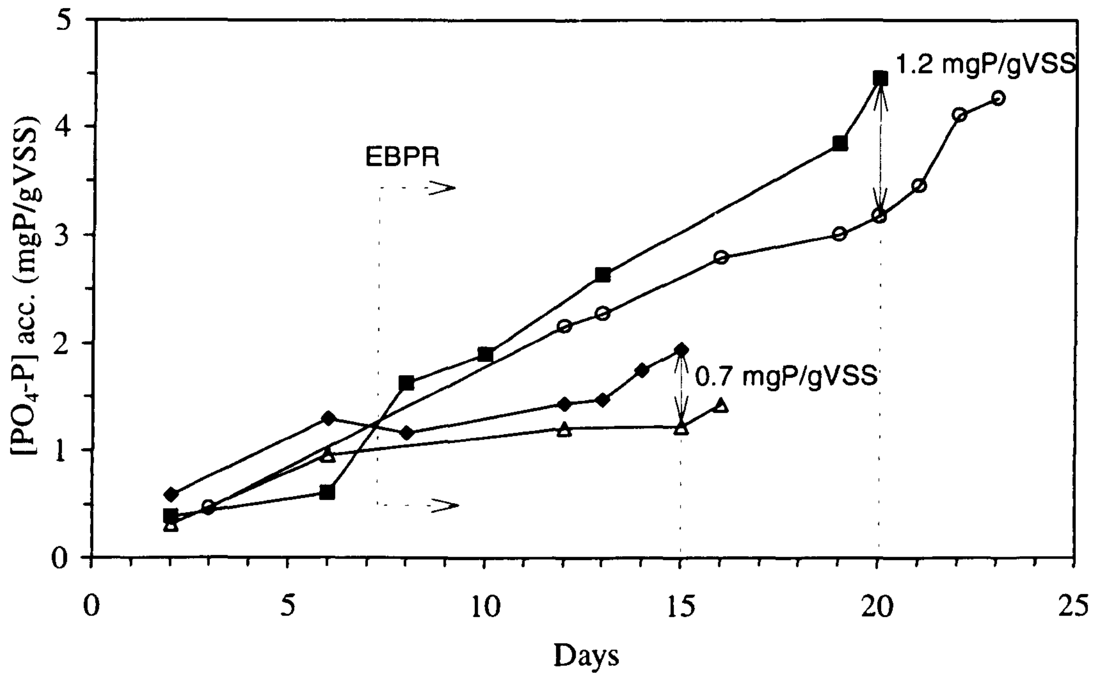


Fig. 4.8. Development of EBPR as shown by the accumulated phosphorus during four bioaugmentation runs. (o) L = 0.76, P/M = 4.1, (■) L = 0.44, P/M = 4.3, (Δ) L = 0.73, P/M = 1.5 and (♦) L = 0.44, P/M = 1.5.

This led to the supposition that lower organic loadings in the anaerobic phase, would lead to shorter start-up times. A lower S_0/X_0 ratio would allow less substrate to pass on to the aerobic phase and thus enhance the switch over from the heterotrophic biomass to the polyP biomass. For this reason the reactor was operated during the next bioaugmentation runs with almost half the organic loading at two influent P/M ratios: 4 and 1.5 mgP/gVSS (Table 4.3.). Influent values and solids concentrations are included in Appendix II, table II.2.

For both influent P/M values the lower organic loadings achieved shorted start-up times (figure 4.8.). Although the difference in the time needed for the achievement of the maximum phosphorus removal rates was not dramatic, the difference in the accumulated phosphorus in the sludge after the attainment of EBPR, was in the range of 1 mgP/gVSS. This was due to the increase in the polyP biomass as a result of faster washout from the reactor of the heterotrophs who were not allowed any substrate at the beginning of the aeration period. This finding agrees with the work of Smolders *et al.* (1995c), who concluded that, in the presence of other heterotrophic organisms, excess substrate in the anaerobic phase during start-up is a disadvantage as the heterotrophs will readily consume it and grow in the subsequent aerobic conditions were they have the advantage because of their higher growth rates (Smolders *et al.*, 1995c; Ghigliazza *et al.*, 1995).

For runs with the same organic loading the higher P/M ratios produced, as expected higher accumulation of phosphorus in the sludge (figure 4.9.).

Recently the significance of *Acinetobacter* in EBPR has been questioned. Streichan *et al.* (1990) and Auling *et al.* (1991), have concluded that *Acinetobacter* is not a dominant species in EBPR. Also, laboratory studies of municipal sludge by Brodisch and Joyner (1983), Hiraishi *et al.* (1989) and Cloete and Steyn (1988), have found that *Acinetobacter* comprised only 1-10 % of the bacterial species.

In this study it was not possible to identify the bacterial species in the sludge and verify the presence of *Acinetobacer* after EBPR establishment. This was due to the fact that the microbiological laboratory available did not have the appropriate methods for bacterial identification, nor the safety grading which would allow handling of the gram positive bacteria identified with the gram staining method.

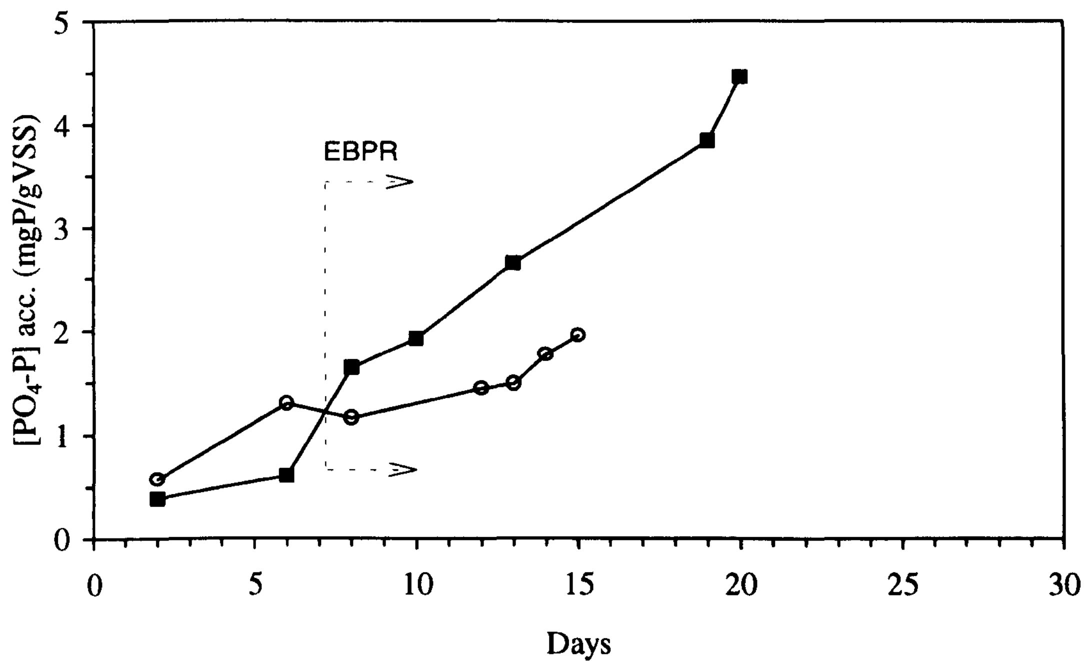


Fig. 4.9. Development of EBPR as shown by the accumulated phosphorus during two bioaugmentation runs. (■) L = 0.44, P/M = 4.3 and (o) L = 0.44, P/M = 1.5.

The impact of the addition of the pure culture was alternatively investigated by adding it at different times during the run (operational parameters shown in Table 4.4. and in Appendix II, Table II.3), always before 50 to 60 days, which was the time EBPR was being established in the control runs. As shown in figure 4.10., similar EBPR development profiles as in figure 4.6. were obtained regardless of the time of the addition of the pure culture. This, proved that quicker EBPR development was the direct result of the addition of the pure culture of *A. lwoffi*.

It must be noted that although run A5a was operating with a lower available phosphorus concentration (P/M=1.5) than the other two runs, this did not affect the time of EBPR onset. As shown in figure 4.9., parameters such as L and P/M, effect the phosphorus removal performance of the reactor after the establishment of EBPR. The only effect the lower P/M ratio during run A5a, was the higher phosphorus removal rates (when expressed as a % of the influent phosphorus - figure 4.10.), observed during the acclimatisation period (days 1-25), as the minimum requirements of the sludge constituted a higher proportion of the influent.

4.2.4.2. Comparison of other sludge properties between the control and bioaugmentation runs

Apart from the shorter start up times, the bioaugmented reactor exhibited a higher capacity for EBPR as shown by the higher release during the anaerobic phase, for comparable phosphorus influent concentrations and biomass content. The anaerobic phosphorus release in the control reactor averaged 18.5 mgP/g dry weight compared with 33.2 mgP/g dry weight in the bioaugmented. This corresponded to a phosphorus sludge content of 160 mgP/gVSS. This value is consistent with the higher values quoted in the literature of 110 mgP/g dry weight (Appeldoorn *et al.*, 1992) and 180 mgP/g dry weight (Wentzel *et al.*, 1988), for lab-scale systems. The control reactor, on the other hand, reached a maximum of 78 mgP/gVSS, after which increasing phosphorus concentrations were detected in the effluent.

TABLE 4.4. OPERATIONAL PARAMETERS OF THE RUNS USED FOR THE DETERMINATION OF THE EFFECT OF THE OF THE TIME OF ADDITION OF THE PURE CULTURE DURING START-UP.

Bioaug/tion (days)	RUN	N/COD (gN/gCOD)	P/M (mgP/gVSS)	L (gCOD/gVSS.d)	STR_E (d)
t = 0	B 1a	0.06	4.1	0.76	9
t = 25	A 5a	0.05	1.5	0.45	8
t = 30	A 4a	0.07	4.7	0.45	10

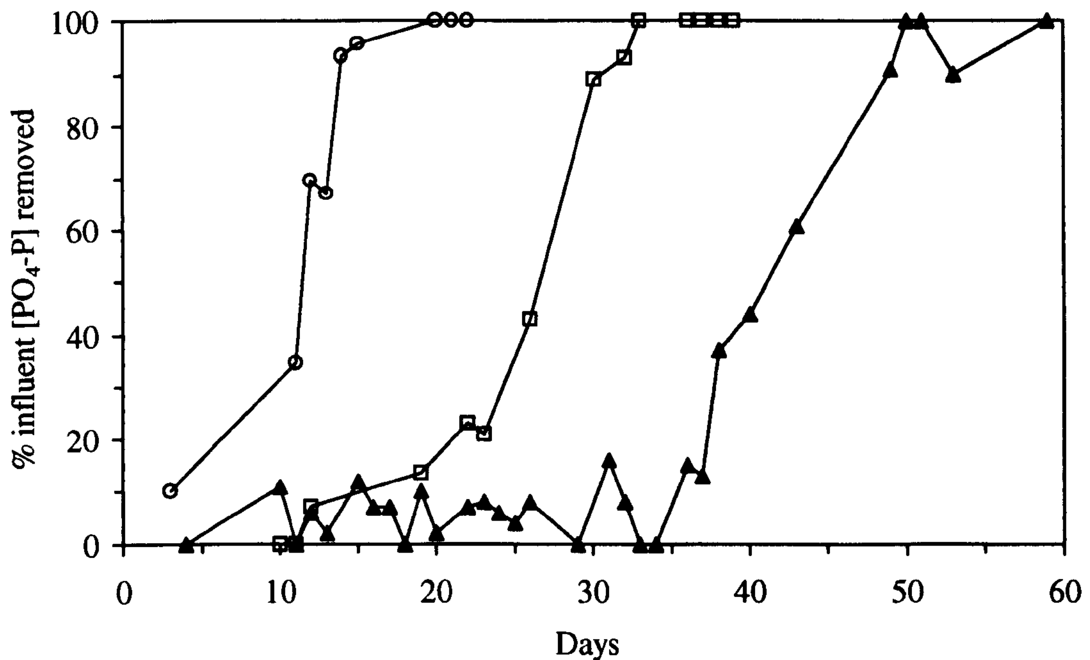


Fig. 4.10. Development of EBPR with bioaugmentation at (o) t=0 days, (□) t=25 days and (▲) at t=30 days.

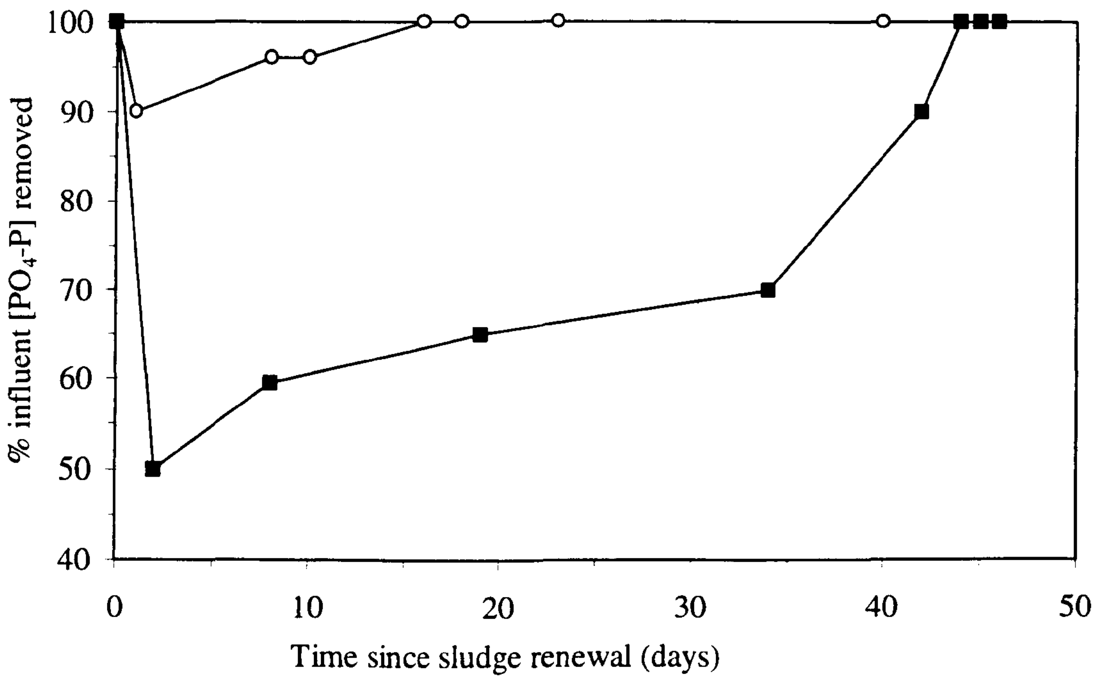


Fig. 4.11. The effect of sludge renewal on phosphorus removal efficiency in the bioaugmented (o) and control (■) reactors.

Microscopic investigations indicated the development of different bacterial communities between the EBPR sludges developed via pure culture addition and via slow sludge acclimatisation. Gram-staining revealed that the control sludge contained a mixture of gram positive and gram negative bacteria in equal proportions, whereas in the bioaugmented sludge over 75% of stained bacteria were gram negative. This pointed to a different polyP population between the two reactors, with different phosphate accumulating abilities.

Differences in the ability of bacteria to accumulate polyphosphate have been observed, which were independent from the age of the culture (Bark *et al.*, 1992). The theory that other bacterial species are involved in biological phosphorus removal, not belonging to the genus *Acinetobacter* is supported by the work of Nakamura *et al.* (1989), Wagner *et al.* (1994) and Bond *et al.* (1995). Bacterial community structure studies have recently revealed the importance of gram-positive bacteria, even in acetate fed reactors (Wagner *et al.*, 1994), although in the past acetate fed reactors were though to select *Acinetobacter* spp. which were found to constitute up to 90% of all the cultured heterotrophs of the sludge sample (Wentzel *et al.*, 1988).

The addition of the pure culture had no significant effect on the COD removal. Both control and test reactors achieved >90% removal rates for the COD loadings of 0.6 to 0.7 gCOD/gVSS·day. The SSVI of both the bioaugmented and control sludge increased over a period of 100 days but did not exceed 150 ml/g.

The ammoniacal nitrogen removal efficiency of the sludges was not significantly different between the control and bioaugmented reactors. The effluent was always highly nitrified, but complete denitrification took place during the settling and idle periods or during the very first moments of feed in the new cycle and no nitrates were present in the anaerobic phase of the following cycle.

When up to 70% of the biomass was removed and replaced with unconditioned sludge, the bioaugmented sludge had only a slight reduction in its phosphorus removal capability and re-gained high phosphorus removal rates within one week. When the control sludge was put through the same test, the reactor took approximately 40 days to reach the same removal rates (figure 4.11.). This also points to high growth rates of

Acinetobacter lwoffi and contradicts previous results quoting slow recovery rates of polyphosphate bacterial populations after disturbances (Okada *et al.*, 1992).

4.2.5. Overview of Results

The first experimental phase of this study demonstrated that the addition of a pure culture of *Acinetobacter lwoffi* to an activated sludge without phosphorus removal capabilities, results in the development of EBPR in 14 days. A control reactor allowed to develop an enhanced culture of polyphosphate bacteria, reached the same removal rates after approximately 50 days.

This finding establishes the success of a rarely used start-up method for EBPR development, which produces the shortest published start-up time. These results also, contradict the recent findings which doubt the contribution of *Acinetobacter* spp to EBPR.

The higher phosphate release rates as well as the higher sludge phosphorus content rates of the bioaugmented reactor as compared to the control, indicated that EBPR was due to different bacterial communities. This was also verified by microscopic investigations which showed that the control reactor had a mixture of gram positive and gram negative bacteria whereas the bioaugmented contained predominantly gram negative bacteria.

Both the control and bioaugmented reactors achieved 90% COD removal rates and both had similar ammonia removal and sludge settling properties (SSVI 80 - 150 ml/g).

Finally the sludge produced through the bioaugmentation procedure shows resilience to biomass replacement of up to 70 % of VSS.

The significance of these results regarding the next step of the experimental work, was the conclusion that bioaugmentation and EBPR sludge inoculation, were to be used for reactor start-up and that the enhanced culture development method, as applied for the control runs, was to be abandoned.

4.3. PHASE 2: PARAMETER SELECTION AND REACTOR OPERATION FOR THE DEVELOPMENT OF EXCESS SLUDGE FOR THE THICKENING AND STORAGE EXPERIMENTS

4.3.1. Objective

The objective of the second experimental phase was to monitor the phosphorus removed by the reactor for varying operational modes and attempt to correlate the phosphate removed and that released during the anaerobic phase with these operational parameters. The ultimate goal of this research phase was to investigate if a link exists between the parameters influencing the phosphorus release in the anaerobic zone, with the phosphorus released during sludge treatment.

The literature states that the main factors affecting phosphorus release in the anaerobic zone of an EBPR plant, are the ORP, level of nitrates present, the substrate composition and the concentration of metals.

In this study, the effect of the variation of the influent COD and P concentrations and the resulting COD/P ratio was chosen as the substrate parameter to be studied and the SRT_E as the operational. The characteristics of the sludge produced as described by its total phosphorus content, metal precipitates and settling properties, were also monitored.

Apart from the influent COD/P ratios and SRT_E, the dissolved oxygen concentration during the aeration period and the extent of nitrification (as expressed by the amount of nitrate in the excess sludge) were also altered. Varying these parameters does not influence the anaerobic phosphorus released directly, as it affects the operational stages following the anaerobic. Nevertheless, the performance of the reactor during these operational changes, as described by the phosphorus removal capabilities of the sludge produced, is included in this section. This was desirable, so

that a complete picture of the sludge parameters used for the storage and thickening experiments, is presented by the end of this chapter.

4.3.2. Methodology

4.3.2.1. Methodology for operating the reactor with different SRT_E

The effect of various aerobic sludge retention times (SRT_E) on the performance of the reactor, was investigated by altering the reactor operating SRT_E range between 5 and 16 days. This was achieved by changing the excess sludge wasting rate at the end of the aerobic period. After each change the reactor was operated for six cycles, to ensure that any resulting change in the biomass had taken place, before recording the daily phosphorus profile.

The variation of the operating SRT_E was kept in the range of 5 to 16 days. No extreme values (less than 5 d and more than 16 SRT_E) were investigated as it was not intended to push the EBPR process to failure by operating at very low or very high SRT_E.

The effect of the operating SRT_E on the phosphorus removal properties of the reactor, was investigated at the influent P/M ratio range of 0.65 - 5.5 mgP/gVSS and at an organic loading range of L = 0.24 - 0.55 gCOD/gVSS.

The operating parameters, influent values and operating solids concentrations of the runs involved are included in table 4.5. and in Appendix II, Tables II.4. - II.7.

4.3.2.2. Methodology for operating the reactor with different influent COD/P ratios

For the determination of the effect of the influent COD/P ratio on the anaerobic phosphorus release the influent characteristics of the different runs were altered. Two sets of experiments were performed. For the first set of experiments the influent COD was varied while the available phosphorus, as expressed by the P/M ratio, was kept constant. These experiments were performed at three influent P/M values, namely 3.2, 4.5 and 6.0 mgP/gVSS (Tables II.8. and II.9. in Appendix II) For the second

experimental set, the increase in the influent COD/P was achieved by increasing the influent phosphorus concentration and keeping the influent COD constant. This was investigated at an organic loading range of L = 0.2 to 0.9 mgCOD/gVSSd (Tables II.10. and II.11.).

The variation in the influent COD/P for the first experimental set was in the range of 5 - 44, which was achieved by varying the influent COD concentration from 150 to 736 mg/l. For a constant VSS concentration this increase of the influent COD resulted in a L range of 0.18 - 0.94 mgCOD/gVSSd (Table II.8).

For the second experimental set the range of the COD/P values investigated was 5 - 102. This was achieved by varying the influent phosphorus concentration from 4 to 32 mgP/l. The resulting P/M ratios were 0.5 - 6.5 mgP/gVSS respectively (Table II.10.).

4.3.2.3. Methodology for operating the reactor with different effluent nitrate levels

In the SBR cycle, when operated for EBPR, nitrification takes place in the aerobic phase, which follows the anaerobic. The amount of nitrate generated in the aerobic zone of the reactor and the amount removed through denitrification, depends on the influent TKN/COD ratio (when the SRT, aeration time and temperature are constant).

To modify the amount of nitrate leaving the reactor with the excess sludge, two methods of operation were employed. The first consisted of varying the influent N/COD ratio, by varying the influent ammonia concentration. Two ammoniacal nitrogen concentrations were used, namely 30 and 50 mg/l as [NH_3-N] (Table 4.6.). For a constant organic loading of 0.45 mgCOD/gVSSd, this gave a N/COD ratio of 0.07 and 0.11 mgN/mgCOD respectively.

For a number of the experimental runs, decreasing the ammonia concentration in the influent from the average operating value of 50 mgN/l to 30 mgN/l, to produce sludges with less nitrate, was considered undesirable. These runs were used for determining the effect of varying influent phosphorus or COD on EBPR and all other influent parameters, including ammonia, had to be constant. For these runs a second method was employed. An anoxic phase was included in the SBR cycle, following the

aerobic, were denitrification took place. The extent of denitrification and consequently the amount of nitrate in the sludge leaving the system, depended on the time allowed during this anoxic stage. The anoxic stages in this study were half and one hour (operational parameters included in Appendix II).

4.3.2.4. Methodology for operating the reactor with low dissolved oxygen during the aerobic phase

Dissolved oxygen levels were varied in the aerobic stage of the cycle by modifying the flow rate of the air pump. During normal operation, the D.O. in the aerobic phase varied from 0 mg/l to 4 mg/l. The flow rate of the air pump was approximately 1.4 l/min. For the low D.O. runs, the oxygen concentration was restricted first to 1 mg/l and then to 0.5 mg/l. For the unrestricted air supply runs, the D.O. was allowed to increase to 7.5 mg/l. The intention was to monitor the phosphorus concentration during the aerobic and the following settling stage, to detect any alterations in the uptake profile or any secondary release during settling. The sludge produced during these runs was used for sludge treatment experiments as described in detail in the following chapter (operational parameters included in Appendix II).

4.3.3. Results and Discussion

4.3.3.1. The effect of SRT_E variation on EBPR

As mentioned in section 4.3.2.1., different excess sludge withdrawal rates were employed in order to achieve different sludge ages in the SBR. This variation of the sludge retention time, directly influences the biomass growth rate (μ). This, in turn, is thought to effect the storage compounds (PHB, polyP, glycogen) of the polyP bacteria and hence the phosphorus release and removal properties of the sludge (Smolders *et al.*, 1995b).

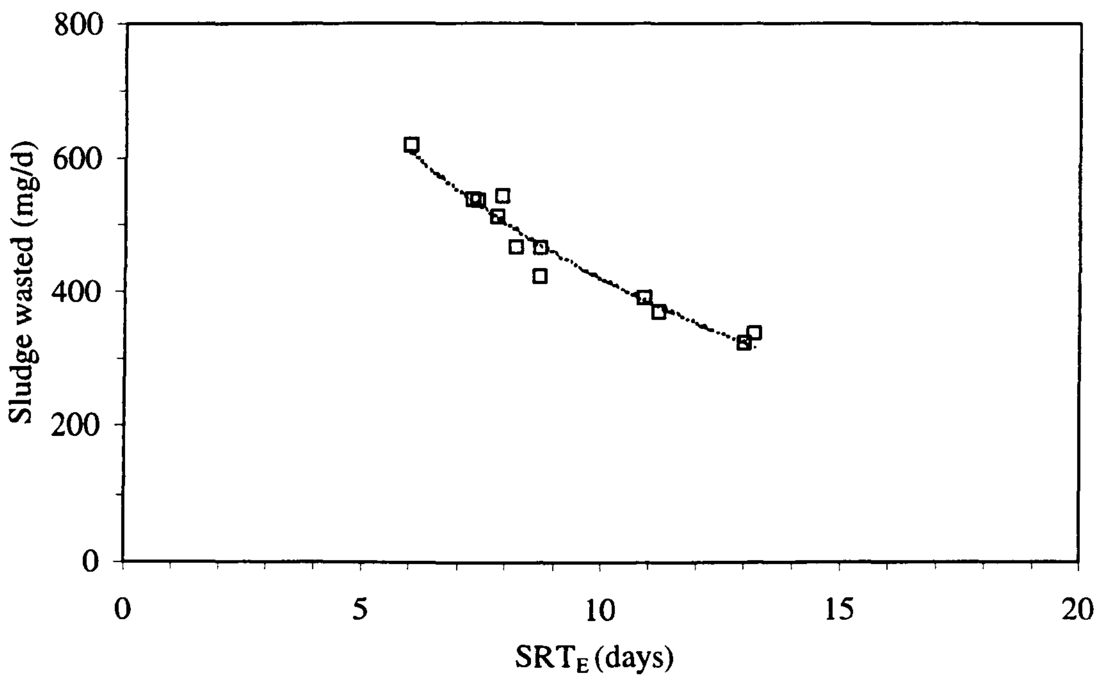


Fig. 4.12. Effect of excess sludge wasting rate on reactor SRT_E.

The effect of varying the sludge wasting rate, on the reactor retention time, is shown in figure 4.12. Reducing the daily wasting rate from 300 ml/d to 100 ml/d (or from 700 mgVSS/d to 340 mgVSS/d, figure 4.12), has the effect of increasing the SRT_E of the sludge from 5 to 13 days (equivalent SRT 16 - 40 days). The slight variations on the calculated sludge retention time occur as a result of varying effluent solids concentrations (detailed results are included in Appendix II, Table II.4.).

As figure 4.13. shows, the change of operating SRT_E in the range of 5 to 13 days did not significantly effect the phosphorus removed per unit biomass in the SBR. The influent phosphorus and carbon concentrations were kept constant at 19 mgP/l and 450 mgCOD/l respectively (Table 4.5. and Table II.5.). A decrease of approximately 1 mgP/gVSS is observed between the amount of phosphorus removed during the 5 day SRT_E run and that of the 13 day SRT_E run (figure 4.13.).

As the change in SRT_E has a direct effect on the sludge concentration in the reactor (figure 4.14, Table II.6.), for constant influent carbon and phosphorus values, this results in decreasing organic loadings and P/M values for increasing SRT_Es (Table 4.5.). The effect of the increasing SRT_E on the phosphorus removal properties of the reactor may therefore be confounded by the decreasing available phosphorus per unit biomass (P/M ratio).

Indeed, as figure 4.15. shows, operating the reactor over the same SRT_E range as in figure 4.14., but with a constant P/M and L has no effect on the amount of phosphorus removed. This was found to be the case for the two combinations of P/M and L values used (Table II.7. and figure 4.15.).

The effect of SRT_E on phosphorus removal was further examined for the P/M value of 3 mgP/gVSS and the organic loading L = 0.4 mgCOD/gVSSd, by linear regression. The null hypothesis (Ho) tested was that the slope (β) was not significantly different from zero. The two tailed test, with H_o: $\beta=0$ and $H_{0.5}$: $\beta\neq0$, gave a rejection region of $t > 2.571$ or $t < -2.571$, based on 5 degrees of freedom (n-2). The calculated t value (-0.456) does not fall in the rejection region therefore we cannot reject the null hypothesis that $\beta=0$.

TABLE 4.5. EFFECT OF SRT_E ON REACTOR PERFORMANCE FOR RUNS WITH INFLUENT COD ≈ 450 mg/l AND [PO_4-P] ≈ 19 mg/l

RUN	SRT_E (d)	MLSS (mg/l)	MLVSS (mg/l)	ESS (mg/l)	P/M (d)	P_{rem} (mg/l)
A3ii - Ph 1	5.3	4418	2280	12	4.04	4.00
A4i - Ph 2	7.3	3648	2390	19	3.50	3.50
A4iii - Ph 1	8.0	3688	2506	12	3.30	3.23
A4vi - Ph 1	9.6	4020	2444	10	3.28	3.28
A3ii - Ph 2	10.9	4503	2592	12	3.13	3.01
A4ii - Ph 1	13.0	3900	2600	19	3.24	2.92
A4i - Ph 1	13.2	3648	2724	19	3.16	3.16

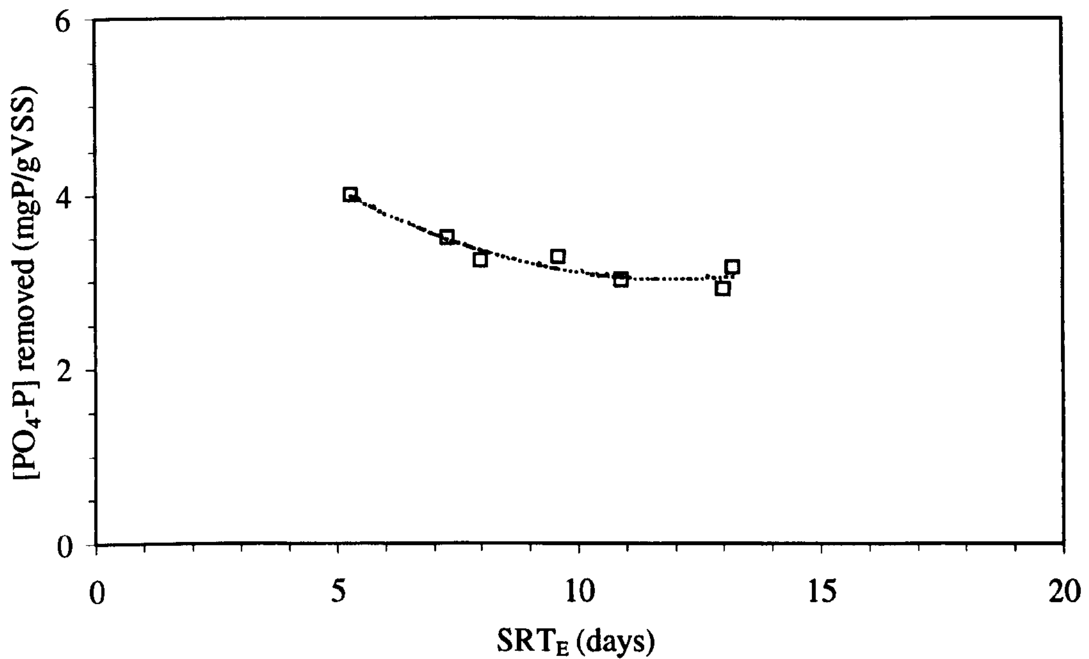


Fig. 4.13. Effect of increasing SRT_E on EBPR.

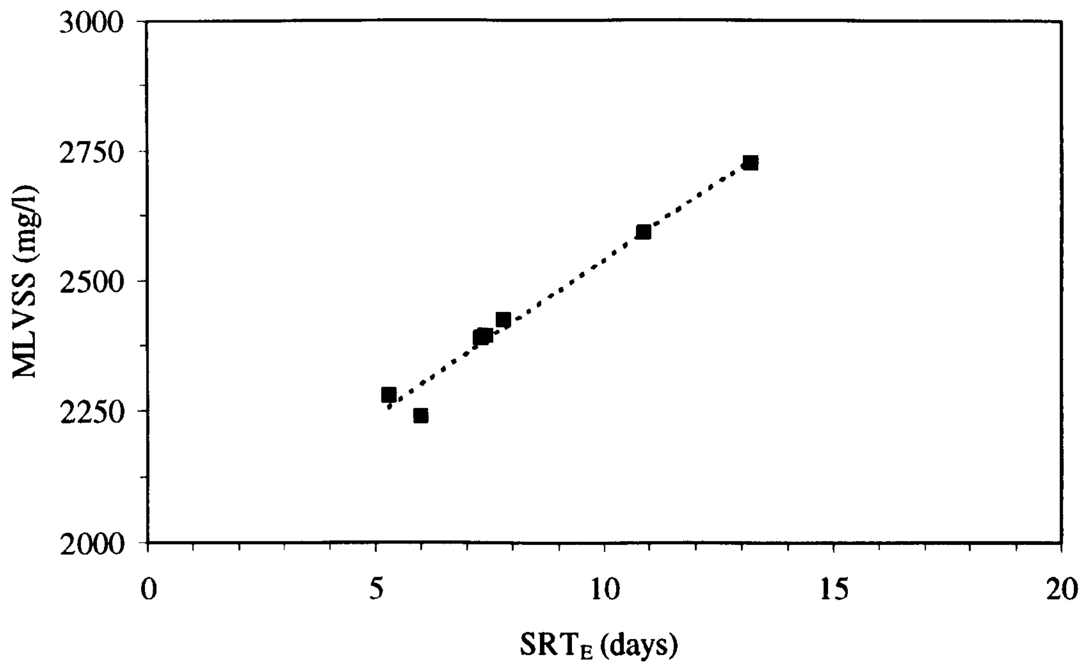


Fig. 4.14. Effect of increasing SRT_E on reactor MLVSS concentration.

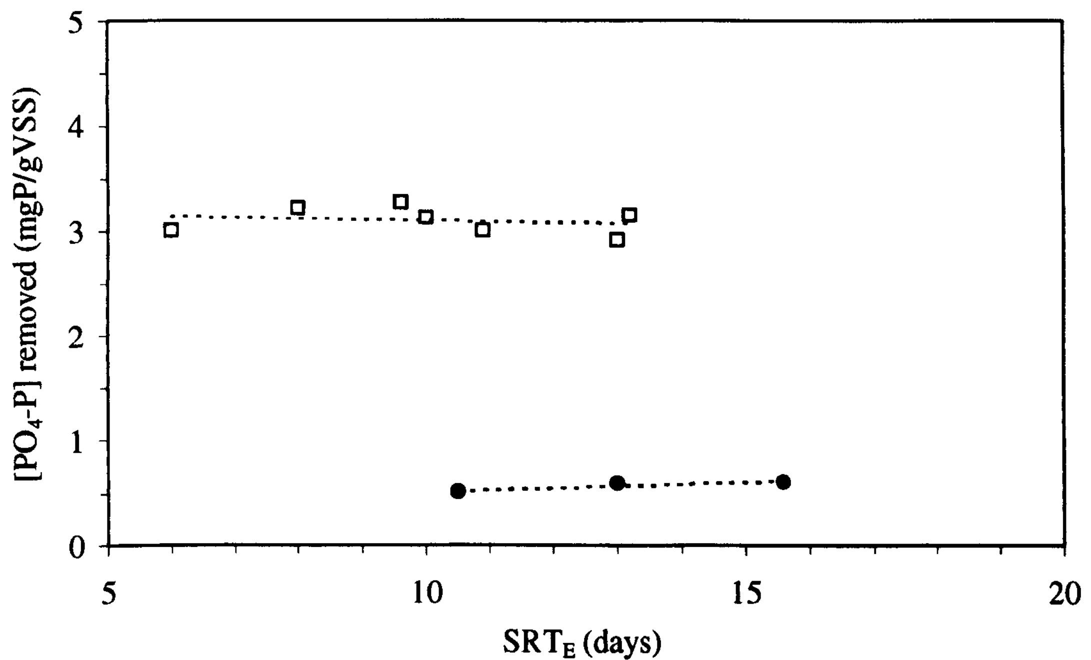


Fig. 4.15. Effect of increasing SRT_E on EBPR, for runs with constant P/M and L values. (□) P/M = 3.0 mgP/gVSS - L = 0.4 mgCOD/gVSS.d and (●) P/M = 0.65 mgP/gVSS - L = 0.24 mgCOD/gVSS.d.

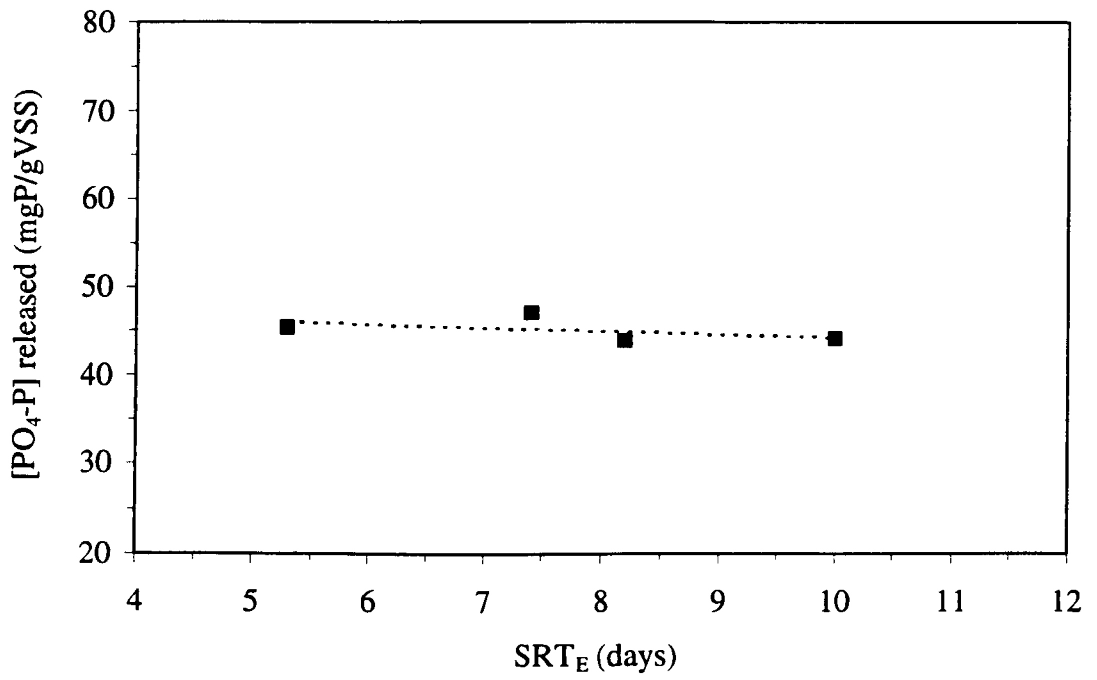


Fig. 4.16. Effect of increasing SRT_E on anaerobic phosphorus release.

Furthermore, a 95% confidence interval for the slope β, the expected change in phosphorus removal for a 1% increase in the SRT is $5.7x10^{-2}$. The interval estimate of the slope parameter β, is -0.067 to 0.0469. We can therefore be 95% confident that the true mean increase in phosphorus removal per 1% increase in SRT (between the values of 5 to 13 days), is in the area of zero.

Full scale studies on the other hand, have indicated that increasing the aerobic SRT from 3 to 8 days results in a 50% decrease in the P/VSS ratio and 23% lower phosphorus removal rates (McClintock *et al.*, 1993). Nevertheless, it is generally accepted that there appears to be a lower limit for the SRT (between 2.9 and 1.5 days) below which EBPR does not operate (Shao *et al.*, 1992; Mamais & Jenkins, 1992; Smolders *et al.*, 1995b). As previously mentioned it was not the purpose of this study to follow EBPR performance to failure by operating in the lower limit of the published SRT values.

Similar results, to the phosphorus removal ones, just described, were obtained when the amount of phosphorus released at the end of the anaerobic phase was correlated to the varying SRT_E. As shown in figure 4.16., for SRT_Es between 5 and 10 days, the phosphate released by the biomass averaged 45.2 ± 1.5 mgP/gVSS (for a constant P/M = 4 mgP/gVSS). This finding indicates that EBPR functions independently of the sludge retention time, between the values of 5 and 13 days.

This finding does not agree with the results of Smolders *et al.* (1995b), who found that an increase in the SRT_E from 5 to 20 days resulted in a 46% increase in the anaerobic phosphorus release (approximately 53 mgP/l - value calculated from data in paper). This was most probably due to the fact that their results were presented on a volumetric basis, i.e. in mg/l, which introduces the confounding effect of the variation in VSS.

4.3.3.2. Effect of varying influent COD and P concentrations

As described in the literature review (chapter 2, section 2.4.2.2.), the phosphate released in the anaerobic stage, is strongly dependent on the available acetate. On the other hand the polyphosphate synthesis rate is dependent on the phosphate present in

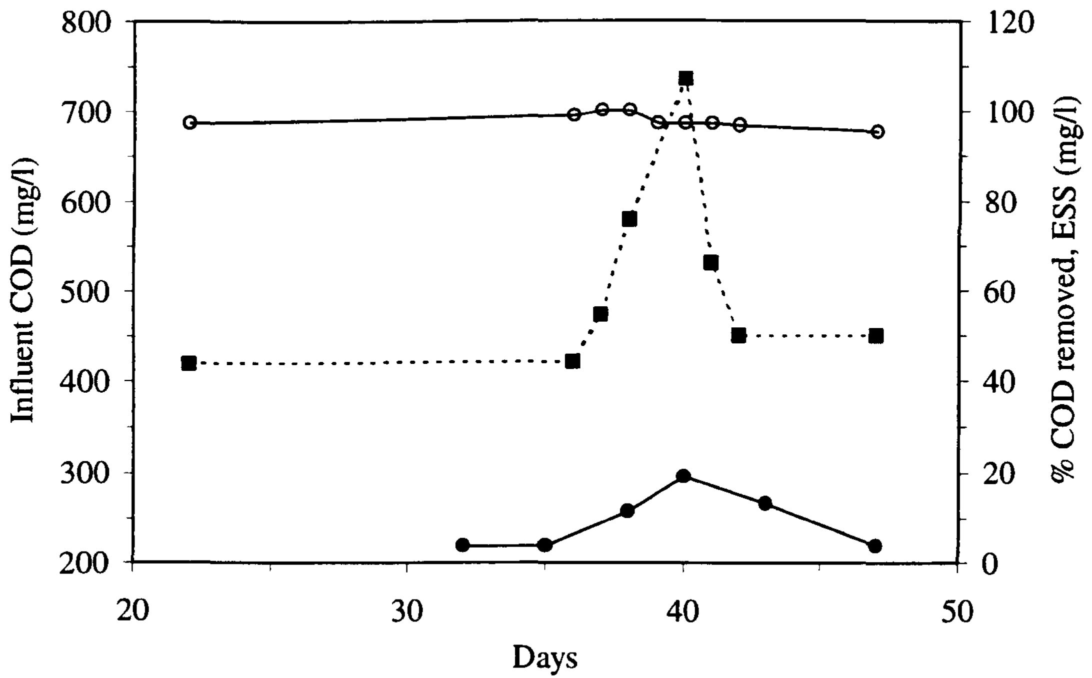


Fig. 4.17. Increase of effluent solids concentration with increasing influent COD. (o) % influent COD removed, (●) ESS and (■) influent COD.

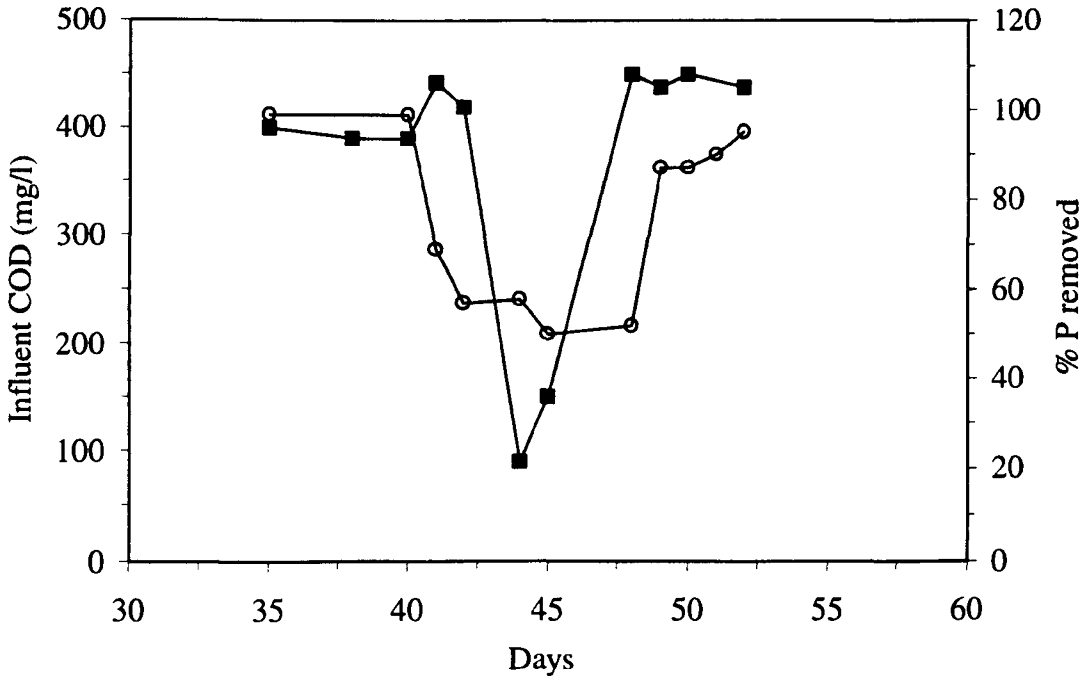


Fig. 4.18. Effect of decreasing COD on phosphorus removal. (o) % influent P removed and (■) influent COD.

solution. Therefore the influent COD/P ratio and more specifically the influent VFA/P ratio, is considered a crucial operational parameter for EBPR (see also chapter 2, section 2.5.4.1.).

The variation of the influent COD/P ratio in full scale treatment plants is primarily due to low COD concentrations or increased rainfall, which affects the composition of the COD, reducing the available VFAs in the influent (Carlsson *et al.*, 1996). The variation of the influent COD values was the focus of the first set of experiments investigating the effect of the influent COD/P ratio on reactor performance.

Effect of influent COD on EBPR

As already mentioned the variation of the influent COD/P ratio was investigated first by altering the COD concentration in the influent. The range of COD/P ratios tested, was limited by the large effect that extreme variations of COD have on the overall performance of the reactor. Increasing the influent COD to 700 mg/l resulted in high organic loading rates and deflocculation of the sludge, increasing the effluent solids concentration (ESS) on the particular run from 4 mg/l to 19 mg/l (figure 4.17). Decreasing the influent COD to below 100 mg/l affected the phosphorus removal efficiency of the reactor reducing it to as low as 50 % (figure 4.18).

This limited the investigated range of COD/P, with this method between the values of 5 - 44. The extreme values were achieved by progressively altering the loading of the reactor and maintained only for two cycles of operation (24 hours) (figure 4.17., 4.18.).

Most researchers agree that increased amounts of RBCOD in the influent of both full and lab scale EBPR plants will result in an increase in the amount of phosphorus released in the anaerobic zone (Winter, 1989; Hascoet *et al.*, 1985b; Carlsson *et al.*, 1996 and many others). Therefore, increasing COD/P ratios (achieved through an increase in COD and a constant P), result in increasing anaerobic phosphorus release. This increased release will result in an increased removal in the reactor, since the amount uptaken during the aerobic phase is closely related to the amount released in the anaerobic phase.

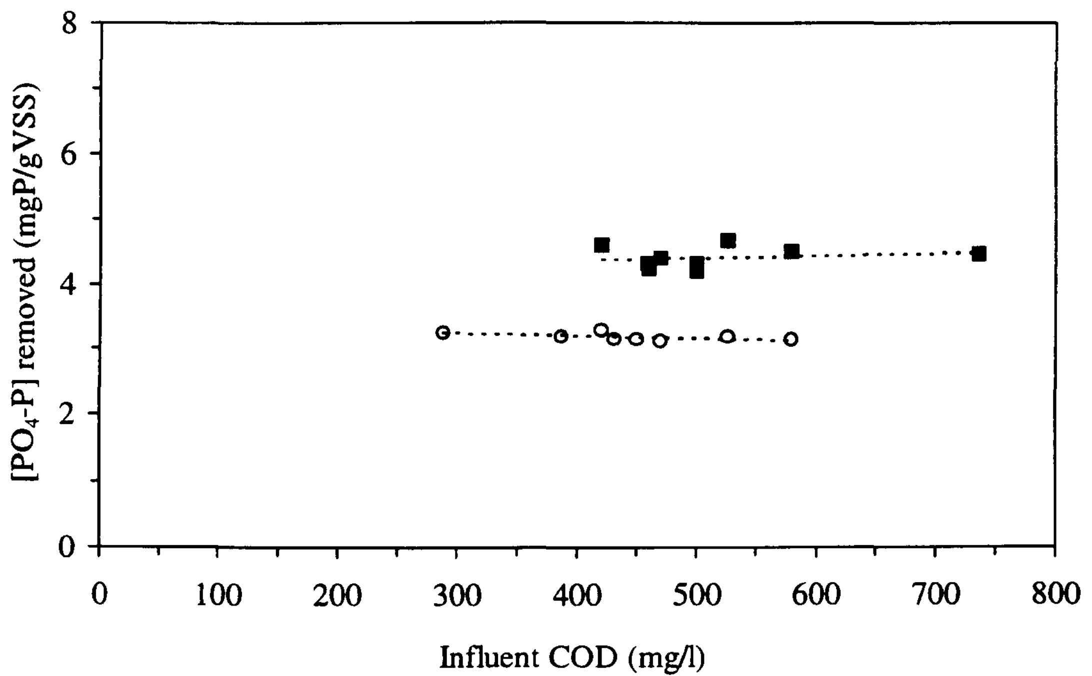


Fig. 4.19. Effect of increasing influent COD on phosphorus removal. (o) P/M = 3.2 mgP/gVSS and (■) P/M = 4.5 mgP/gVSS.

According Bundgaard and Pedersen (1990), a COD/P ratio above 50 can be considered favourable for EBPR and pure strains of *A.lwoffi* have been shown to have a comparable optimum COD/P ratio of 67 (Pauli and Kaitala, 1995). It is widely accepted (Siebritz *et al.*, 1980; Pitman *et al.*, 1992 and many others) and more recently demonstrated (Carlsson *et al.*, 1996), that it is the VFA fraction of the influent that is of crucial importance to anaerobic phosphorus release (see also Literature Review, section 2.3.6.). In full or lab scale plants receiving municipal wastewater as feed, this fraction constitutes between 2-10% of the total COD of a primary effluent (Henze *et al.*, 1995). In this study, the sole carbon source added was acetate, therefore all COD was available for assimilation in the anaerobic zone.

As can be seen from figure 4.19., for the two of the three P/M investigated (4.5 and 3.2 mgP/gVSS), altering the influent COD between 290 and 750 mg/l had no effect on the amount of phosphorus removed by unit biomass. This was supported by linear regression. The null hypothesis (Ho) tested was that the slope (β) was not significantly different from zero. The two tailed test, with H_0: $\beta=0$ and $H_{0.5}$: $\beta\neq0$, gave a rejection region of $t > 2.365$ or $t < -2.365$, based on 7 degrees of freedom (n-2). The calculated t value (-1.891) does not fall in the rejection region therefore we cannot reject the null hypothesis that $\beta=0$. Furthermore, the 95% confidence interval for the slope is -9×10^{-4} to 0.0001. We can therefore be 95% confident that the true mean increase in phosphorus removal per 1% increase in influent COD is in the area of zero. A similar result was obtained for the P/M = 4.5 (figure 4.19.).

The results obtained for the influent COD were echoed in the organic loading L and COD/P graphs for the two P/M values (figures 4.20. and 4.21.).

For the higher phosphorus value of P/M=6 mgP/gVSS, a different profile emerged. As shown in figure 4.22, the removal capacity of the reactor increased from 3.24 mgP/gVSS to 5.76 mgP/gVSS by increasing the influent COD from 150 mg/l to 500 mg/l. This resulted in an increase in the organic loading from 0.2 mgCOD/gVSSd to 0.7 mgCOD/gVSSd (figure 4.23).

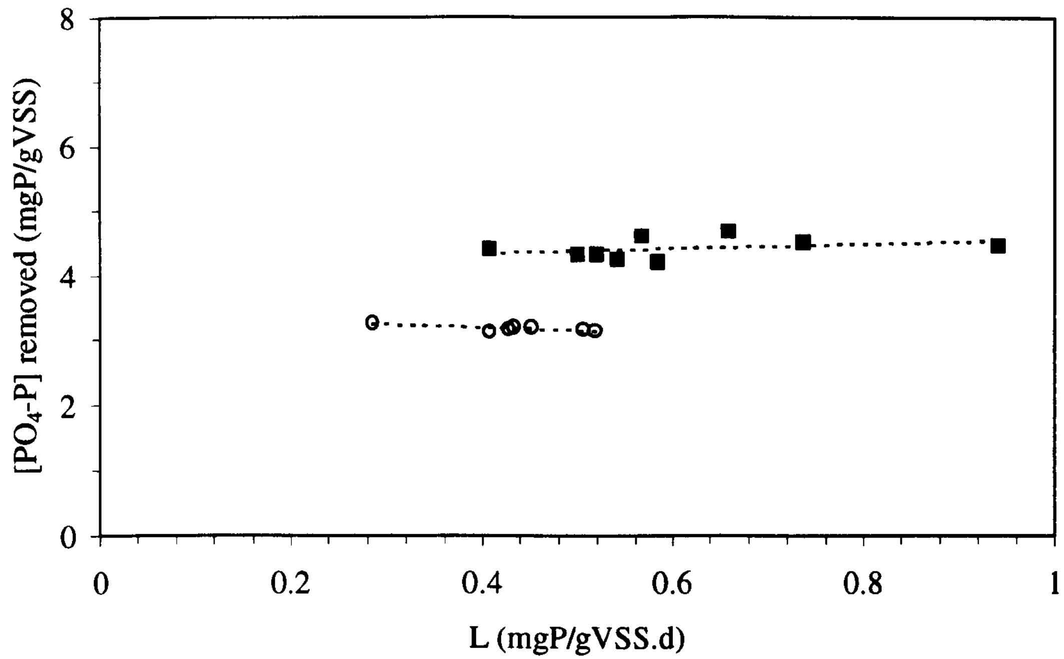


Fig. 4.20. Effect of increasing organic loading (L), on phosphorus removal. (o) P/M = 3.2 mgP/gVSS and (■) P/M = 4.5 mgP/gVSS.

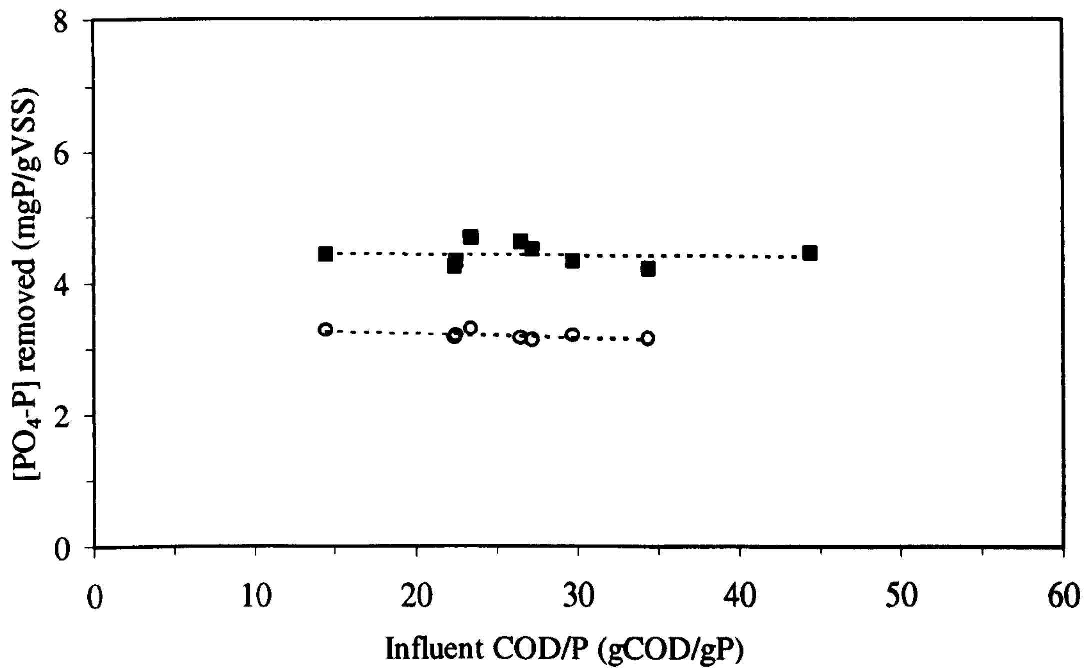


Fig. 4.21. Effect of increasing influent COD/P on phosphorus removal. (o) P/M = 3.2 mgP/gVSS and (■) P/M = 4.5 mgP/gVSS.

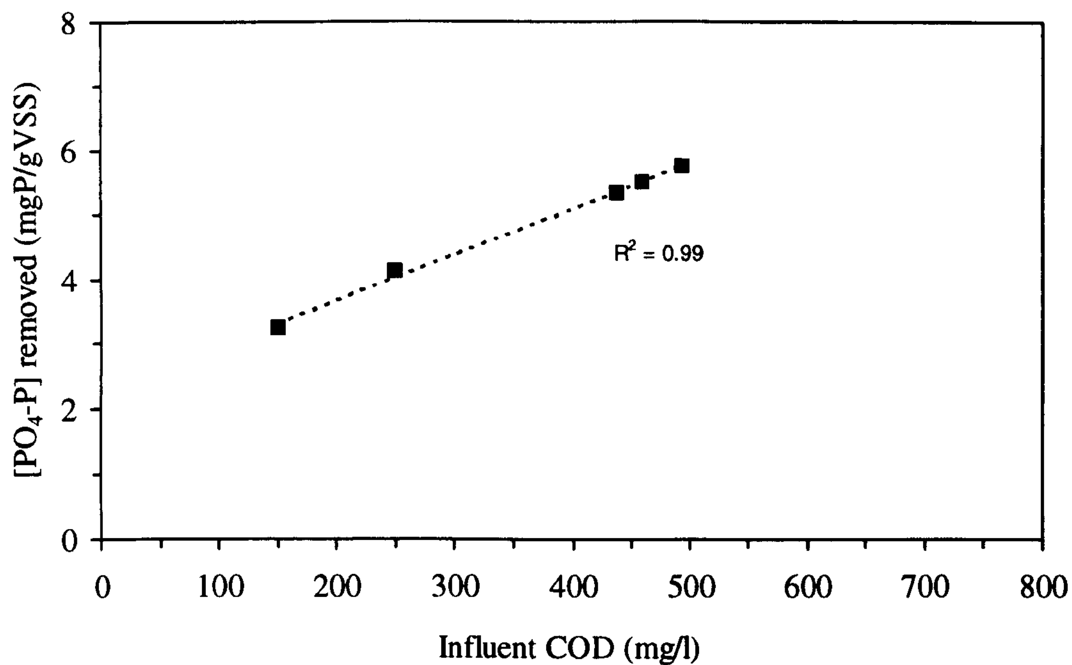


Fig. 4.22. Effect of increasing influent COD on phosphorus removal at P/M = 6 mgP/gVSS.

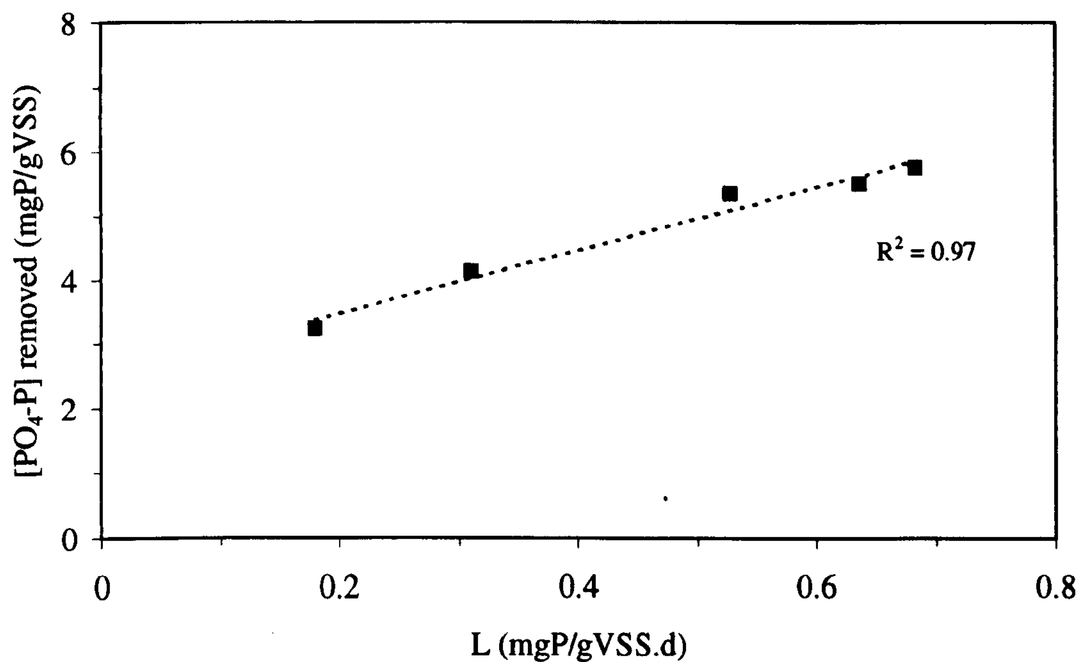


Fig. 4.23. Effect of increasing influent organic loading (L), on phosphorus removal at P/M = 6 mgP/gVSS.

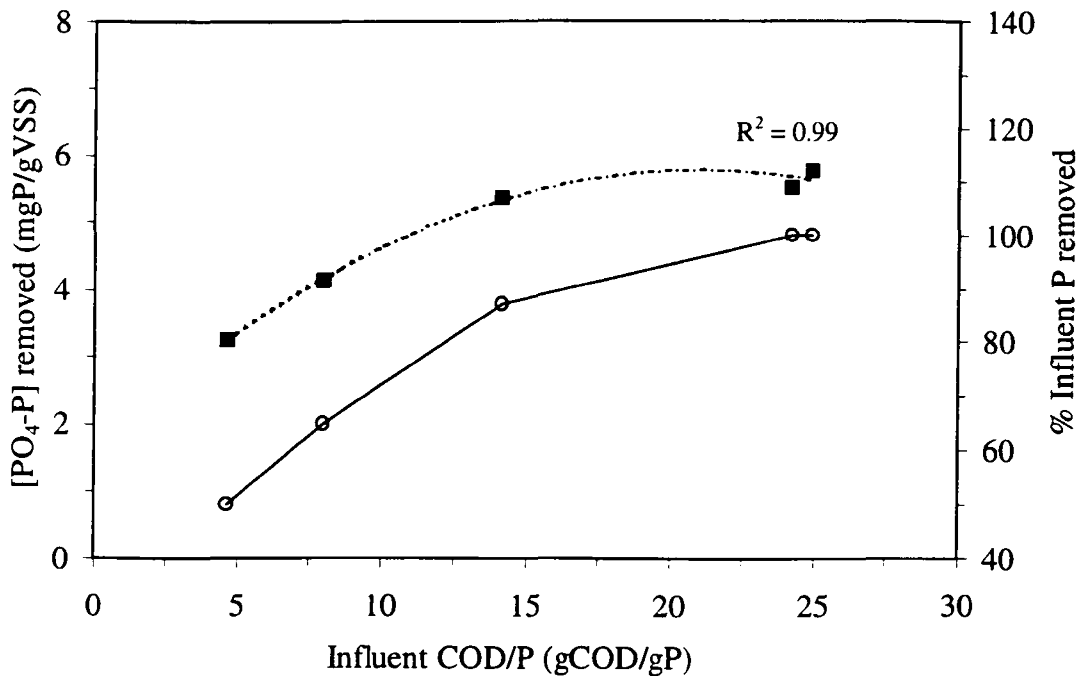


Fig. 4.24. Effect of increasing influent COD/P on phosphorus removal for P/M = 6 mgP/gVSS. (o) % influent P removed and (■) P removed per unit VSS.

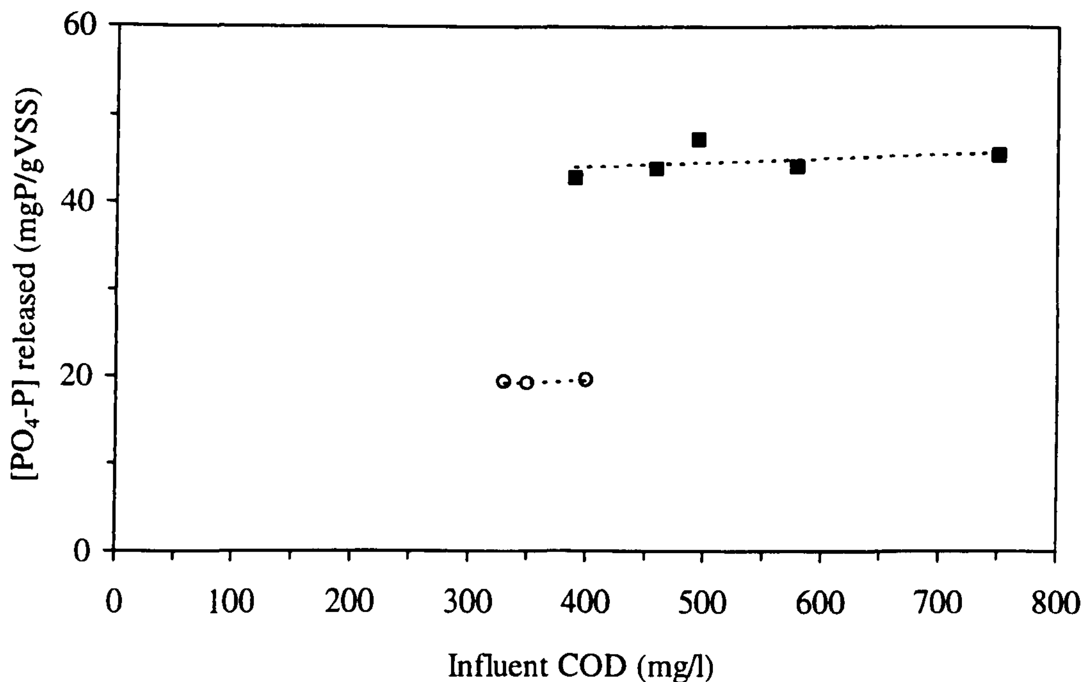


Fig. 4.25. Effect of increasing influent COD on anaerobic phosphorus release. (o) P/M = 0.6 mgP/gVSS and (■) P/M = 4.0 mgP/gVSS.

It must be noted that during the runs with P/M =3.2 and 4.5 mgP/gVSS, the phosphorus removal efficiency of the reactor was always above 90 %. During the P/M=6 runs, the equivalent efficiency values varied from 50% to 100%, increasing with increasing organic loadings. This indicated that the reactor during these trials was working on the verge of EBPR failure for the low COD values. The effect of the resulting influent COD/P ratio on the removal properties of the reactor is shown in figure 4.24. For this particular influent phosphorus value, linear regression revealed that all three parameters (influent COD in mg/l, organic loading L in mgCOD/gVSSd and influent COD/P in mgCOD/mgP) successfully described the effect of increasing influent carbon on the phosphorus removal properties of the reactor. The correlation coefficient obtained for all three parameters was $R^2 > = 0.97$, indicating that they may predict EBPR efficiency for the P/M value of 6 mgP/gVSS.

The effect of the increasing influent COD on anaerobic phosphorus release was investigated only for the P/M values which gave removal efficiencies above 90%. As for the phosphorus removal results, for the two P/M values investigated, namely 0.6 and 4 mgP/gVSS, no increase in the amount of phosphorus released anaerobically was observed for increasing COD values (figure 4.25.). Once again this was supported by linear regression. The null hypothesis (Ho) tested was that the slope (β) was not significantly different from zero. The two tailed test, with H_o: $\beta=0$ and $H_{0.5}$: $\beta\neq0$, gave a rejection region of $t > 3.182$ or $t < -3.182$, based on 3 degrees of freedom (n-2). The calculated t value (0.735) does not fall in the rejection region therefore we cannot reject the null hypothesis that $\beta=0$. Furthermore, the 95% confidence interval for the slope is -0.016 to 0.025. It is therefore 95% confident that the true mean increase in phosphorus removal per 1% increase in influent COD is in the area of zero. It must be noted that the rather large width of the confidence interval reflects the small number of observations available.

For the P/M value of 4 mgP/gVSS the variation in the influent COD between 390 to 750 mgP/gVSS, resulted in an organic loading range of 0.4 to 0.9 mgCOD/gVSSd (figure 4.26.). The resulting COD/P values and their effect on phosphorus removal are shown in (figure 4.27.).

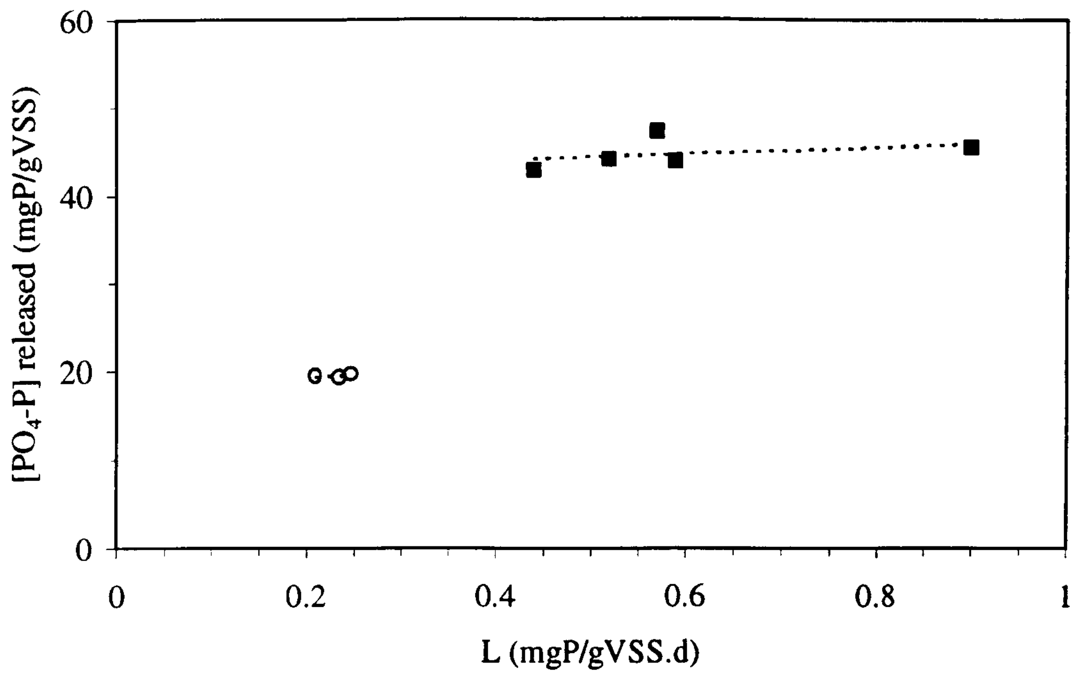


Fig. 4.26. Effect of increasing organic loading (L), on anaerobic phosphorus release. (o) P/M = 0.6 mgP/gVSS and (■) P/M = 4.0 mgP/gVSS.

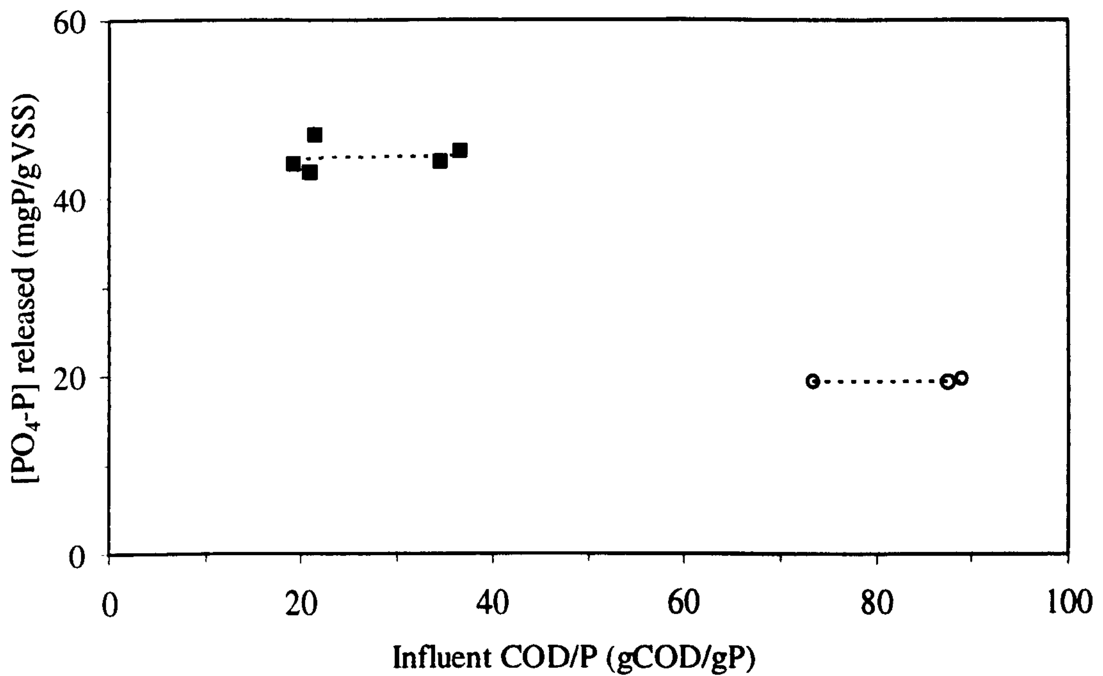


Fig. 4.27. Effect of increasing influent COD/P on anaerobic phosphorus release for: (o) P/M = 0.6 mgP/gVSS and (■) P/M = 4.0 mgP/gVSS.

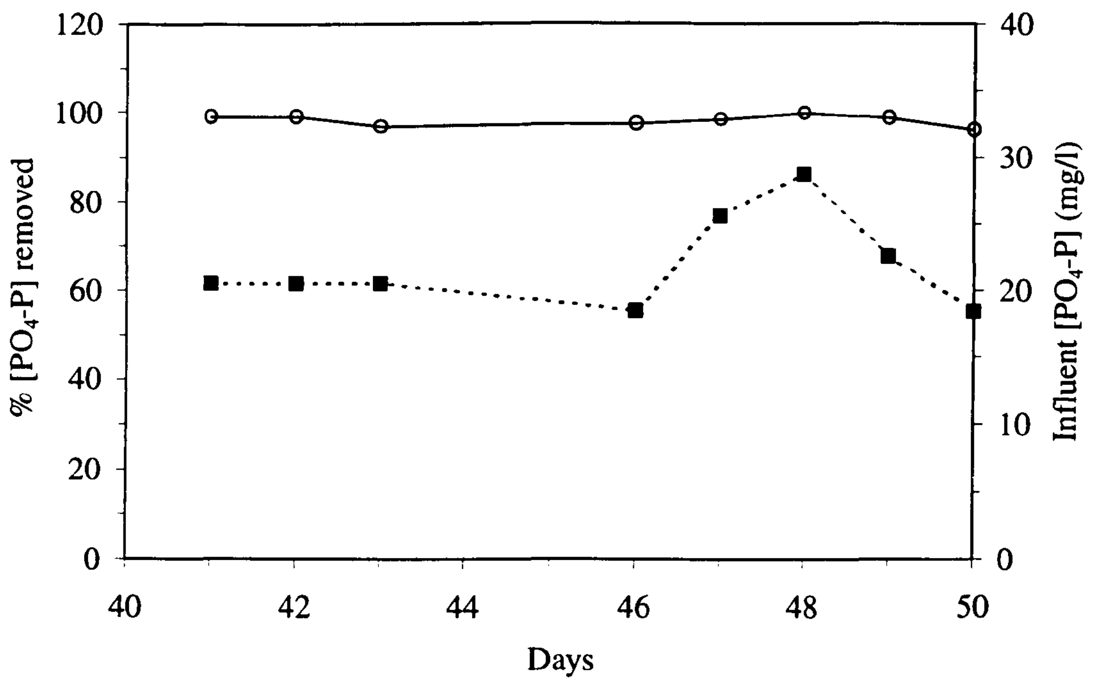


Fig. 4.28. Effect of increasing influent phosphorus on phosphorus removal. (o) % influent P removed and (■) influent [PO_4-P].

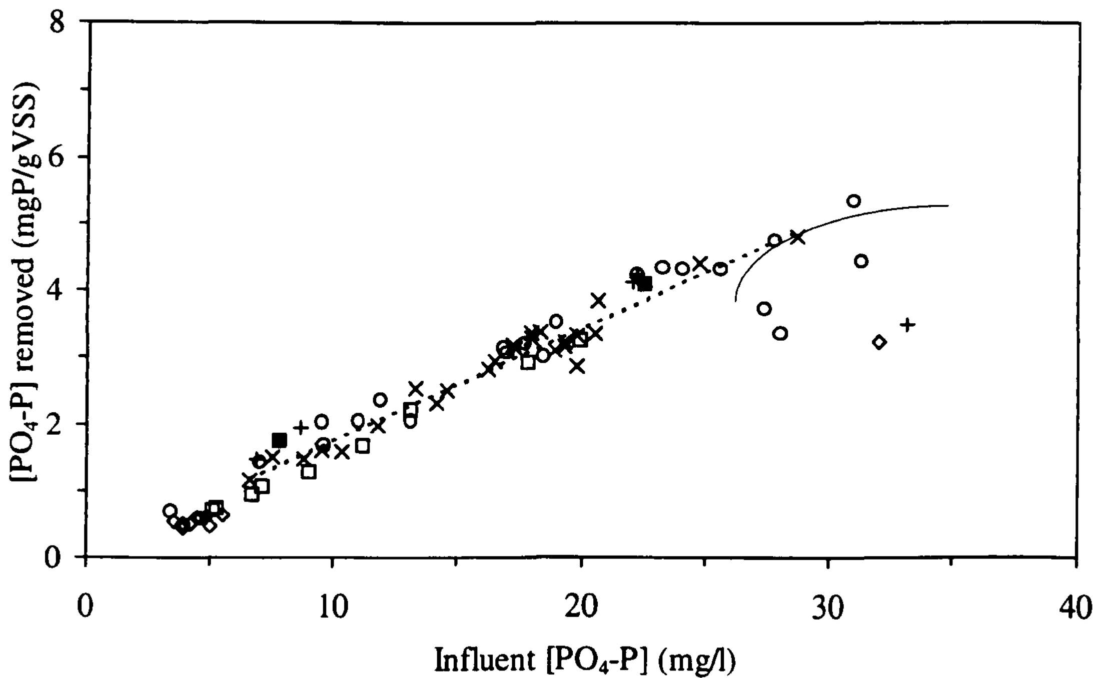


Fig. 4.29. Effect of increasing influent [PO_4-P] on phosphorus removal for various organic loadings. (◊) L=0.2, (□) L=0.3, (×) L=0.4, (o) L=0.5, (+) L=0.6, (■) L=0.7. All L values in mgCOD/gVSS·d.

Effect of influent phosphorus on EBPR

For the second experimental set, the increase in the influent COD/P was achieved by increasing the influent phosphorus concentration and keeping the influent COD constant. This was investigated at an organic loading range of L= 0.2 to 0.9 mgCOD/gVSSd. Unlike the problems encountered with the extreme influent COD values (as described in the previous section), decreasing the influent phosphorus concentration has no effect on the reactor performance. Increasing it above the removal capacity of the sludge will only result in higher effluent phosphorus concentrations, but the COD and ammonia removal processes remain unaffected.

This allowed a more extensive range of COD/P ratios to be investigated than with the previous method (10 - 160).

As with the high influent COD values, for the high influent phosphorus values investigated the influent phosphorus was progressively increased as shown in figure 4.28. The resulting investigated phosphorus range was 4 to 30 mgP/l. This increase in the influent phosphorus had no effect in the removal efficiency of the reactor as long as the P/M ratio was kept below 5.5 mgP/gVSS. Above that value the efficiency of the reactor depended on the available influent COD, with 90% phosphorus removal rates being achieved if the COD/P ratio was above 25 (see results of previous section).

The effect of the increasing influent phosphorus is clearly demonstrated in figure 4.29. The points separated and falling below the solid line, correspond to P/M values above 5.5 mgP/gVSS with organic loadings of below 0.8 mgCOD/gVSSd. The limitations of the L and P/M combinations are better illustrated in figure 4.30. All points representing phosphorus removal efficiencies above 90% fall in a straight line, since all the available influent phosphorus per unit biomass, is removed. The points that represent removal efficiencies between 70 and 50%, fall below the solid lines.

The effect of the influent COD/P for the various organic loadings on phosphorus removal is shown in figure 4.31. The points corresponding to phosphorus removal below 90% have not been included.

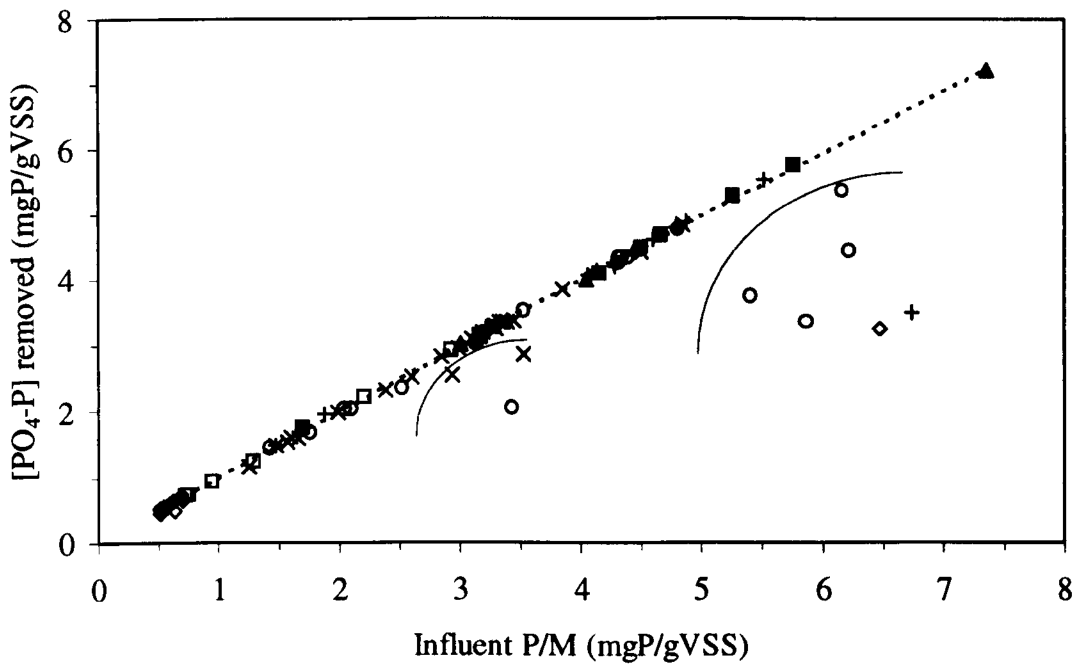


Fig. 4.30. Effect of increasing P/M on phosphorus removal for various L. (◊) L=0.2, (□) L=0.3, (×) L=0.4, (o) L=0.5, (+) L=0.6, (■) L=0.7, (▲) L=0.9.

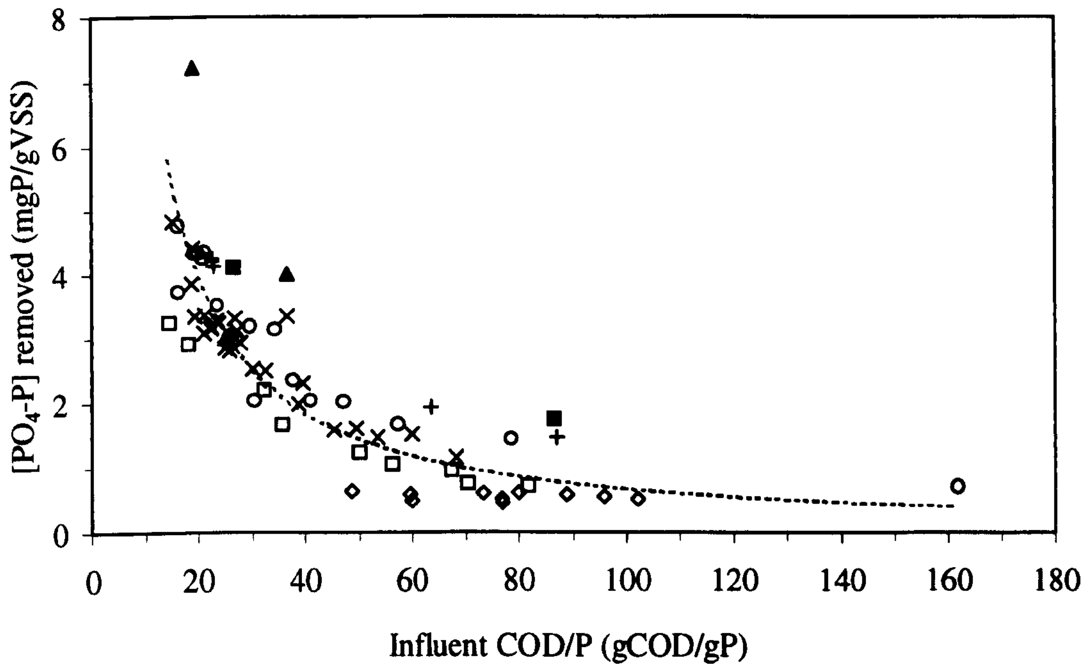


Fig. 4.31. Effect of increasing influent COD/P on phosphorus removal for various L. (◊) L=0.2, (□) L=0.3, (×) L=0.4, (o) L=0.5, (+) L=0.6, (■) L=0.7, (▲) L=0.9. For both figures organic loading values (L) in mgCOD/gVSS·d.

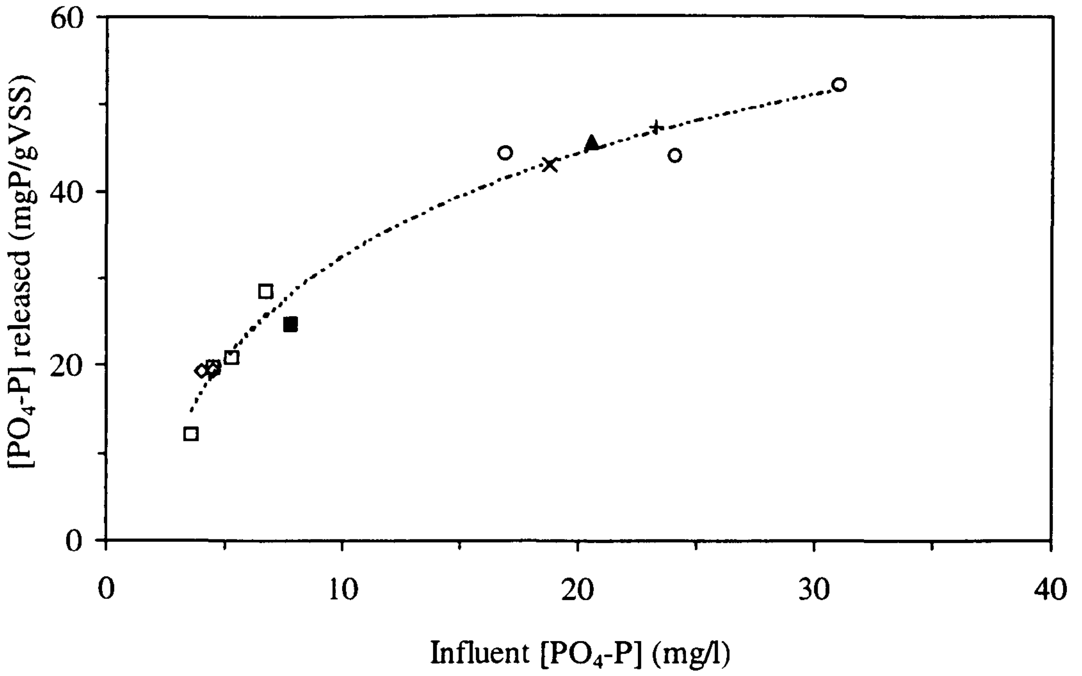


Fig. 4.32. Effect of increasing influent [PO_4-P] on anaerobic phosphorus release for various L.

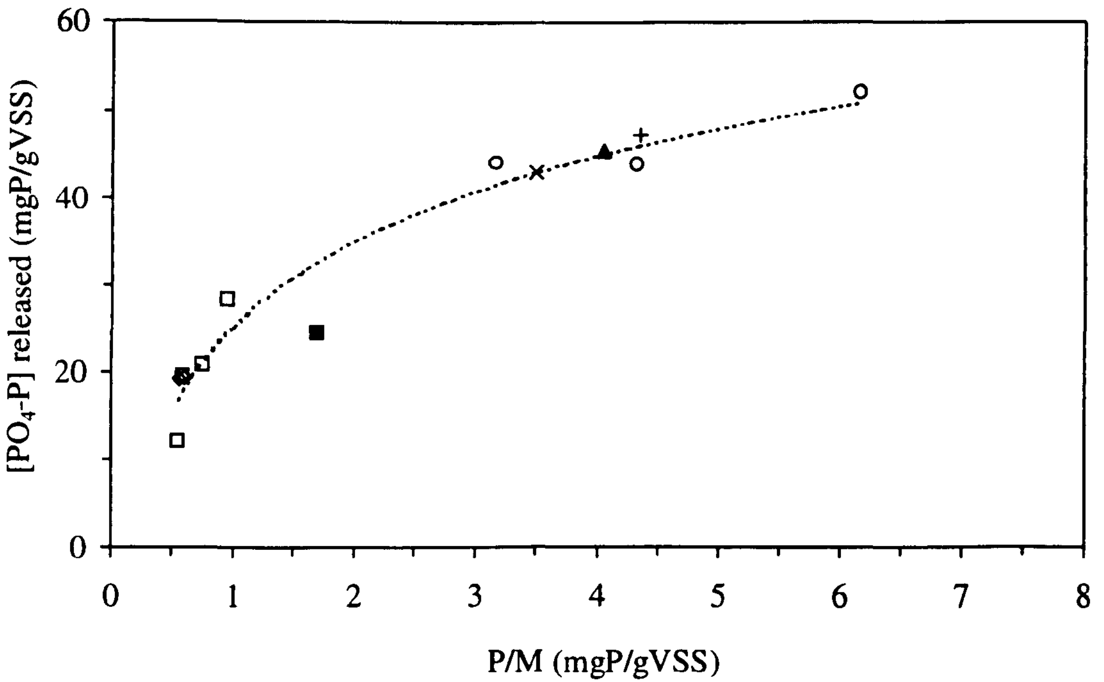


Fig. 4.33. Effect of increasing P/M on anaerobic phosphorus release for various L.

(◊) L=0.2, (□) L=0.3, (×) L=0.4, (o) L=0.5, (+) L=0.6, (■) L=0.7, (▲) L=0.9.

As the graph indicates variation of the COD/P ratio through phosphorus increase or decrease has a significant effect on phosphorus removal for all the investigated organic loadings.

As with phosphorus removal, increasing influent phosphorus concentrations result in increased anaerobic phosphate release (figure 4.32.). Comparing the graphs depicting the effect of increasing influent phosphorus on P removal and release, it appears that although the amount of phosphorus removed is directly proportional to the amount of influent P (for runs with no carbon limitation), this is not the case for the phosphorus released in the anaerobic zone. The relationship describing the effect of influent P on the amount of P released, appears to be asymptotic with a maximum value of 60 mgP/gVSS. This limit could either indicate a maximum release value for the range of L investigated (0.2 - 0.9 mgCOD/gVSS d), or it could point to an absolute maximum release capacity of the type of sludge accumulated in this study (conventional activated sludge inoculated with *A. lwoffi*).

For the range of investigated organic loadings and for phosphorus removal efficiencies above 90%, the influent [PO_4-P] concentration describes the anaerobic release in the reactor well ($R^2 = 0.96$). The same is true for the resulting P/M ratio (figure 4.33.). The influent COD/P shows greater variation (figure 4.34.).

For all the experiments performed, irrespective of their organic loading and influent phosphorus concentration, the amount released during the anaerobic phase of the cycle was directly proportional to the amount taken up during the subsequent aerobic phase (figure 4.35.).

An important note is that all values quoted in the phosphorus release graphs had an anaerobic pH of 7.0 - 7.5. This must be kept in mind for comparative studies as it has been recently demonstrated that the anaerobic phosphorus release / acetate uptake ratio depends on the pH value, in both lab scale (Smolders *et al.*, 1994) and full scale studies (Carlsson *et al.*, 1996).

It must also be noted, that among the values included in the above experiments were runs with varying SRT_E (6-13 days). The only experiments that were not included were the extreme 5 day runs and 16 day runs.

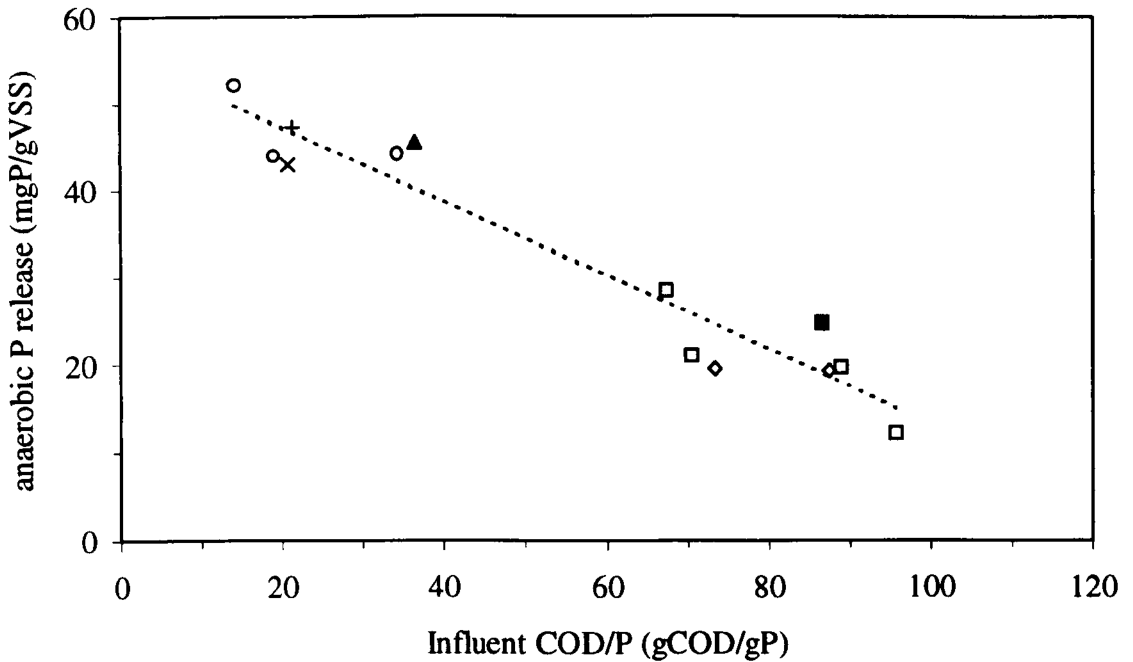


Fig. 4.34. Effect of increasing influent COD/P on phosphorus release for various L. (◊) L=0.2, (□) L=0.3, (×) L=0.4, (o) L=0.5, (+) L=0.6, (■) L=0.7, (▲) L=0.9. All organic loading values (L) in mgCOD/gVSS·d.

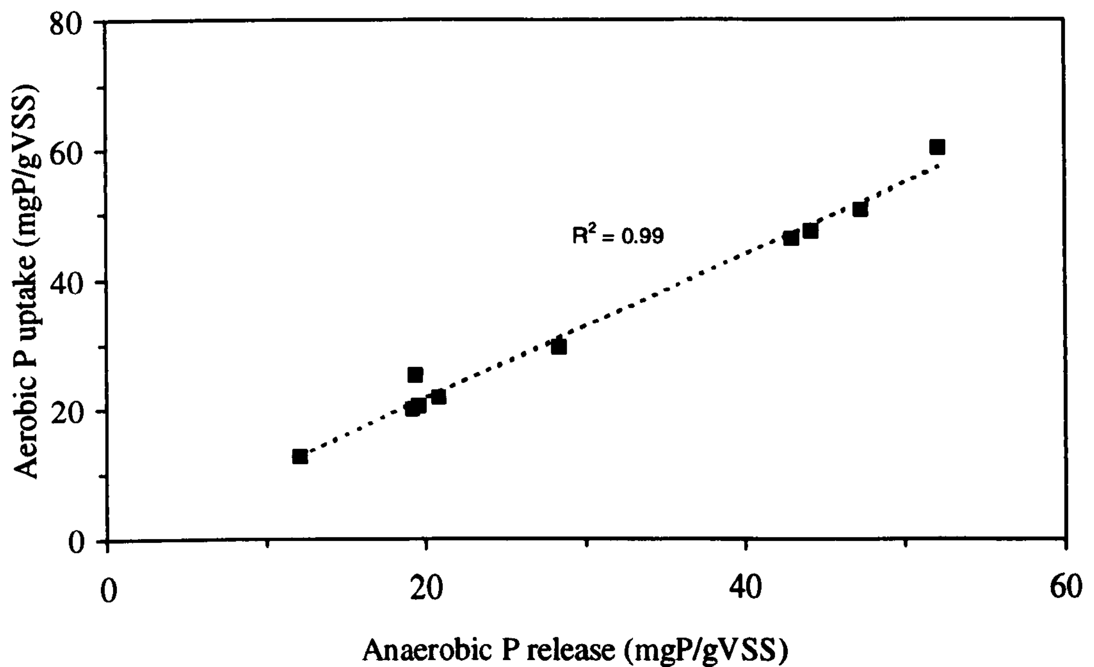


Fig. 4.35. Aerobic phosphors uptake as a function of anaerobic phosphorus release.

TABLE 4.6. EFFLUENT NITRATE CONCENTRATIONS FOR VARYING INFLUENT AMMONIA

Run	Phase	$[NH_3\text{-}N]_{in}$ (mg/l)	SRT_E (d)	TKN/COD (gN/gCOD)	VSS (mg/l)	$[NO_3\text{-}N]_{eff}$ (mg/l)	r_N (gN/gVSS·d)
A1ii	2	35	13	0.09	2376	6.3-10.6	0.002-0.003
A4i	1	52	13	0.13	2724	12.7-19.9	0.02

The inclusion of these points was deemed desirable since in the section 4.3.3.1. it was shown that variations in the operating SRT_E in the range of 5-16 days had no effect on phosphorus removal or release.

Before concluding this part of the discussion, a drawback of the analysis presented so far has to be pointed out. The VSS values used for the calculation of phosphorus release per unit biomass were the values measured at the end of the aerobic phase. The use of the aerobic VSS is the commonly used biomass fraction for most calculations in activated sludge systems. However, a more appropriate fraction for the phosphorus release studies, since release takes place in the preceding anaerobic phase, would have been the anaerobic sludge mass (VSS at the beginning of the anaerobic phase), or even the initial X_0 concentration, measured before the addition of feed. As these values were available for only a fraction of the experiments, the aerobic VSS concentration was used instead.

4.3.3.3. Reactor operation with different effluent nitrate levels

In a SBR operated for EBPR, the aerobic stage provides the environment for combined carbon oxidation and nitrification. In this study, an influent COD/TKN range of 7 - 13 gN/gCOD and an aeration period of 4 to 5 hours, proved adequate for complete ammonia removal, resulting in effluent [NH_3-N] levels below 1 mg/l. On the other hand complete denitrification within the operational cycle was rarely achieved, leaving the effluent highly nitrified. This was not a problem for the performance of EBPR, since by the beginning of the next cycle complete removal of nitrate took place. The last traces of all oxidised nitrogen species were denitrified during the first moments of the anoxic fill of the following cycle.

For the sludge treatment experiments though, it was desirable to have varying amounts of nitrate in the excess sludge. The first method employed for producing varying nitrate sludge contents, was to alter the ammonia concentration in the influent. Reducing the influent ammoniacal nitrogen from 52 mgN/l to 35 mgN/l, reduced the amount of nitrate at the end of the aerobic phase to half (Table 4.6.). As

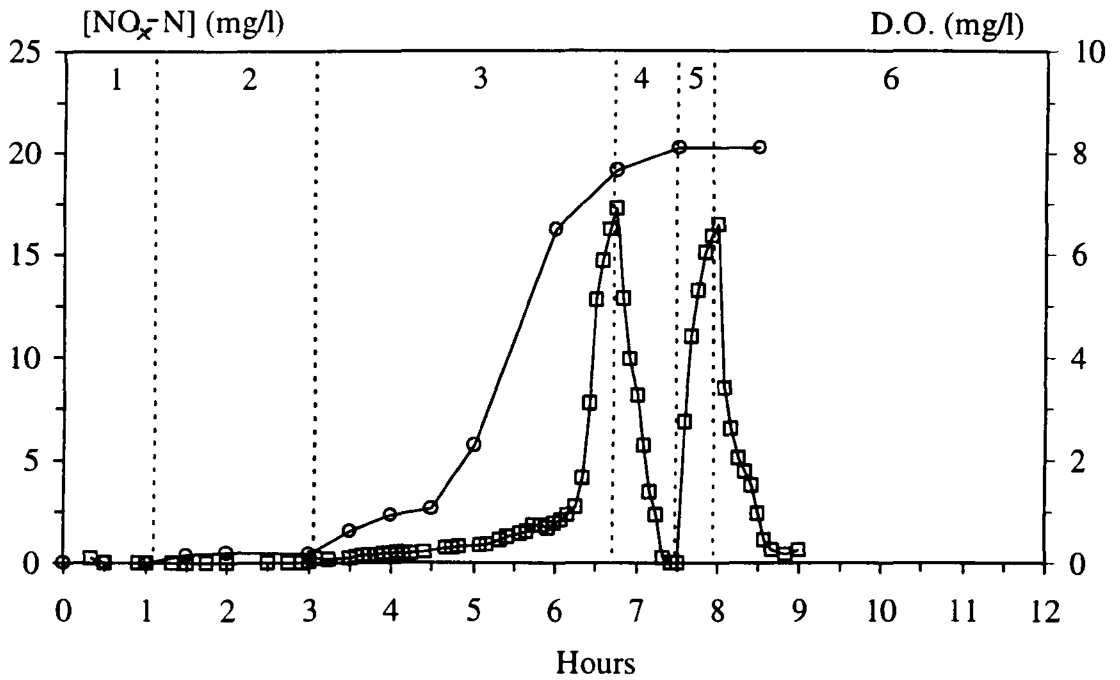


Fig. 4.36. [NO_x-N] and D.O. profile during a full SBR cycle, employing a 30 minute anoxic phase. (o) [NO_x-N] and (□) D.O.

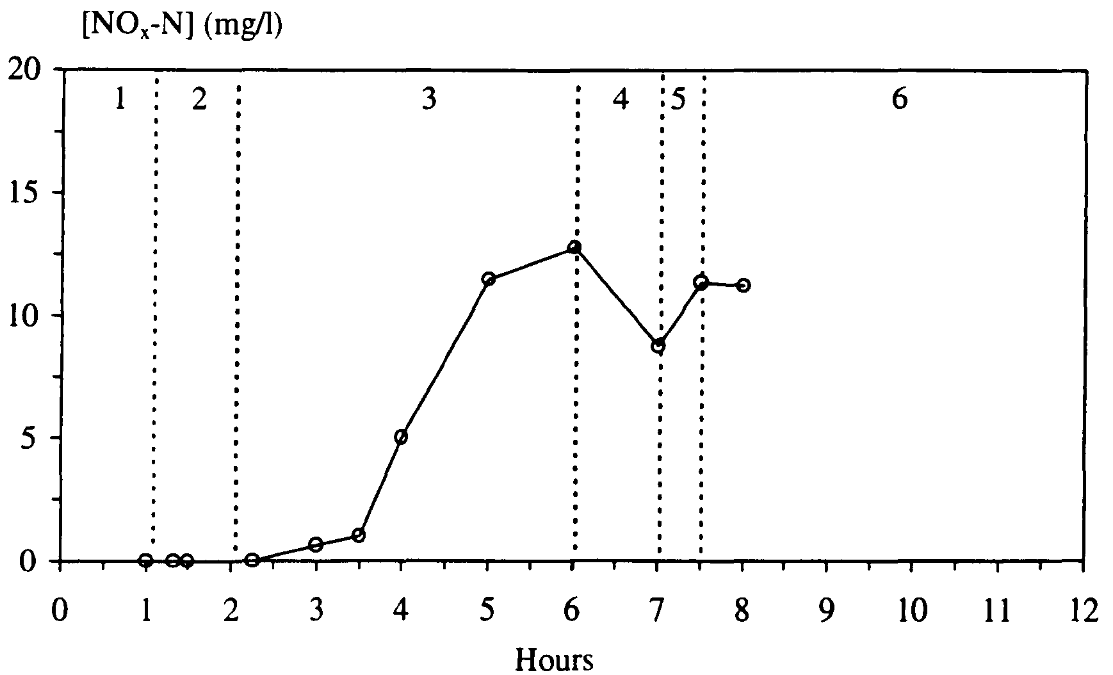


Fig. 4.37. [NO_x-N] profile during a full SBRcycle, employing a one hour anoxic phase.

(1) Feed (2) Anaerobic (3) Aerobic (4) Anoxic (5) Post-aerobic (6) Settle/Draw/Idle.

The second method employed was the introduction of an anoxic zone after the aerobic one. This would allow denitrification to take place after ammonia oxidation and phosphorus removal were completed. The initial anoxic length employed was half an hour (figure 4.36.), but that was not adequate time for the D.O. concentration to fall below 0.5 mg/l and for denitrification to commence (Irvine and Busch, 1979). Increasing the anoxic stage from half to one hour gave better results (figure 4.37.).

During all the runs that employed an anoxic stage, a post-aerobic short phase was also added to ensure that secondary phosphorus release would not occur (figures 4.36. and 4.37.). This post-aerobic stage, resulted in further nitrification but the nitrate sludge content was less than at the end of the aerobic stage (figure 4.37.).

The development of nitrification and denitrification in the reactor was also monitored during the start-up phases. Figure 4.38. shows the development of EBPR during a bioaugmentation run with *Acinetobacter* spp. at t=30 days. Figure 4.39. shows the development of ammonia removal over the same operating period. Within two weeks from seeding the reactor with fresh sludge, complete removal of ammoniacal nitrogen was achieved. From the concentrations of nitrate and nitrite in the effluent it appears that for the first 50 days, only the first step of nitrification was operating and as a result nitrite was accumulated in the reactor. The increase in the effluent nitrate concentration, which progressively replaced nitrite as the oxidised nitrogen form in the reactor, coincided with the establishment of high rates of EBPR. Daily profiles taken over this period show this exact progress (figure 4.40.).

It is not clear why this nitrite built-up took place in the reactor and as the ability of the chromatographer to detect nitrite was due to a new analytical column bought at the start of this final run, no data from other similar runs are available for comparison.

It is believed that high concentrations of free ammonia inhibit *Nitrobacter* spp, which are responsible for converting nitrite to nitrate. It has been shown that as a result of high concentrations of [NH_3-N], ammonia is converted only to NO_2^- without further oxidation to NO_3^- (Orhon and Artan, 1994; Balmelle *et al.*, 1992).

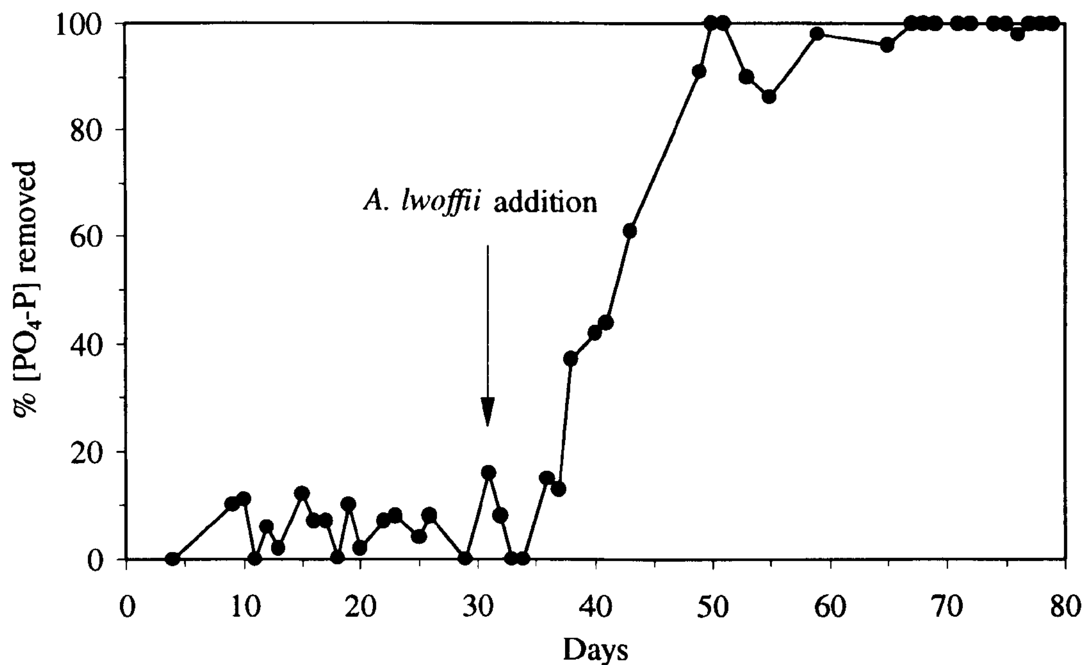


Fig. 4.38. Development of EBPR during a bioaugmentation run at t=30 days.

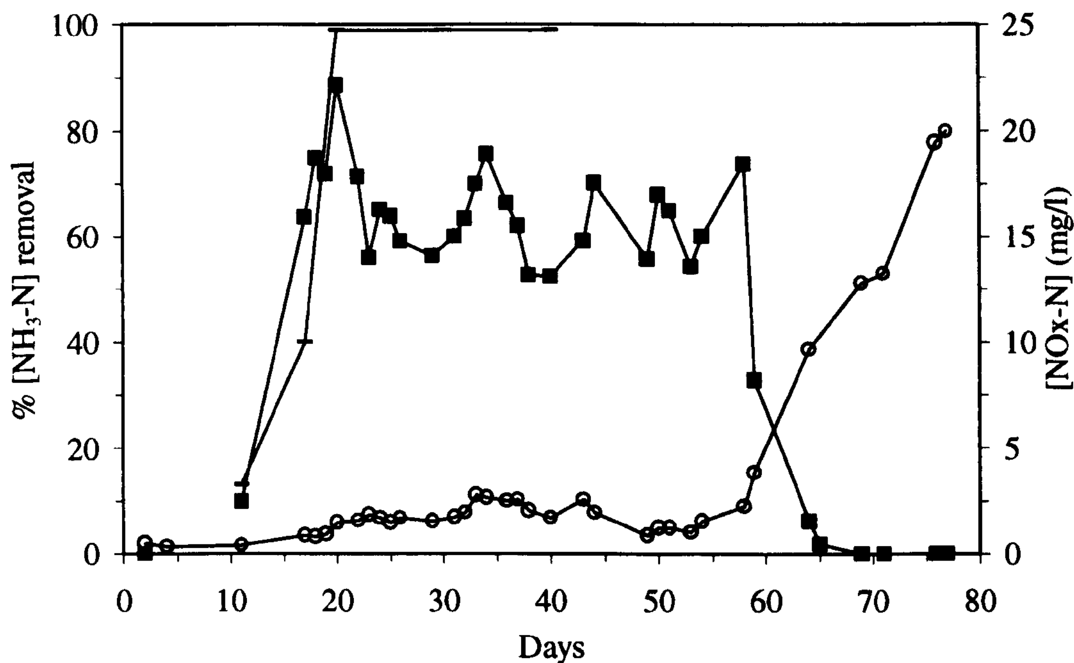


Fig. 4.39. Development of ammonia removal and nitrification during a bioaugmentation run at t=30 days. (–) [NH_3-N], (o) [NO_3-N] and (■)[NO_2-N].

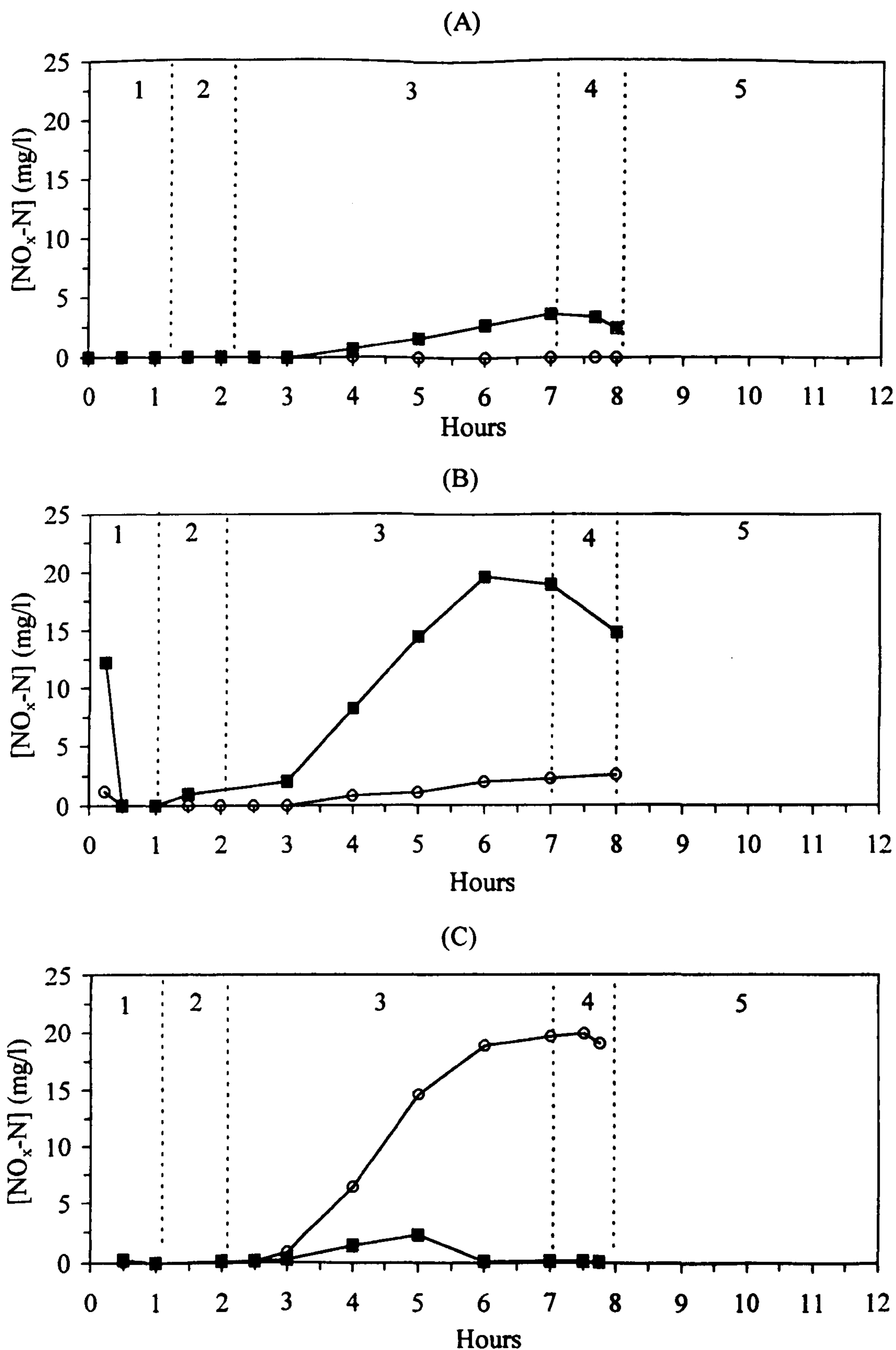


Fig. 4.40. Development of [NO_x-N] profiles during a bioaugmentation run at t=30 days. (o) [NO_3-N] and (■) [NO_2-N]. (A): day 11, (B): day 43, (C): day 78. (1) Feed (2) Anaerobic (3) Aerobic (4) Settle (5) Draw/Idle.

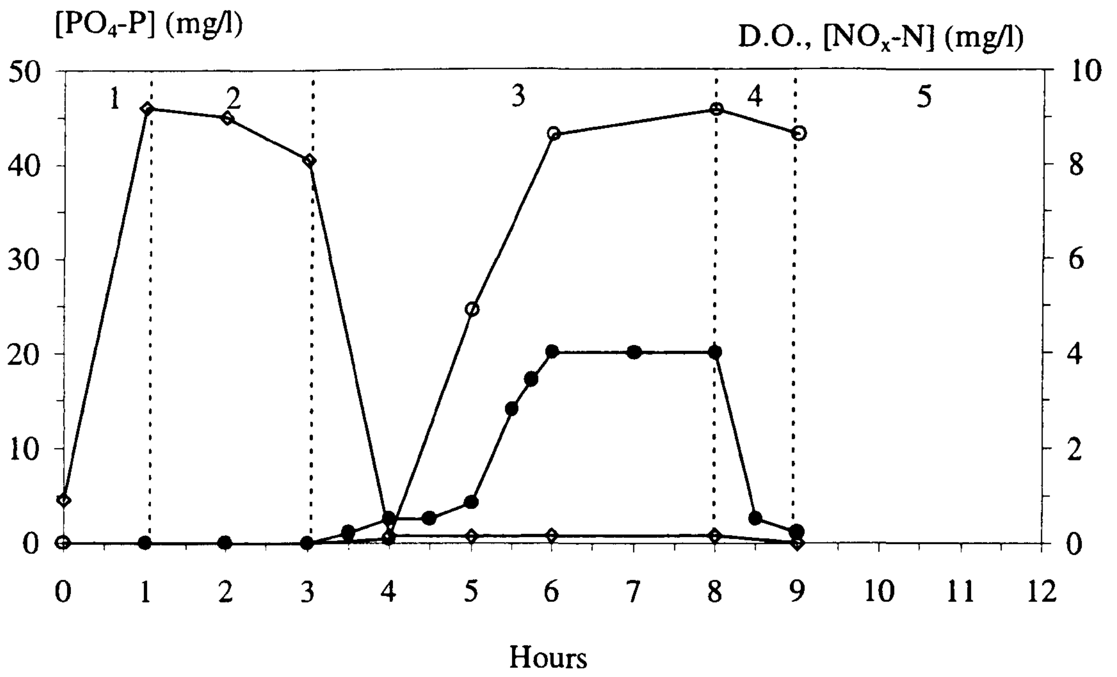


Fig. 4.41. (◊) [PO_4-P], (o) [NO_x-N] and (●) D.O. profiles during a full SBR cycle.

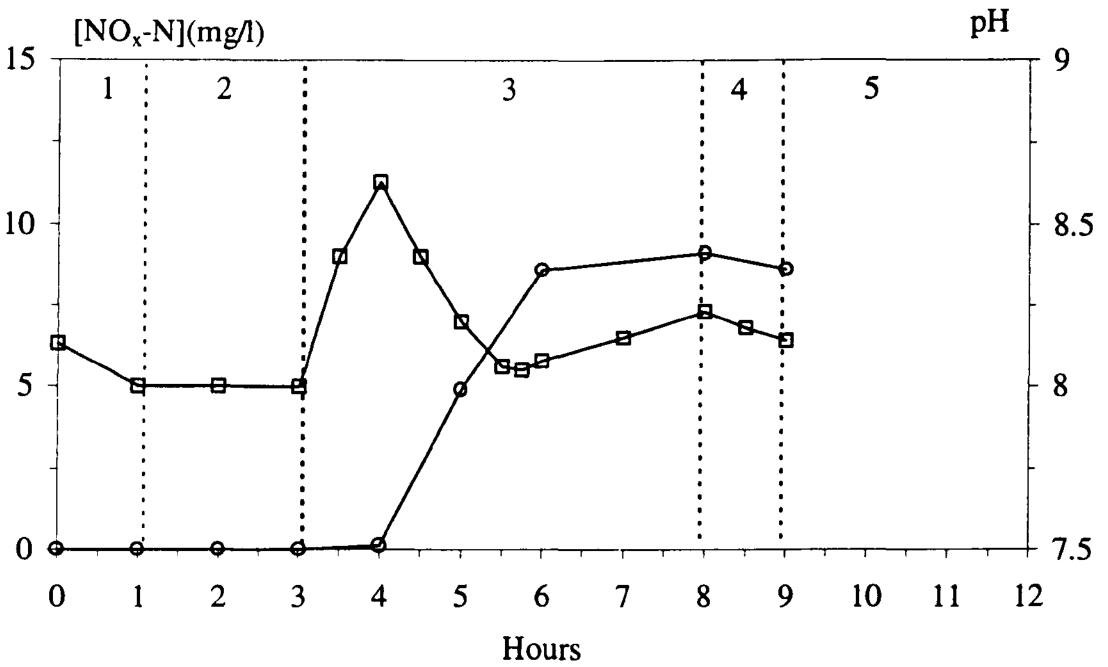


Fig. 4.42. (o) [NO_x-N] and (□) pH profiles during a full SBR cycle.

SBR phases: (1) Feed, (2) Anaerobic, (3) Aerobic, (4) Settle, (5) Draw/Idle

Other factors associated with nitrite built-up are a variation in temperature, a decrease in the dissolved oxygen concentration, solids washout, acute process loading and finally simultaneous nitrification denitrification which results in a hidden nitrate reduction (Yang and Alleman, 1992). Of the above factors in this study the most probable cause of the nitrite built-up was the presence of free ammonia and low D.O. levels in the initial stages of the aerobic phase. During the aerobic stage, phosphorus is taken up by the polyP bacteria and ammonia is utilised for cell synthesis.

High oxygen uptake rates at the start of the aerobic stage do not allow the dissolved oxygen concentration to rise to detectable levels until most of the phosphate has been taken up (figure 4.41.). As phosphate uptake nears completion, the D.O. starts to increase and at the same time nitrification starts to take place. This is accompanied by the decrease in the pH, which had previously risen during phosphorus uptake (figure 4.42.). It must be noted, that since in this particular run the pH values were above 8.0, some ammonia removal due to volatilisation and stripping cannot be excluded.

The observed late increase in the D.O. due to demands for cell synthesis by the polyP bacteria, may be the reason for the accumulation of nitrite in the reactor, but it does not explain why this build-up seased after a number of days had passed (figure 4.39.). There is a possibility that the nitrite oxidising biomass is slowly acclimatised to the operating conditions, but the literature does not support this.

4.3.3.4. Reactor operation with low dissolved oxygen during the aerobic phase

The dissolved oxygen requirements for biomass growth, phosphate uptake and ammonia oxidation lie within the range of 2-6 mg/l (Yeoman *et al.*, 1988a) and should never be allowed to drop to below 1 mg/l. Although it is well established that D.O. concentrations below 1 mg/l are detrimental for nitrification (Irvine, 1979), the effect of operating EBPR with an aerobic stage in oxygen limited conditions is unclear. On the other hand over aeration, with D.O. concentrations above 5 mg/l, has been shown to be detrimental to phosphorus removal, causing aerobic digestion of solids, excessive nitrification and re-release of phosphorus from the sludge (Yeoman *et al.*, 1988a).

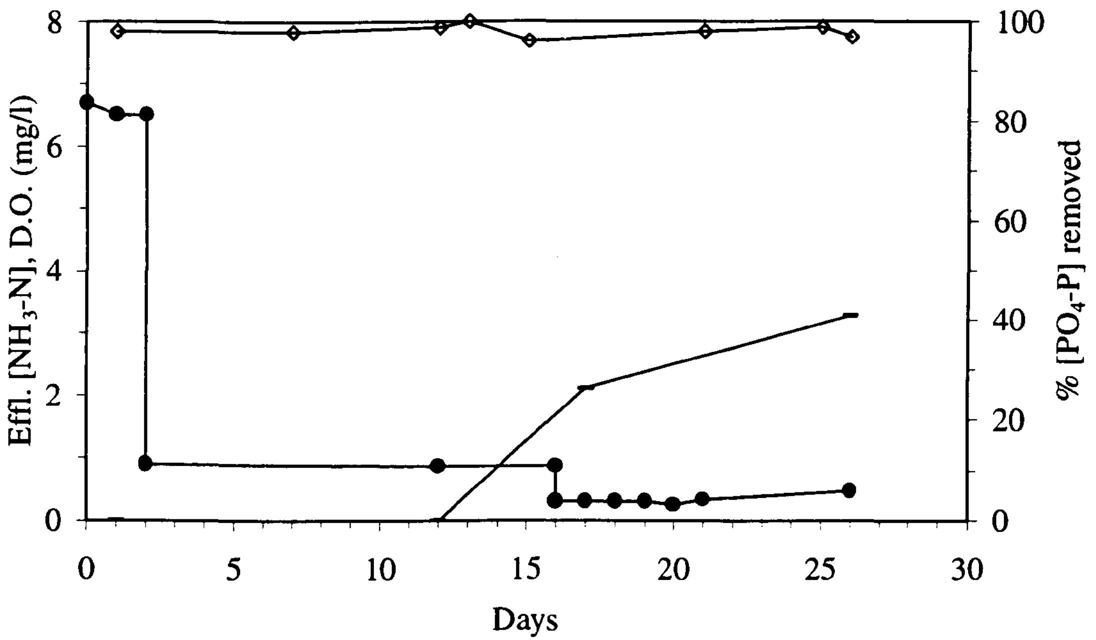


Fig. 4.43. Reactor operation with decreasing (●) D.O. levels. Days 1-2 unrestricted air supply, days 2-16 D.O. < 1 mg/l, days 16-25 D.O. < 0.5 mg/l. (-) effluent [NH_3-N] and (◊) % influent [PO_4-P] removed.

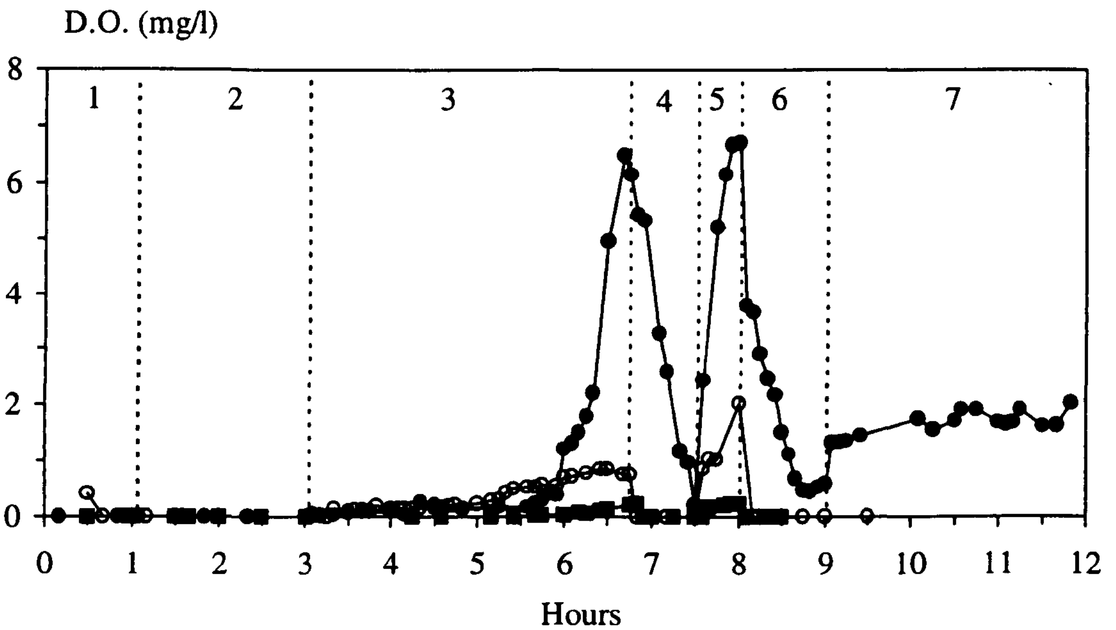


Fig. 4.44. D.O. profiles during a full SBR cycle for reactor operation as shown in Fig. 4.43. (●) unrestricted air supply, (o) D.O. < 1 mg/l, (■) D.O. < 0.5 mg/l. SBR phases: (1) Feed, (2) Anaerobic, (3) Aerobic, (4) Anoxic, (5) Post-aerobic, (6) Settle, (7) Draw-Idle

In this study the effect of operating the reactor in a wide range of dissolved oxygen concentrations (maximum aerobic D.O. 0.5 - 8 mg/l), was investigated over a month of operation. The intention was to explore the effect of varying D.O. concentrations on the excess sludge redox levels, which would in turn effect the phosphorus release rates during sludge treatment.

When the oxygen supply to the aeration stage of the SBR cycle was not restricted, the D.O. increased from an undetectable concentration to 5-8 mg/l in the space of 4 to 5 hours. For the oxygen limited runs the D.O. concentration was maintained at 1 mg/l for the first half of the run and at 0.5 mg/l for the remaining half (figure 4.43.). This decrease had no effect the overall phosphorus and COD removal properties of the reactor, although an increase in the effluent ammonia from an undetectable concentration to 3 mg/l, over the 25 day period was noted.

The D.O. and pH profiles for the three operating D.O. levels, (unrestricted, 1 mg/l and 0.5 mg/l) are shown in figures 4.44. and 4.45. respectively. The first effect of the decrease in the air supply, demonstrated from the pH profile (figure 4.45.), was the delay in the increase of pH with decreasing D.O., indicating a delay in phosphate uptake and polyphosphate synthesis (figure 4.46.). The increase in pH during phosphate uptake has been explained as a net decrease in the acidity of the mixed liquor due to $H_2PO_4^-$ transfer into the cell (Wentzel *et al.*, 1986), or CO_2 stripping (Comeau *et al.*, 1987).

The maximum pH value, signalling the near completion of phosphorus uptake and the beginning of nitrification is shifted from 1.5 hours after the initiation of aeration, for the no oxygen limitation run, to 2 hours for the 1 mg/l D.O. run, to 3.5 hours for the 0.5 mg/l run. The following pH suppression (CO_2 evolution due to biomass synthesis and ammonia oxidation) is also delayed and in the 0.5 mg/l D.O. runs, is severely curtailed. This lack of pH suppression, which allowed the overall pH to be maintained around neutral values, resulted in a net increase in the reactor pH, which eventually operated wholly in the alkaline region (figure 4.45.).

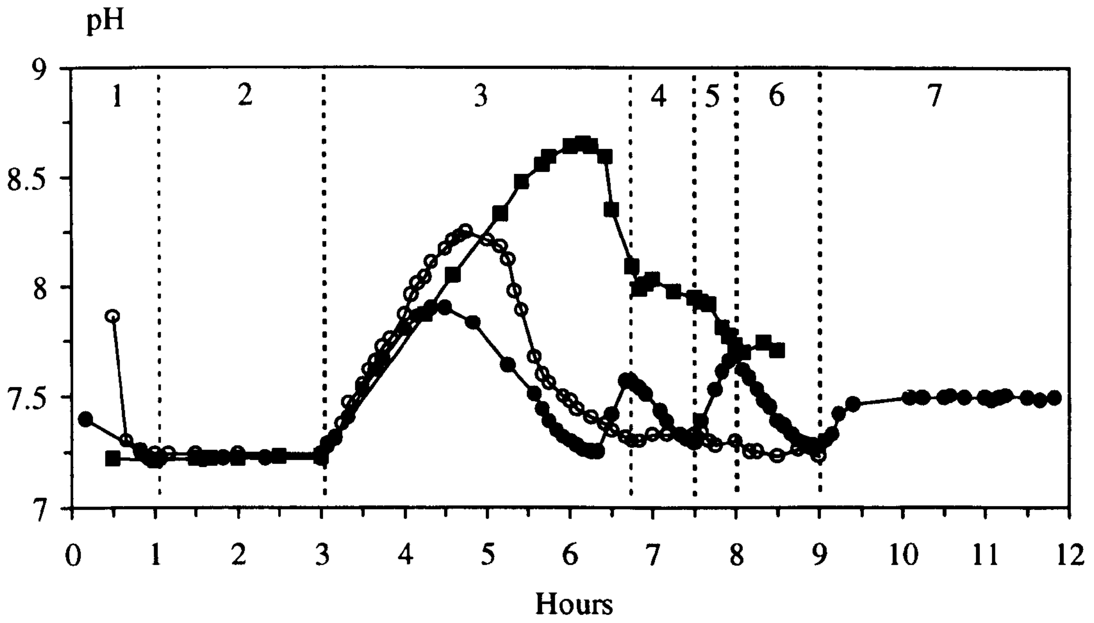


Fig. 4.45. pH profiles during a full SBR cycle for reactor operation as shown in Fig. 4.43. (●) unrestricted air supply, (○) D.O. < 1 mg/l, (■) D.O. < 0.5 mg/l

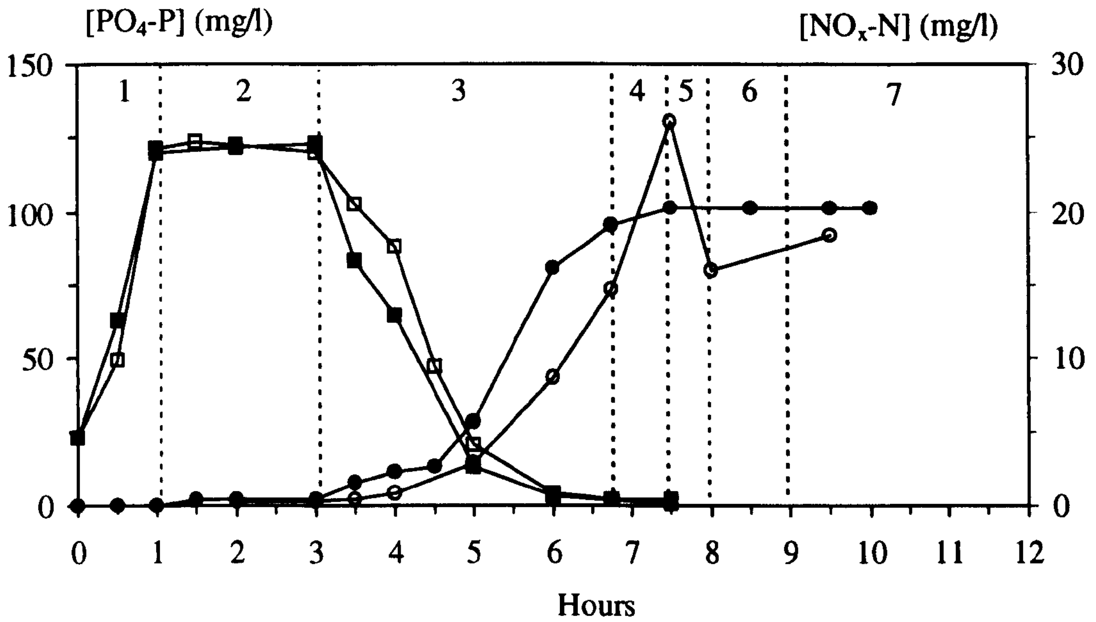


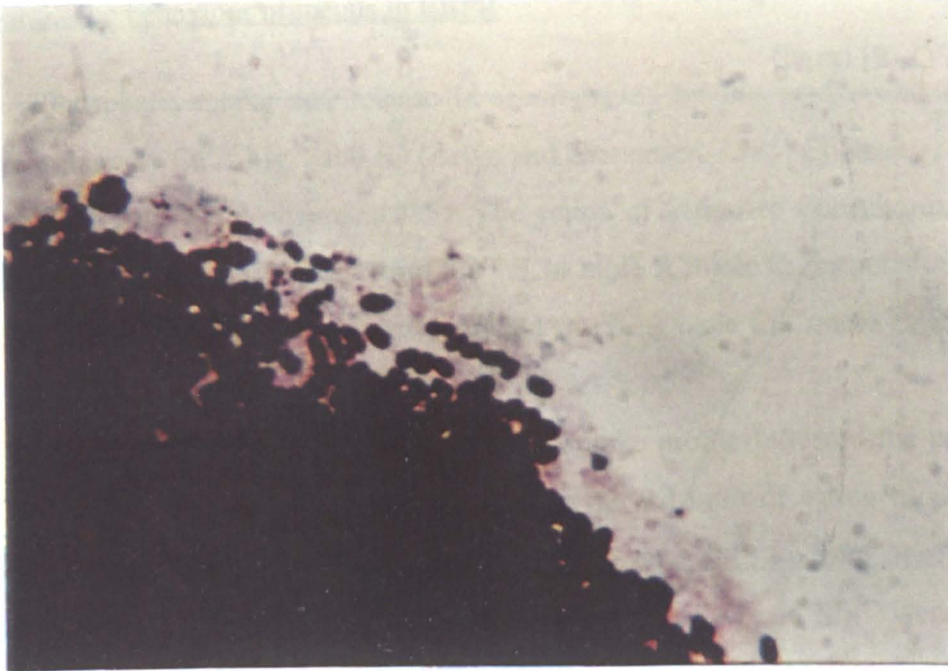
Fig. 4.46. [PO_4-P] and [NO_x-N] profiles during a full SBR cycle for reactor operation as shown in Fig. 4.43. (●)[NO_x-N] unrestricted air supply, (○) [NO_x-N] D.O. < 1 mg/l, (■) [PO_4-P] unrestricted air supply, (□) [PO_4-P] D.O. < 1 mg/l.
SBR phases: (1) Feed, (2) Anaerobic, (3) Aerobic, (4) Anoxic, (5) Post-aerobic, (6) Settle, (7) Draw-Idle

A second effect of the decrease in the available air, is the disappearance of the secondary pH increase observed in the unrestricted air supply runs (figure 4.45.) occurring during the last half hour of the first aerobic stage and again repeated in the post-aerobic stage. This can be explained either as further CO_2 stripping, or as simultaneous nitrification - denitrification with an increase in pH due to released alkalinity. Denitrification may be taking place inside the microenvironment of the flocs in spite of the high D.O. concentration in the bulk liquid. This is especially true for sludges dominated by *Acinetobacter* spp. who form dense, compact flocs (photograph 4.4.).

The low D.O. (1 mg/l) in the aeration phase also affected nitrification (figure 4.46.). At the end of the aerobic phase (t = 6:45 hours), the NOx-N concentration in the bulk liquid was 5 mg/l less than in the unrestricted air supply reactor. The nitrification rate decreased from 0.071 d^{-1} for the unrestricted air supply to 0.053 d^{-1} for the D.O. limited runs.

Monitoring the ORP profile of the reactor revealed the depression of the redox values of the sludge for the low D.O. runs (figure 4.47.). During the oxygen limited operation, the reactor ORP continues to decrease during the aeration period, remains constant in the anoxic and post aerobic stages and starts to increase only in the supernatant. The breakpoint in the unlimited air runs coincides with the appearance of free residual D.O. (> 0.1 mg/l) in the bulk liquid and a rapid increase in the nitrification rate. In the air restricted runs this feature is delayed and is far less pronounced (figure 4.47.).

It is important to point out that the ORP profiles presented in figure 4.47. should only be compared as profiles and not as actual mV values. This is a result of the lack of reliability and most importantly reproducibility of the ORP platinum electrode used for the measurements. These shortcomings are a direct result of the effect that parameters such as pH, D.O., the ionic forms of nitrogen and electrode treatment have on the electrode potential (Heduit and Thevenot, 1992). This potential nevertheless seems to provide an overall indication of the redox state of the biomass especially on a comparative basis and has therefore been included in the results presented here.



Photograph 4.4. Neisser stained compact sludge floc, typical of *A. lwoffi* (Oil immersion light microscopy, magnification x1000).

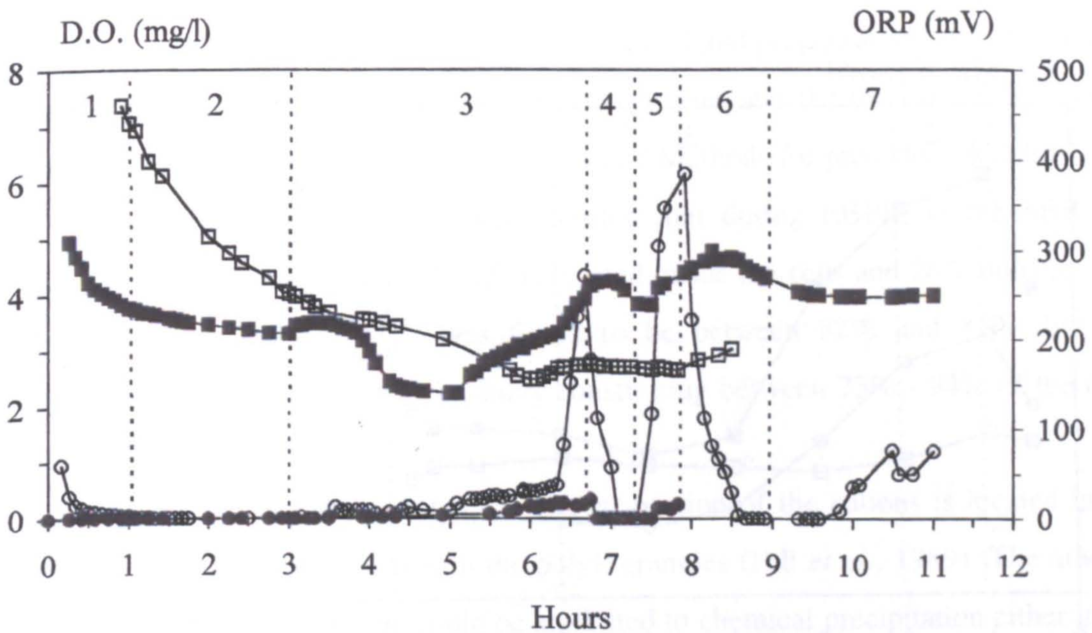


Fig. 4.47. ORP profiles during a full SBR cycle for reactor operation as shown in Fig. 4.43. (●) D.O. unrestricted air supply, (○) D.O. < 0.5 mg/l, (■) ORP unrestricted air supply, (□) ORP D.O. < 0.5 mg/l. SBR phases: (1) Feed, (2) Anaerobic, (3) Aerobic, (4) Anoxic, (5) Post-aerobic, (6) Settle, (7) Draw-Idle

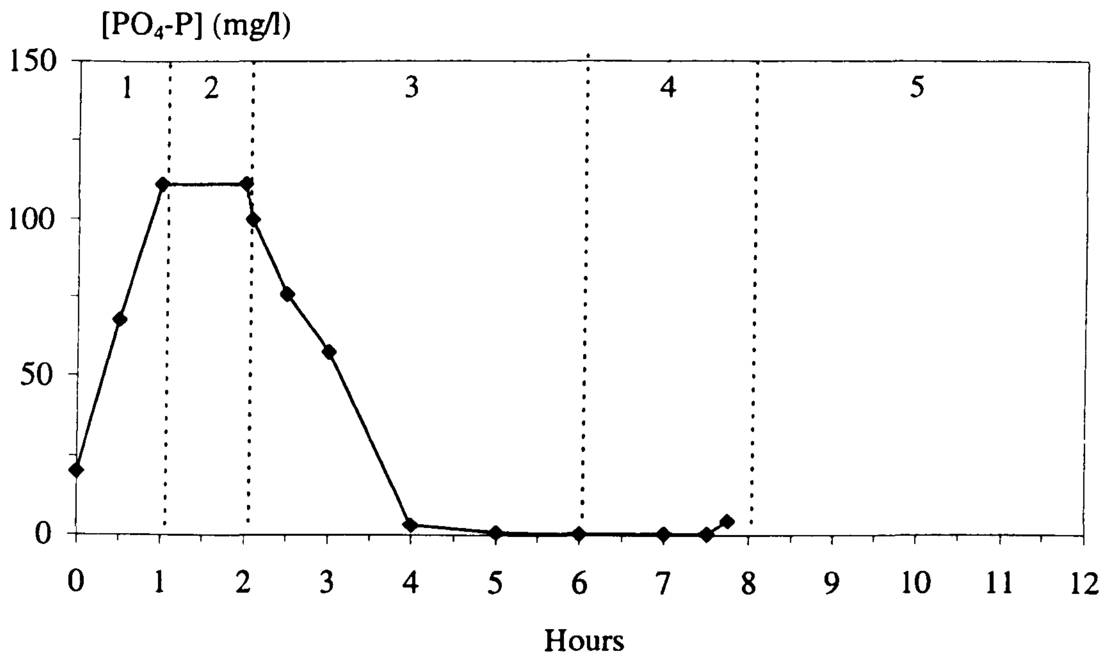


Fig. 4.48. [PO_4-P] profile during a full SBR cycle.

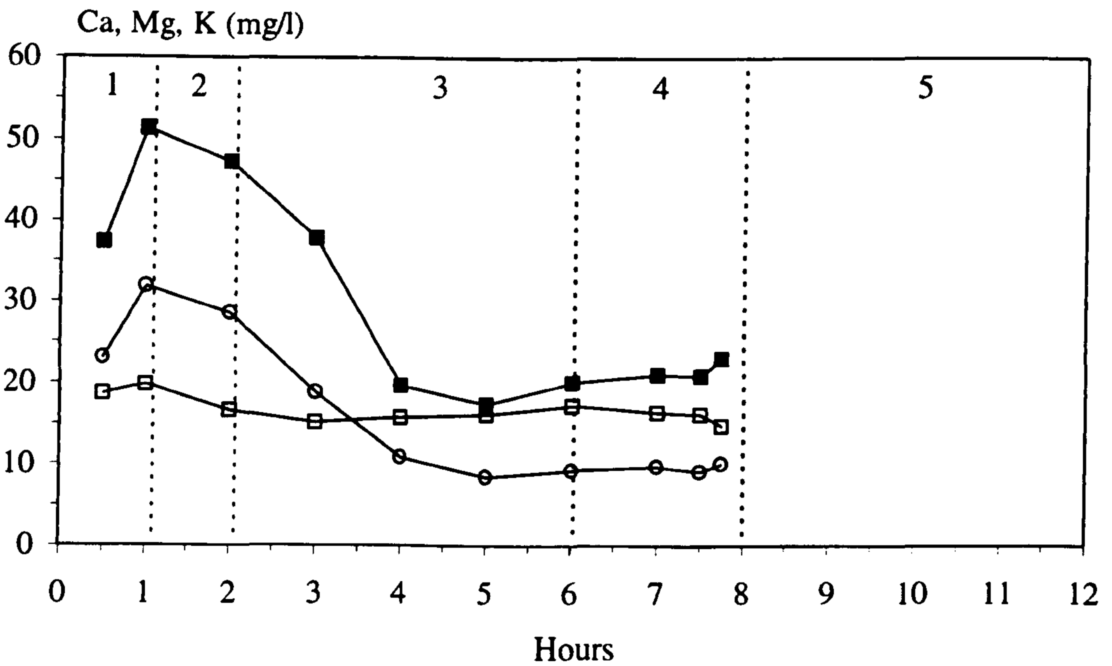


Fig. 4.49. Release and uptake of cations during a full SBR cycle. (○) Ca, (■) K, (□) Mg.

SBR phases: (1) Feed, (2) Anaerobic, (3) Aerobic, (4) Settle, (5) Draw-Idle.

4.3.3.5. The behaviour of metals in EBPR

Phosphate uptake and release is accompanied by the uptake and release of cations, namely Ca^{2+}, Mg^{2+} and K^+ (Arvin and Kristensen, 1985; Comeau *et al.*, 1986; VanGroenestijn and Deinema, 1985). The ratios of cation to phosphorus released, published in the literature vary from 0.2 - 0.34 mole K^+/mole P, for potassium, 0.26 - 0.32 mole Mg^{2+}/mole P, for magnesium and 0 - 0.12 mole Ca^{2+}/mole P, for calcium (for references see Literature Review, section 2.3.4.).

In this study, the Mg^{2+} and K^+ release/uptake profile followed the phosphorus profile closely (figure 4.48. and 4.49.). The observed ratio of cation to phosphorus released was 0.04 mgCa^{2+}/mgP, 0.3 mgK^+/mgP and 0.19 mgMg^{2+}/mgP. For the particular biomass selected in this study, it appears that K^+ and Mg^{2+} were the most important counter ions of polyP as they correlated well with P release/uptake (figure 4.50.). This was not the case for Ca^{2+}, as shown by figure 4.51.

To investigate whether the origin of the released cations was inside the cytoplasm (bound to polyP), or outside (e.g. bulk liquid precipitation), the sludge was separated into extracellular and intracellular fractions and the total metal content of each fraction was determined (see Materials and Methods for procedure details).

From this investigation it was revealed that during EBPR, in the SBR, on average 74% of the sludge total Ca^{2+} is located inside the cells and 26% outside. The fraction of intracellular Mg^{2+} was found to be between 82% and 81%, but the intracellular K^+ showed more variability constituting between 73% - 94% of the total sludge K^+.

These results indicated that the largest portion of the cations is located inside the cell, probably comlpexed with the polyP granules (Hill *et al.*, 1989). The amount found outside the cytoplasm, could be attributed to chemical precipitation either in the bulk liquid or inside the flocs. A strong possibility exists that the extracellular content of the cations is bound in the ECPs of the sludge (Morgan *et al.*, 1990; Karapanagiotis *et al.*, 1989) or is adsorbed at the outer cell surface of *Acinetobacter* cells (Streichan and Schon, 1991; Bark *et al.*, 1992).

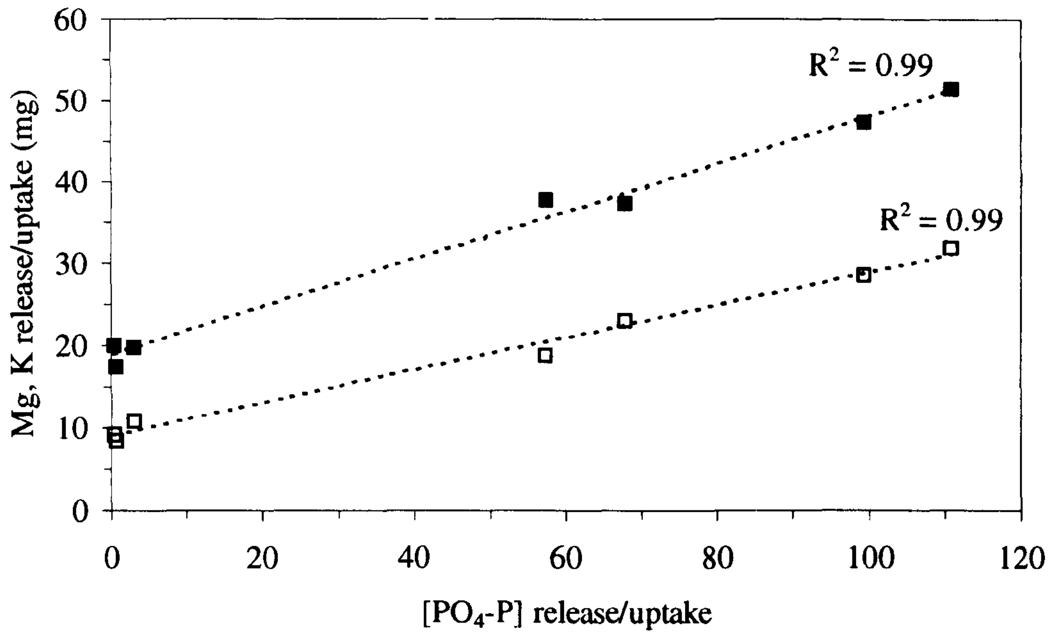


Fig. 4.50. Release and uptake of cations as a function of phosphorus transport. (■) K, (□) Mg.

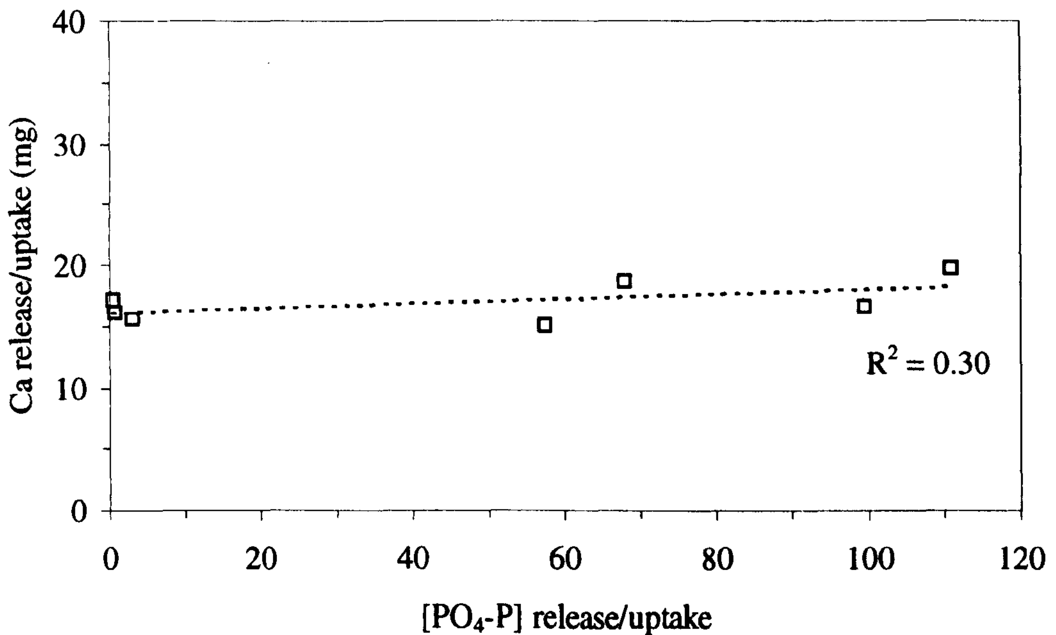


Fig. 4.51. Release and uptake of Ca^{2+} as a function of phosphorus transport.

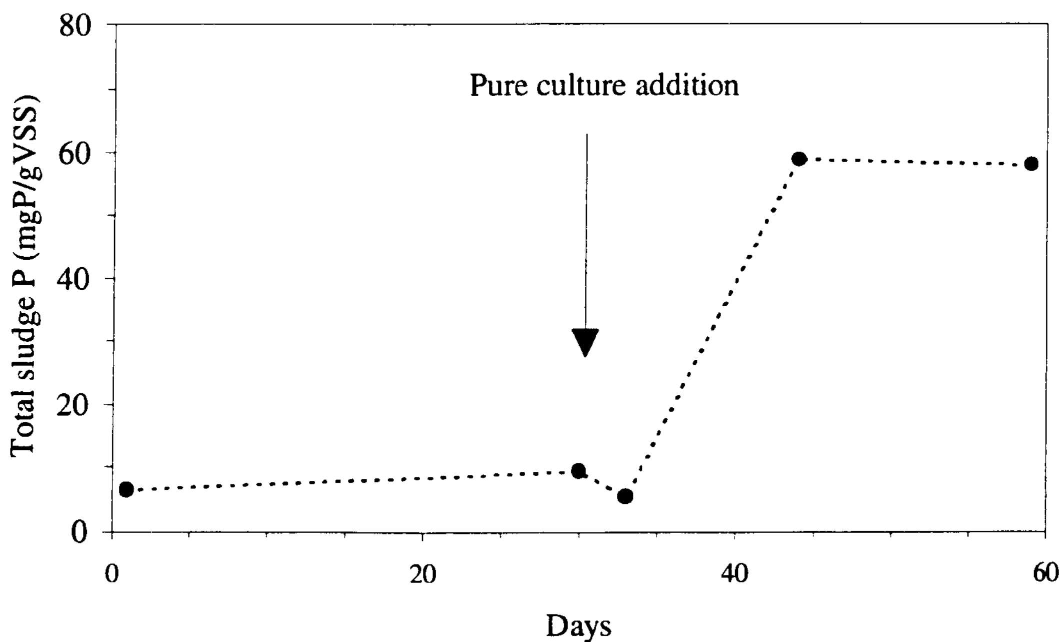


Fig. 4.52. Increasing phosphorus sludge content with increasing days in run.

4.3.3.6. Total phosphorus sludge content and phosphorus fractionation results

The development of EBPR in the reactor, resulted in an increase in the sludge phosphorus content (figure 4.52). The total phosphorus of the sludge increased from 6.32 mgP/gVSS in the fresh, unacclimatised sludge, to as high as 194 mgP/gVSS for the higher phosphorus loaded runs (influent [PO_4-P] = 30 mg/l). During good EBPR operation (phosphorus removal efficiency >90%), the sludge phosphorus content varied between 60 mgP/gVSS and 194 mgP/gVSS.

Not all of phosphorus contained in the sludge was released during the anaerobic phase in the daily cycles. Between 22 and 47 mgP/gVSS were released in the anaerobic stage which constituted 29% to 54% of the total phosphorus sludge content. A comparison with values published in the literature is shown in Table 4.7.

As with the cations (section 4.3.3.5.), an estimation of the phosphorus fractions in the sludge, will determine the extent, if any, of the contribution of the chemical precipitants to phosphorus removal. The increased phosphorus concentration in the anaerobic zone of a EBPR plant, is believed to lead to chemical phosphate precipitation (Arvin, 1983; Beccari *et al.*, 1985; Miya, 1987). Even when calcium phosphate precipitation is not possible in the bulk liquid of a treatment plant, it may still take place inside the bioflocs (Arvin, 1979, 1983), during denitrification. This is a result of the decreasing CO_2 concentration and increasing pH, which favours precipitation (Menar and Jenkins, 1969, Ferguson *et al.*, 1969).

Attempts to quantify the contribution of precipitated phosphates to EBPR in the literature have produced various results, ranging from 60% (Arvin, 1985) to 15-27% (Lan *et al.*, 1983), to as little as 3.4% (Appeldoorn *et al.*, 1992) (percentages of total sludge phosphorus content). Pure cultures of various *Acinetobacter* strains, have shown even lower precipitation (Appeldoorn *et al.*, 1992).

In this study, sludge fractionation at the end of the aerobic stage (see Materials and Methods for procedure details), revealed that the cold PCA fraction, constituted between 37% and 51 % of the total phosphorus in the sludge and the HPP fraction, constituted 20% - 36% of the total phosphorus.

TABLE 4.7. TOTAL PHOSPHORUS VALUES AND FRACTION RELEASED DURING ANAEROBIC CONDITIONS PUBLISHED IN THE LITERATURE

Reference	Total Phosphorus (mgP/gVSS)	P released (mgP/gVSS)	% TP released
Kuba *et al.*, 1993	90-110[1]	65[1]	60
Comeau *et al.*, 1990	-	33[1]	55
Kang *et al.*, 1991	-	-	40
Mino *et al.*, 1987	35.6-84.8	-	-
Matsuo, 1994	75-160	-	-
Blonda *et al.*, 1994	128	-	-
Appeldoorn *et al.*, 1992	110[1]	95[1]	97
Fukase *et al.*, 1985	110[1]	-	
Wentzel *et al.*, 1988	180[1]	-	-
Manning & Irvine, 1985	60[1]	-	-
This study	60-194	22-47	29-54

1. Calculated on the basis of MLSS.

TABLE 4.8. PHOSPHORUS FRACTIONS OF EBPR SLUDGES PUBLISHED IN THE LITERATURE

		Phosphorus fraction (%TP)				
Reference	Origin of Sludge	Dissolved $[PO_4\text{-}P]$	Metal P	LPP	HPP	Organic[1] + Residue[2]
Mino *et al.*, 1984[3]	Full scale	-	33.2	20.9	22	23.9
Mino *et al.*, 1984[3]	Lab-scale cont.	-	1.8	75	13.1	10.1
Bortone *et al.*, 1992	Lab-scale SBR	10	31	43		16
Converti *et al.*, 1993	Lab-scale SBR	60	25	10		-
This study	Lab-scale SBR.	0.3	0-22	29-51	19-36	29.5-36.3

1. Proteins, nucleic acids, lipids
2. Unextractable fraction
3. Calculated from paper

The cold PCA fraction includes phosphorus originating from metal complexes and from the low polymeric polyP inside the cells.

Assuming that all phosphorus released in the anaerobic zone originates from LPP, as shown by Mino *et al.* (1985) and taking into account that the observed phosphorus released during anaerobic conditions was between 29 - 54 % of the total sludge P, the phosphate involved in precipitation (which would be the part of the cold PCA fraction that is not involved in the formation of LPP), varied between 22% - 0 % of the total phosphorus in the sludge. These results are in agreement with values published in the literature (Table 4.8.), but the comparison should be regarded with caution as the location and mobility of the various phosphorus fractions has been shown to vary according to the substrate used (Jing *et al.*, 1992).

4.3.4. Overview

The objective of the second experimental phase was to operate the reactor with varying influent phosphorus and COD concentrations and varying SRTs. The D.O. level in the aeration tank and the percentage of nitrification were also altered. The effect of the above changes on the phosphorus removal efficiency and the anaerobic phosphorus release was monitored. By combining these results with other sludge characteristics such as its phosphorus and metal fractions, the properties of the sludge (derived from the operation of the reactor as shown in chapter 4) used for the storage and thickening studies (results to follow in chapter 5) were defined.

The results from the varying SRT_E experiments indicated that between the values of 5 and 13 days (13 - 40 days equivalent SRT), EBPR functions independently of the sludge retention time. These findings do not agree with published data by Smolders *et al.* (1995), who for a lab scale SBR, found an increase in phosphorus removal with increasing SRT_Es. From this study it has been made clear that the effect of SRT on the reactor biomass must be taken into account when evaluating the phosphorus removal properties of activated sludges. Presenting the results on a volumetric (or concentration) basis, i.e. mg/l, as in the case of Smolders *et al.* (1995b), introduces the confounding effect of the variations in VSS. It is more

appropriate to quote results as phosphorus per unit biomass (mgP/gVSS or mgP/gSS).

The effect of varying the influent COD and subsequently the organic loading of the sludge, was investigated in the range of 100 mg/l - 700 mg/l. It was concluded that altering the COD between that range had no effect on any of the characteristics of EBPR (% influent P removed, anaerobic P release), for P/M values of 3 - 4.5 mgP/gVSS, indicating that the reactor was operating with an adequate supply of carbon for the requirements of EBPR. On the other hand for a P/M ratio of 6 mgP/gVSS, increasing the influent COD from 100 mg/l to 500 mg/l, improved the phosphorus removal in the reactor by 60%. This indicated the importance of the influent COD/P ratio.

The effect of varying the influent phosphorus concentration was investigated in the range of 4 to 30 mgP/l. This increase in the influent phosphorus had no effect in the overall removal efficiency of the reactor therefore increasing influent concentrations resulted in increasing amounts of phosphorus removed by the sludge. This was true for P/M ratios below 5.5 mgP/gVSS. For higher values the efficiency of the reactor depended on the available influent COD, with 90% phosphorus removal rates being achieved if the COD/P ratio was above 25. As with phosphorus removal, increasing influent phosphorus concentrations resulted in increased anaerobic phosphate release.

The COD/P values offering high phosphorus removal in this study, were as low as 15 mgCOD/mgP. Although at first this appears to be far below the quoted lower favourable value of 50 (Carlsson *et al.*, 1996), it must be taken into account that the most important parameter of the influent wastewater is the VFA/P ratio, as opposed to the more general COD/P ratio. As already mentioned the only carbon source in the feed in this study was sodium acetate, therefore the hole influent COD could be readily assimilated in the anaerobic phase. This would indicate that the quoted values in the literature are not representative of the true potential for EBPR of the wastewater.

An overall conclusion that can be drawn from the above analysis is that the performance of EBPR depends on the combination of influent COD and P values. The

stoichiometry of phosphorus release indicates that around 0.4-0.5 mg of phosphorus are released per mg COD (acetate) consumed, at neutral pH (Smolders *et al.*, 1994, Wentzel *et al.*, 1988, Carlsson *et al.*, 1996). Therefore, for each influent COD value there is a maximum concentration of phosphorus that can be released. Until reaching this critical phosphorus concentration the influent phosphorus is the deciding parameter of the process, because it regulates the amount of stored polyphosphate which in turn controls the amount of available energy for acetate transport and storage during the anaerobic conditions. In order to increase the capacity of the sludge beyond that maximum value an increase in the influent COD is necessary, which will increase the amount of stored PHB in the cell.

When considering COD/P fluctuations in wastewater it is of crucial importance to determine which parameter is the cause of the variation, as a standard dilution or concentration of the wastewater is not always the reason (Carlsson *et al.*, 1996).

Operating the reactor with low D.O. levels, over a period of 30 days, had no effect on the phosphorus removal properties of the sludge, although a progressive deterioration in the ammonia removal rates was observed. A progressive depression of the redox state of the sludge was also noted.

Finally, sludge fractionation results revealed that the greatest proportion of the sludge phosphorus was in the form of LPP (30-50% of the TP) and HPP (19-36 % of the TP).

The analysis presented so far established the effect of the chosen reactor operations, on various aspects of EBPR and nitrogen removal. The next step of the experimental work, presented in the following chapter, deals with establishing the effects of the same operational alterations, on the excess sludge properties during storage and thickening. The most important aspect investigated was the phosphorus release patterns observed, for each mode of reactor operation.

CHAPTER 5. PHOSPHORUS RELEASE DURING SLUDGE STORAGE AND THICKENING

5.1. INTRODUCTION

The release of the biologically bound phosphorus during anaerobic handling of excess sludge, is one of the major problems of enhanced biological phosphorus removal (EBPR) plants. The return of phosphorus rich process waters to the head of the works may lead to plant overload and eventually increased phosphorus concentrations in the effluent.

This chapter discusses the process of phosphorus resolubilisation during the treatment of excess sludge. The sludge used was derived from the lab scale SBR, operating as described in chapter 4.

In this study, the problem of phosphorus release during sludge processing was investigated by comparing different sludge handling methods and by comparing the phosphorus released, for each of the sludge handling methods, from sludges that had been derived from runs with different operational parameters.

The sludge handling methods investigated were quiescent anaerobic storage and gravity thickening. Anaerobic storage was selected as it routinely takes place at some stage in a treatment plant, albeit for varying lengths of time. Gravity thickening, is a popular and widely used dewatering method.

Additional experiments employing fully mixed aerobic storage and fully mixed anaerobic storage were also performed.

For identical batches of sludge, the comparison of the two sludge processing methods focused on the length of the anaerobic retention, as it has been shown to affect the resolubilisation of organically bound phosphorus.

As far as the operational parameters of the plant from which the sludge originated are concerned, it has been shown that sludge age and nitrate concentrations affect phosphate release (for more details and references the reader is referred to chapter 2, section 2.6.).

In this study, the parameters investigated included the reactor influent and operational characteristics, e.g. influent COD, D.O. concentration, as well as sludge properties like phosphorus content, oxidised nitrogen concentration and sludge extraction point (aeration stage as opposed to settling stage).

5.2. OBJECTIVE

The first objective of this research was to determine the different phases describing phosphorus release during short term (0-24 hours) and long term (2-7 days) batch storage and gravity thickening of EBPR excess sludge.

A second objective was to determine any difference in the release observed between the two sludge handling methods employed, namely quiescent anaerobic storage and gravity thickening.

The third objective was to determine any difference in the release patterns observed between sludges that had been derived from runs with different operational parameters. The ultimate goal was to determine wheather a set of operational parameters exists, which produces excess sludge with minimal release during the handling of EBPR sludges.

The phosphorus release patterns were compared with those observed when return activated sludge from a conventional activated sludge plant, was put through the same treatments.

5.3. METHODOLOGY

5.3.1. Methodology for the Determination of Phosphorus Release Patterns During Short and Long Term Sludge Treatment

For both the anaerobic storage and thickening experiments, a litre of excess sludge was withdrawn from the SBR at the end of the cycle and placed in the respective container. For the storage experiments a 1 litre glass volumetric cylinder was used and for the thickening experiments a stirred sludge volume index apparatus (for details see Materials and Methods, chapter 3, section 3.4.1. and 3.4.2.).

For the determination of the phosphorus release phases (first objective), the dissolved orthophosphate concentration in the supernatant was measured. For the first 12 hours the measurements took place at one hour intervals and thereafter every 24 hours.

The phosphorus release rates for short term (0-24 hours) and long term (1-7 days) storage and gravity thickening were determined by linear approximation of the response curve between selected intervals. Regression analysis was performed and the release rates and the nitrate uptake rates observed during the experiments, were calculated as the slope of the best fit line for the chosen time intervals.

5.3.2. Methodology for the Comparison of Phosphorus Release Patterns Between Sludge Storage and Thickening

The same experimental procedure as in section 5.3.1. was followed. An identical sludge sample was used for both storage and thickening experiments and the phosphorus release observed at similar time intervals was compared between the two methods.

As a bench scale gravity thickener a SSVI, WRC apparatus was used. The procedure is described in detail in chapter 3 (Materials and Methods, section 3.4.2.). To the best of the author's knowledge this method has not been used for the investigation of phosphorus release patterns during anaerobic sludge treatment.

5.3.3. Methodology for the Determination of the Effect of SBR Operational Parameters on Phosphorus Release During Sludge Treatment

For the determination of the effect of the operation of the SBR on phosphorus release during sludge treatment, thickening and storage experiments were performed involving batches of sludge that had been derived from the SBR when operating under various strategies.

The operational parameters investigated included influent parameters (e.g. influent COD and phosphorus concentrations) and sludge properties (e.g. total sludge phosphorus and sludge oxidised nitrogen content).

For the investigation of the effect of the influent parameters, the sludges used had similar sludge properties, namely total sludge phosphorus and nitrate concentrations. Similarly, for the investigation of the effect of the sludge properties the sludge batches used had comparable influent characteristics.

The effect of reactor operation under oxygen limitation was also investigated as well as the effect of the point of sludge extraction for wastage (aeration tank versus sedimentation tank).

The effect of the reactor SRT on phosphorus release during sludge handling was not investigated. As shown from the results of chapter 4, the range of SRTs investigated in this study (13 - 40 days) had no effect on the phosphorus accumulating properties of the sludge. It was therefore decided to perform all experiments at the SRT_E of 9 ± 1 days (SRT = 20-30 days) and at the temperature of 20 ± 1 °C.

For all of the experiments, the amount of phosphorus released was compared as mg/l (concentration measured in the supernatant), mgP/gVSS (calculated as the mass of dissolved orthophosphate detected in the supernatant divided by the initial biomass concentration of the sludge used for the experiment) and as a percentage of the total phosphorus content of the sludge as determined by acid digestion.

The mass of released phosphorus was calculated as the concentration measured in the supernatant multiplied by the combined volume of the sludge blanket and supernatant (for detailed example of calculations see Materials and Methods, section 3.4.4.).

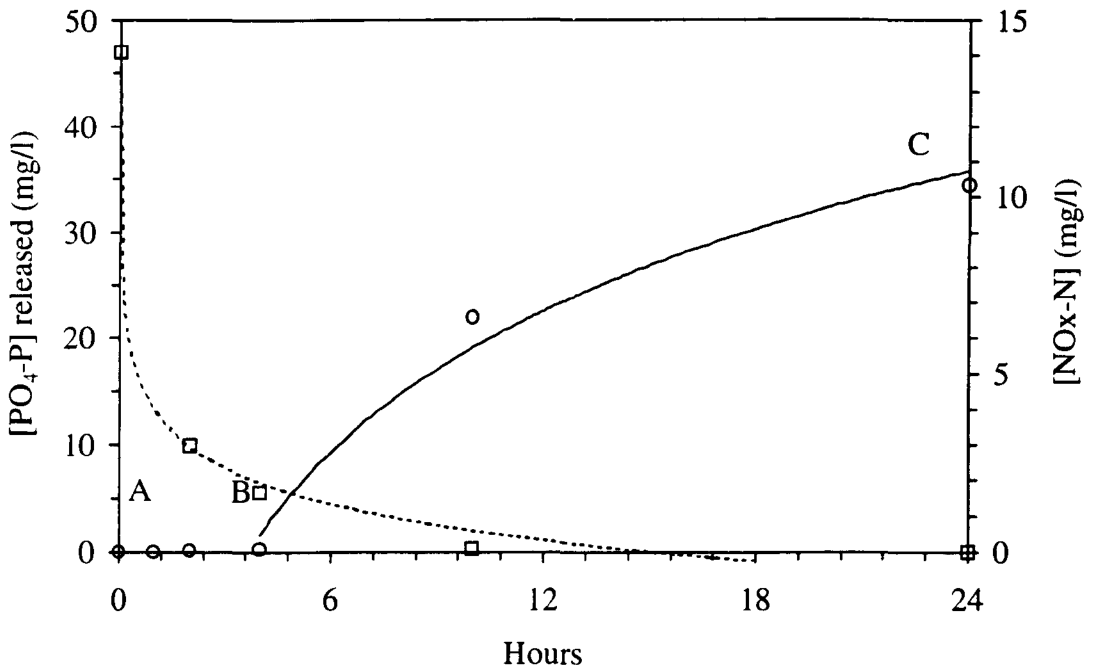


Fig. 5.1. Phosphorus release (O) and nitrate uptake (□) during the first 24 hours of a typical quiescent storage experiment.

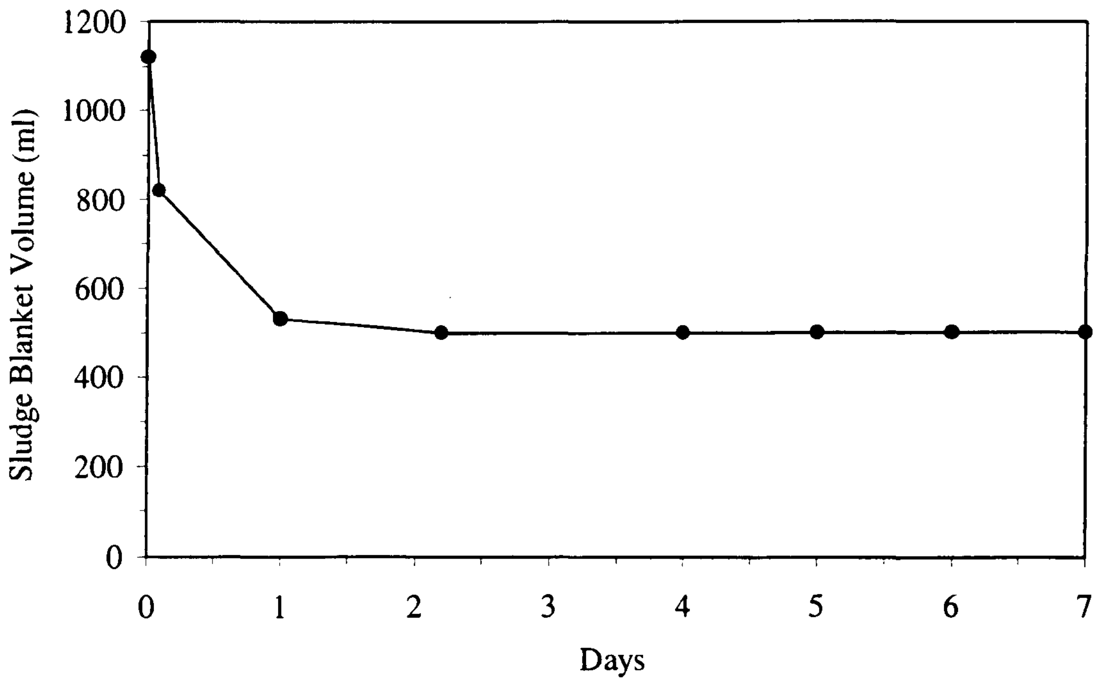


Fig. 5.2. Sludge settlement profile during a long term quiescent storage experiment.

As already mentioned, the effect of influent parameters was investigated for sludges with comparable sludge phosphorus and oxidised nitrogen content. Achieving similar total phosphorus concentrations proved difficult and the time scale of the experimental phase of this work did not allow for repetition of the sludge treatment experiments. Therefore, only a very small number of experiments for each of the operational factors investigated was possible. The results obtained were considered representative of the behaviour of the sludge under the conditions investigated, as it was shown that sludge batches with almost identical characteristics produced identical release patterns (see results and discussion section).

5.4. RESULTS AND DISCUSSION

5.4.1. The Determination of Release Patterns During Short and Long Term Sludge Treatment

5.4.1.1. Quiescent anaerobic sludge storage

The phosphorus release patterns obtained during the storage of excess sludge, were investigated for retention times between 1 to 24 hours and over a period of 7 days.

During short-term storage (0-24 hours), two distinct phases can be seen in the phosphorus release profile (figure 5.1.). The first phase, which in this study varied between 4 and 20 hours according to the sludge properties, showed no or negligible phosphorus release (section AB of solid line in figure 5.1.). In most of the experiments, the oxidised nitrogen present in the sludge was denitrified during this first phase.

The next phase was initiated once the oxidised nitrogen concentration had dropped below 1.5 - 2 mgN/l, and was characterised by a variable phosphorus release

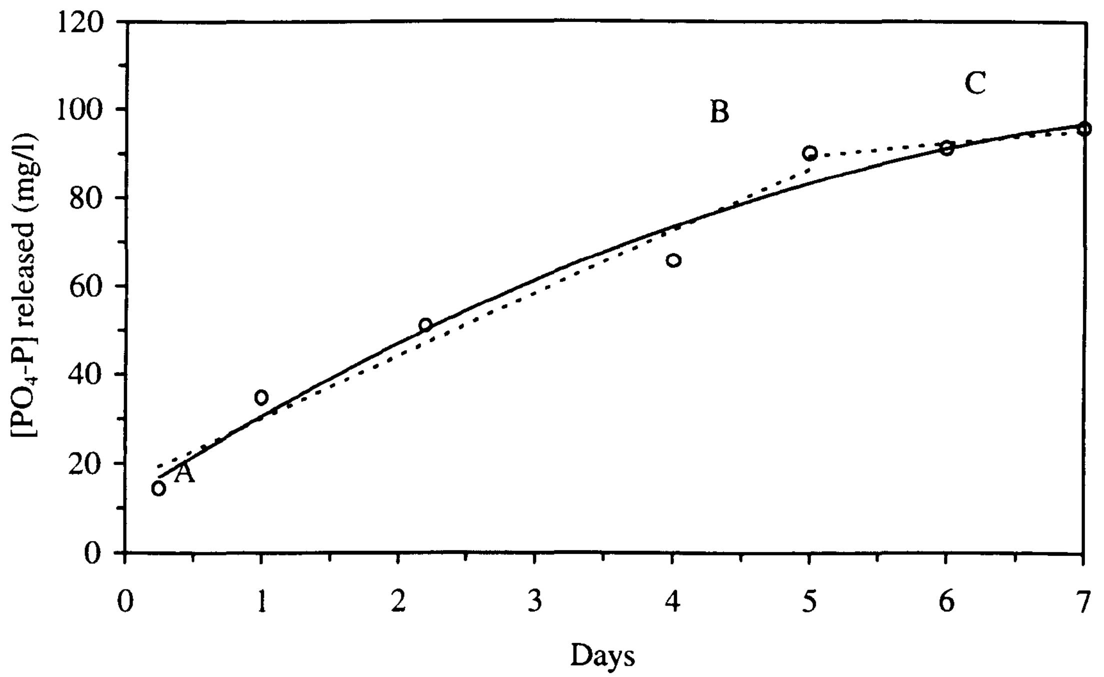


Fig. 5.3. Phosphorus release profile during a long term quiescent storage experiment. Phosphorus release rate (slope of the best fit line between sections) AB: 0.6 mgP/l·h, BC: 0.12 mgP/l·h.

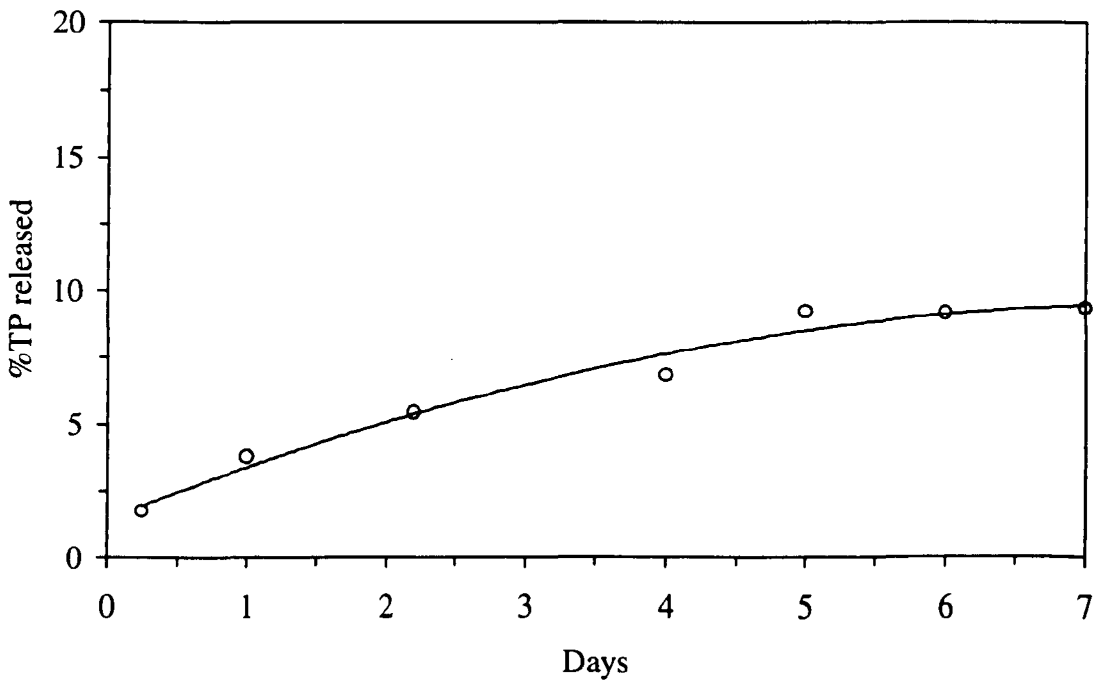


Fig. 5.4. Phosphorus release profile, expressed as a percentage of the total phosphorus sludge content, during long term quiescent storage.

rate (section BC of solid line in figure 5.1.). By the end of the first 24 hours denitrification had been completed (figure 5.1.) and most of the sedimentation had taken place (figure 5.2.).

If the sludge was allowed to remain in storage for a period of a few days, phosphorus continued to be released at an increasing rate until day 5 was reached, at which point the release rate dropped to very slow levels again (figure 5.3.). The rate of release for the first 5 days, calculated as the slope of the best fit line (section AB in figure 5.3.), varied between experiments in the range of 0.6 - 1.4 mgP/l·h. This variation was due to the different properties of the sludges used and is discussed further in section 5.4.3.

The maximum amount of releasable phosphorus during the storage experiments, was reached at approximately 5 days, with minimal release occurring beyond this time (figure 5.4.). In the experiment shown in figure 5.4. the maximum concentration amounted to 10% of the total phosphorus in the sludge. This value represented the lowest figure obtained among the storage experiments, the maximum being 45% of the total phosphorus.

For all quiescent storage experiments performed, three phases could be distinguished and are summarised in table 5.1.

The phosphorus release profile of the excess EBPR sludge was compared to the one obtained after storage of conventional activated sludge. As shown in figure 5.5., the first phase of negligible release observed during the storage of the EBPR sludge is absent and the second phase is characterised by very low, constant release rates in the range of 0.11 mgP/l·h.

5.4.1.2. Gravity Thickening

Similarly to the storage results, the thickening profiles describing short-term phosphorus release, could be divided in two phases (figure 5.6.). The first phase, constituting the first 3 - 10 hours showed negligible or no release (section AB of solid line in figure 5.6.).

TABLE 5.1. PHASES OBSERVED DURING QUIESCENT ANAEROBIC STORAGE OF EXCESS EBPR SLUDGE

Phase	Duration	Phosphorus released (mgP/l·h)	Denitrification rate (mgN/l·h)
I	4 - 20 hours	0 - 0.02	1.5 - 5.6
II	5 days	0.6 - 3.6	0.22 - 0.01
III	undetermined	0.04 - 0.12	no oxidised nitrogen present

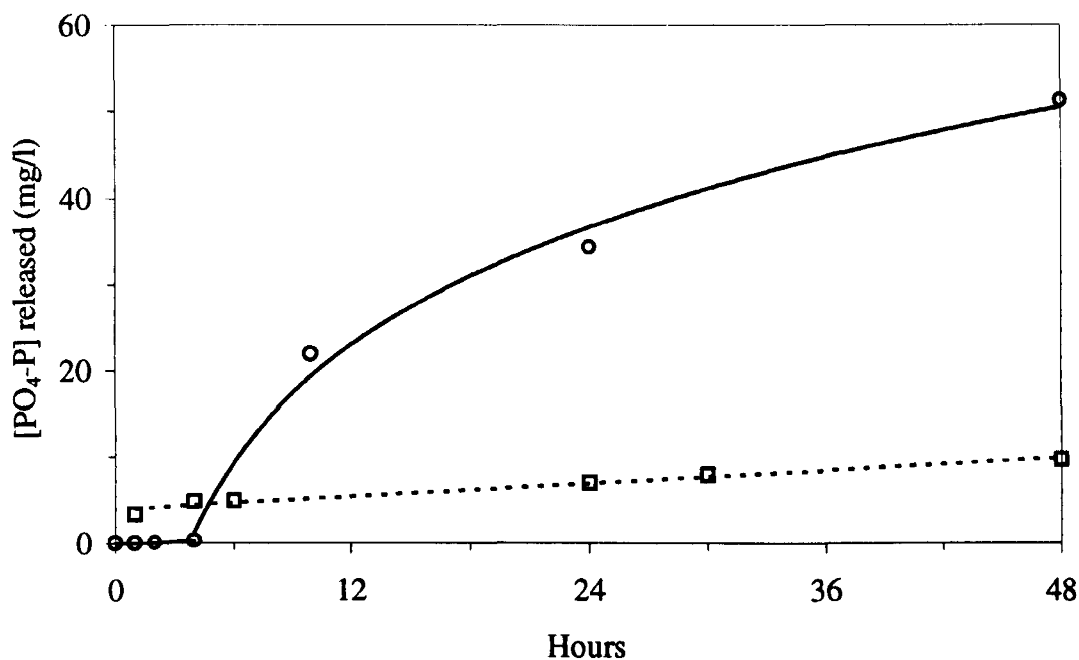


Fig. 5.5. Phosphorus release profile during quiescent storage of (O) EBPR sludge and (□) sludge from a conventional treatment plant.

The second phase was characterised by variable release rates which in this study were in the order of 0.6 mgP/l h to 1.5 mgP/l h (section BC of solid line in figure 5.6.). During these first 24 hours most of the nitrates were denitrified (figure 5.6.) and most of the sludge sedimentation took place (figure 5.7.).

A typical phosphorus release profile over a long term (10 days) thickening experiment is shown in figure 5.8. After the first 24 hours, phosphorus continues to be released (section AB in figure 5.8.) at a rate which varied between experiments in the range of 0.6 - 4.2 mgP/l.h according to the sludge characteristics (further discussion in section 5.4.3).

After approximately 7 days, most of the releasable phosphorus concentration was in solution. This maximum value constituted between 15 to 60% of the total phosphorus sludge content with an average of 40% of the total sludge phosphorus (figure 5.9.).

As shown in figure 5.10., after an initial increase due to high denitrification rates (see figure 5.6.), the supernatant pH remained constant during the first phase of minimal phosphorus release. In the days that followed, it progressively decreased reaching neutral values after approximately a week (figure 5.10.).

As the release of phosphorus progressed during the thickening experiment, a simultaneous increase in the COD value of the supernatant was also observed (figure 5.11). This has been attributed to sludge deflocculation as bacteria move from the flocs to the bulk liquid and to accumulation of non-biodegradable cell lysis products (Rasmussen *et al.*, 1994; Lishman and Murphy, 1994).

The decrease in the sludge biomass during the thickening experiment, indicating endogenous metabolism, was constant as shown by the decrease in the volatile solids fraction of the sludge (figure 5.12.).

As in the anaerobic stage of the SBR operating cycle, the release of phosphorus during thickening is associated with the release of cations, with potassium and magnesium showing the closest correlation to the phosphorus release profile as indicated by the high correlation coefficients of the best fit lines (fig 5.13.).

The observed molar ratio of Mg/P released, varied between 0.17 - 0.30 mgMg/mgP. The range for potassium was 0.4 - 0.64 mgK/mgP.

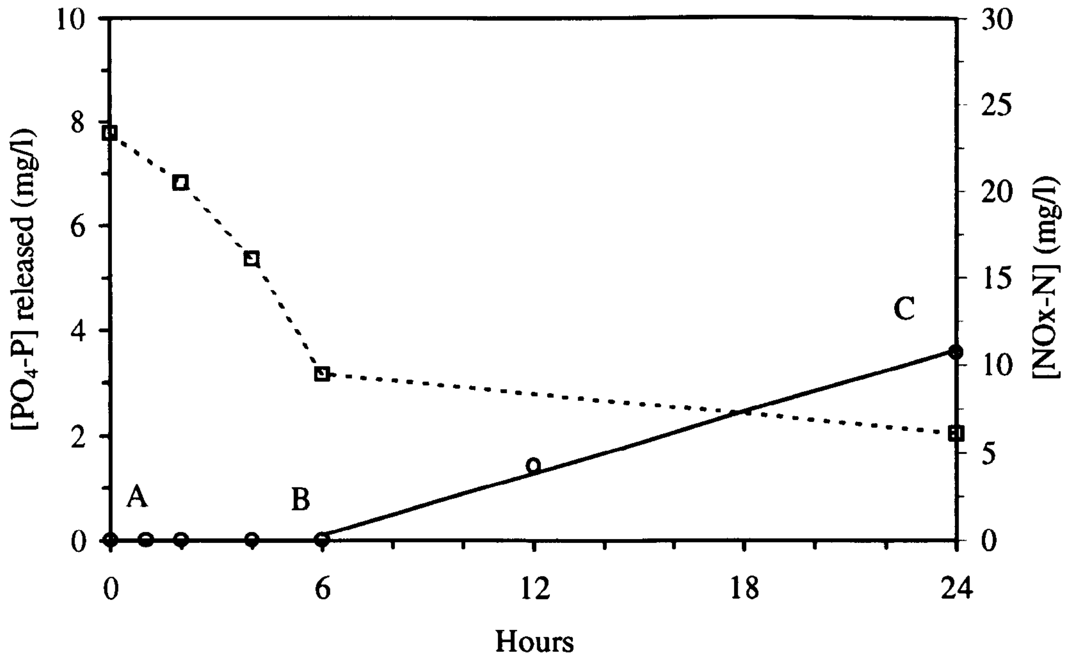


Fig. 5.6. Phosphorus release (O) and nitrate uptake (□) during the first 24 hours of a typical thickening experiment.

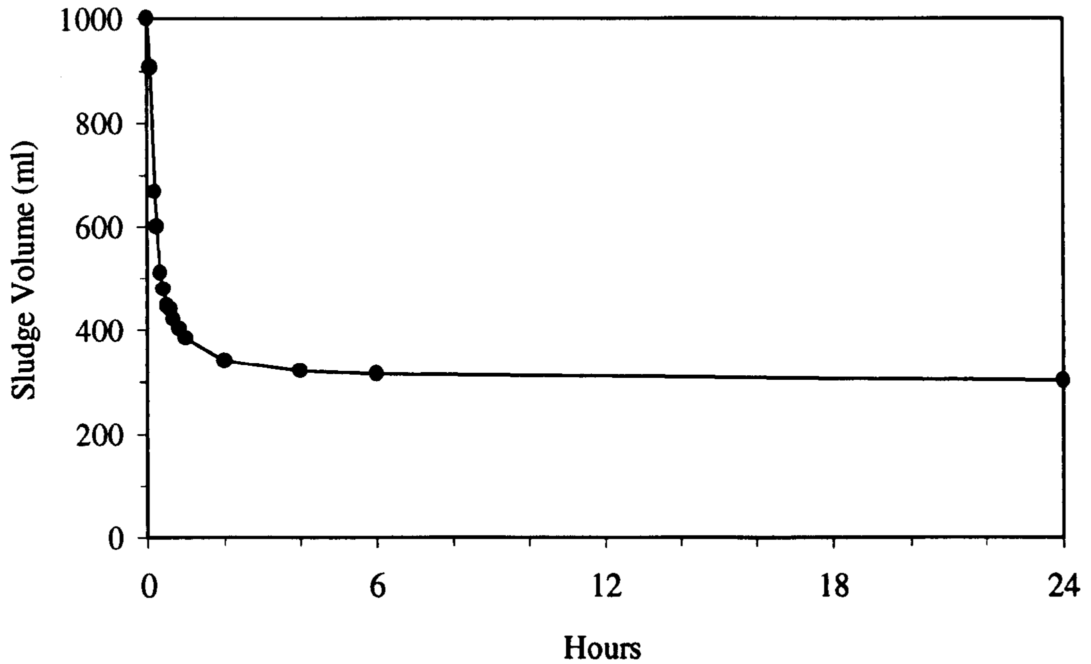


Fig. 5.7. Sludge settlement profile during the first 24 hours of a typical thickening experiment.

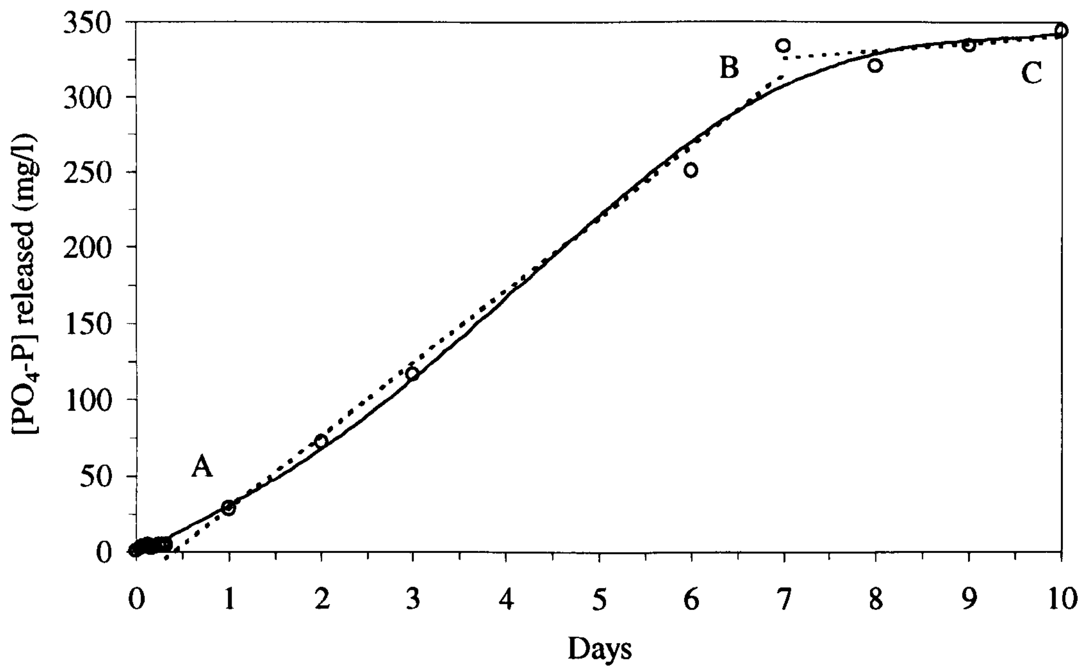


Fig. 5.8. Phosphorus release profile during a long term thickening experiment. Phosphorus release rate (slope of the best fit line between sections) AB: 2 mgP/l·h, BC: 0.19 mgP/l·h.

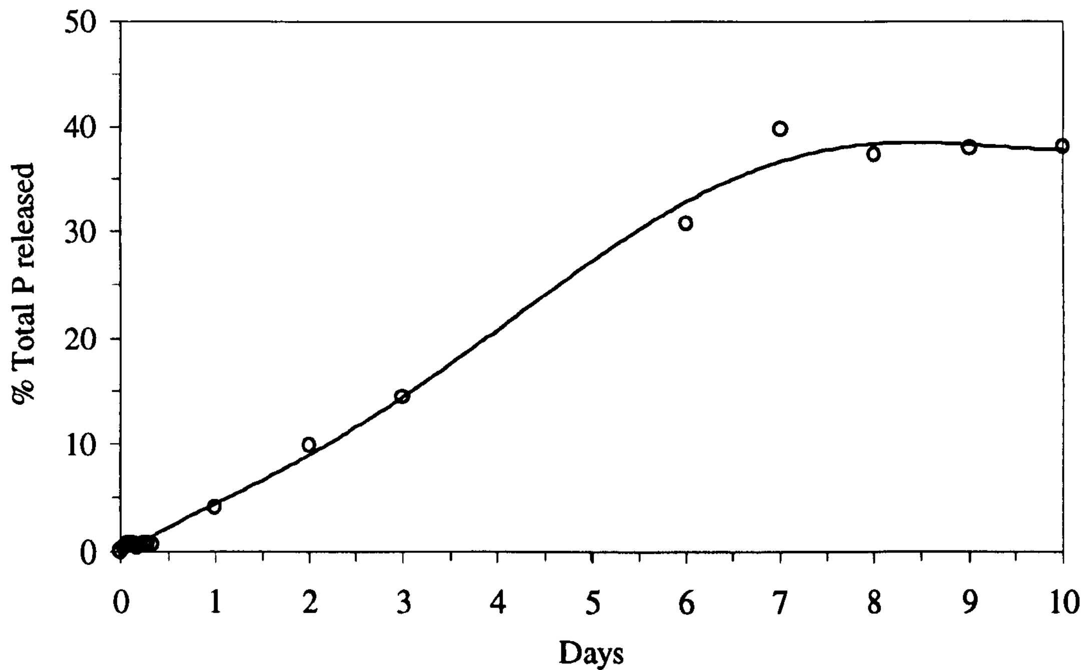


Fig. 5.9. Phosphorus release during a long term thickening experiment, expressed as a percentage of the total phosphorus sludge content.

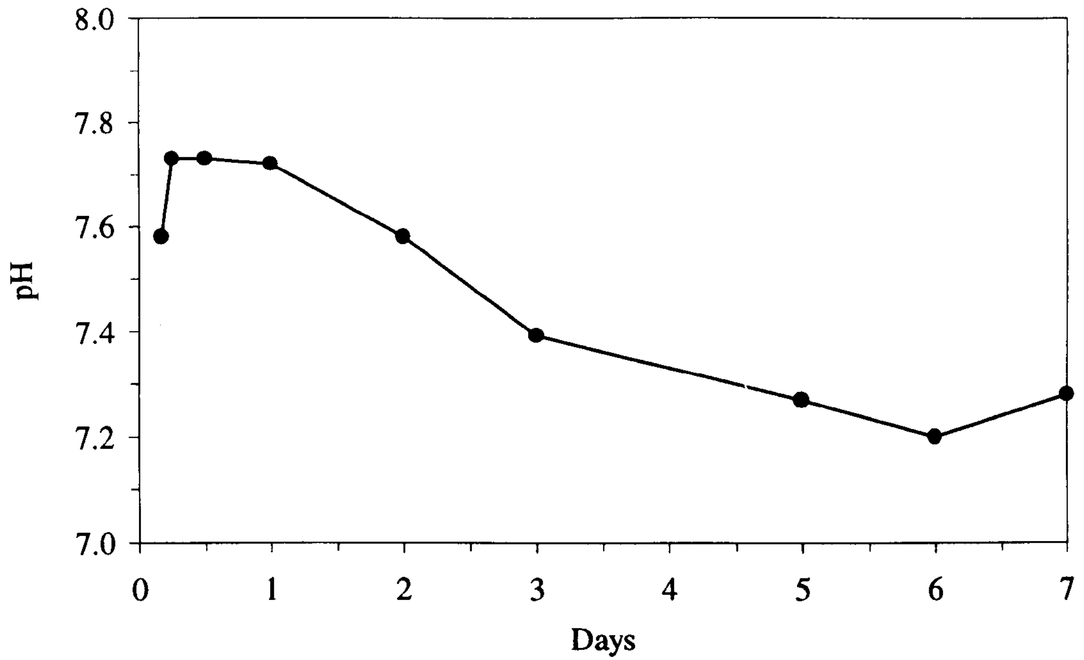


Fig. 5.10. Supernatant pH during a long term thickening experiment.

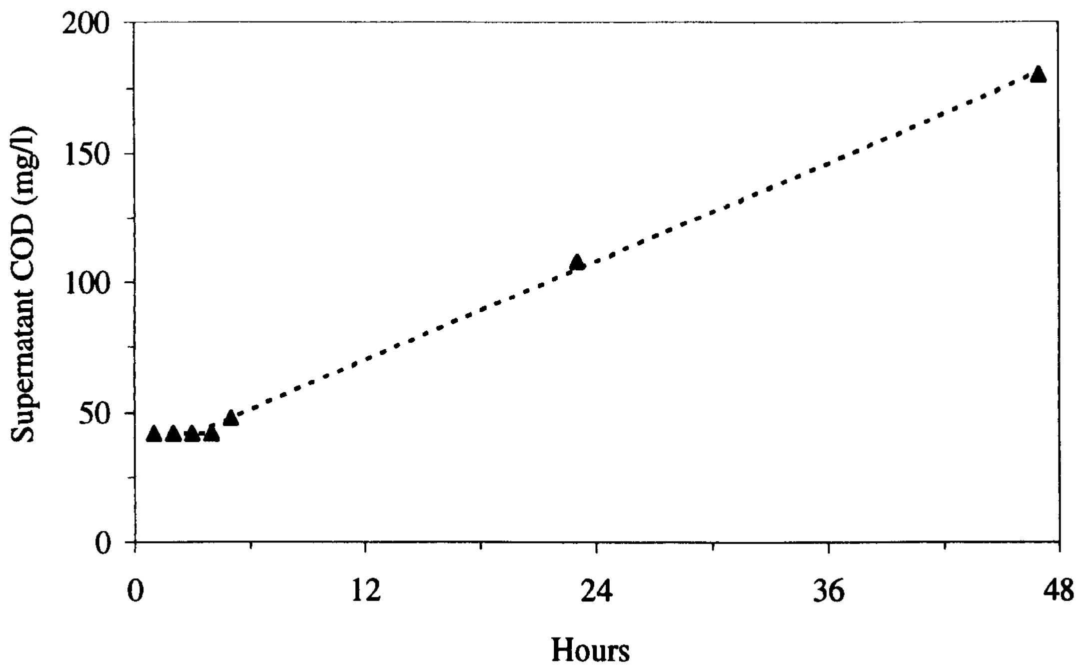


Fig. 5.11. Supernatant COD during the first 48 hours of a thickening experiment.

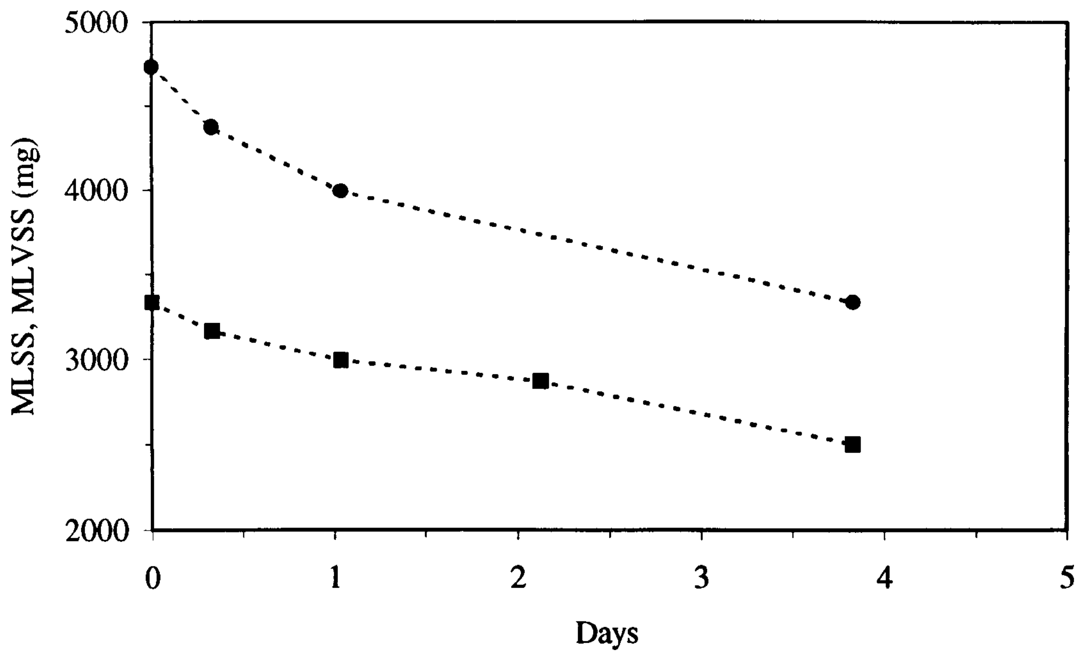


Fig. 5.12. Decrease in (●) MLSS and (■) MLVSS mass during thickening.

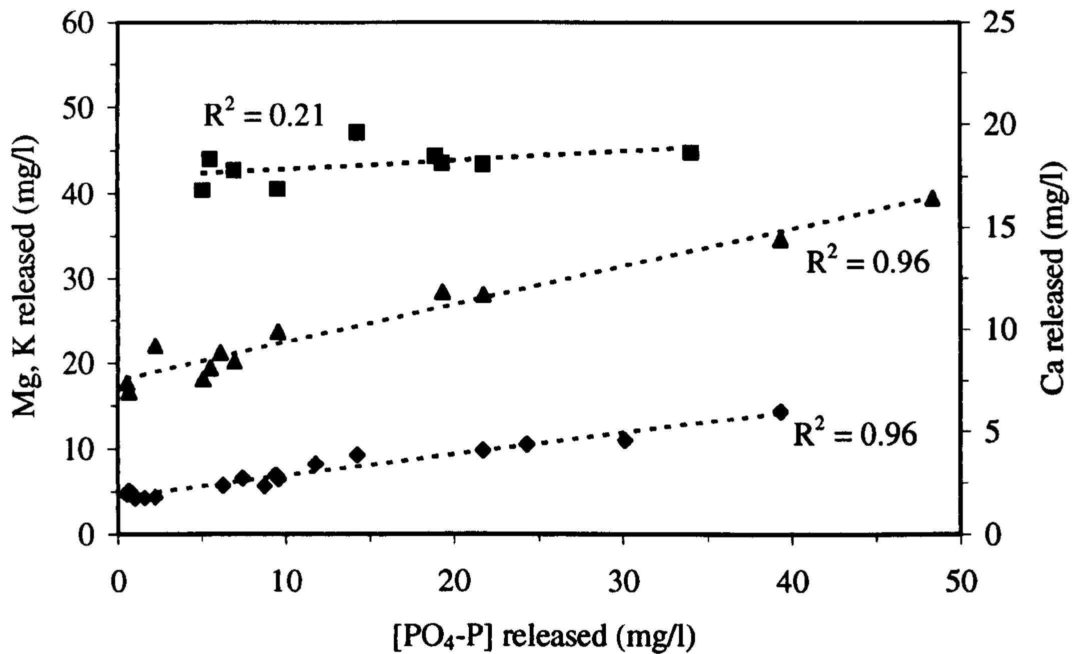


Fig. 5.13. Release of cations as a function of phosphorus release during thickening of excess EBPR sludge. (■) Ca^{2+}, (▲) K^+, (◆) Mg^{2+}.

The molar ratio of Mg/P released during anaerobic digestion quoted by Sen and Randall (1988) was a comparable 0.26 mgMg/mgP but the equivalent molar ratio of potassium released (0.24 mgK/mgP) was lower than the one observed in this study.

Microscopic investigations of the thickened sludge showed all the changes taking place during long term anaerobic thickening. Photograph 5.1. shows the sludge after one hour of thickening during which period no release of phosphorus has been observed. The flocs were still compact and the majority of polyphosphate (stained dark purple areas) was present within the cells. After seven days of anaerobic thickening (photograph 5.2.), the stained area of the flocs had been drastically reduced and the structure of the floc was looser, with isolated bacteria visible. Looking at the sludge with a higher magnification (1000x as opposed to the 200x magnification used in photographs 5.1. and 5.2.), reveals the deflocculation present in the water surrounding the flocs as well as the reduction in the polyphosphate content of the cells (Photograph 5.3.).

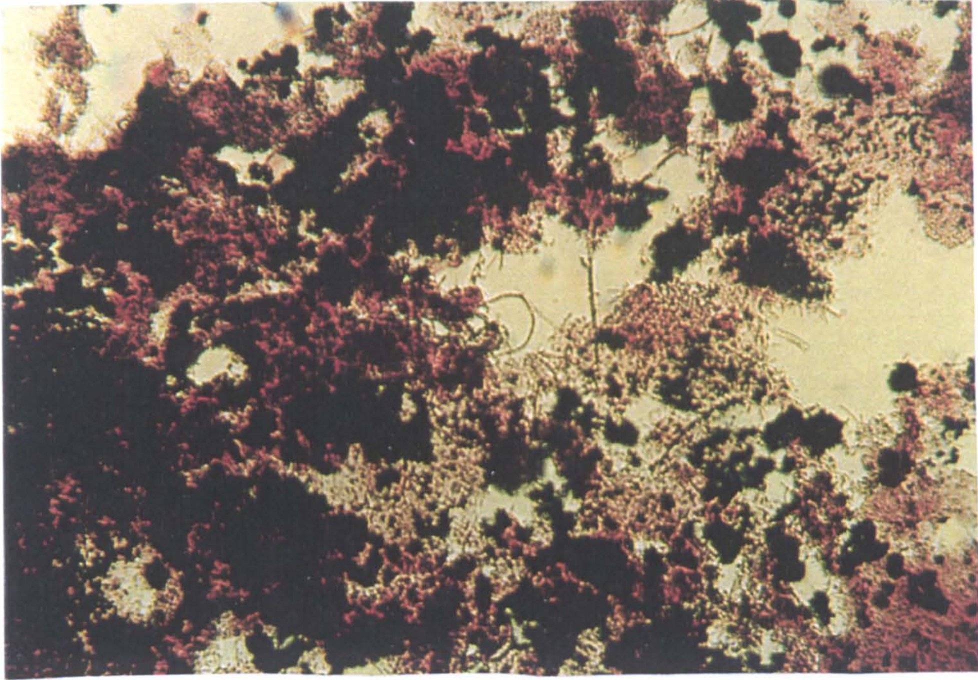
These changes in the floc morphology are typical of sludges in periods of starvation and have been known to occur after 4 to 7 days. Large, firm and round flocs are steadily replaced by small, weak and irregularly shaped ones as described by Horan and Shanmugan (1986).

Table 5.2. presents an overview of the phosphorus release phases observed during the batch gravity thickening of EBPR sludge.

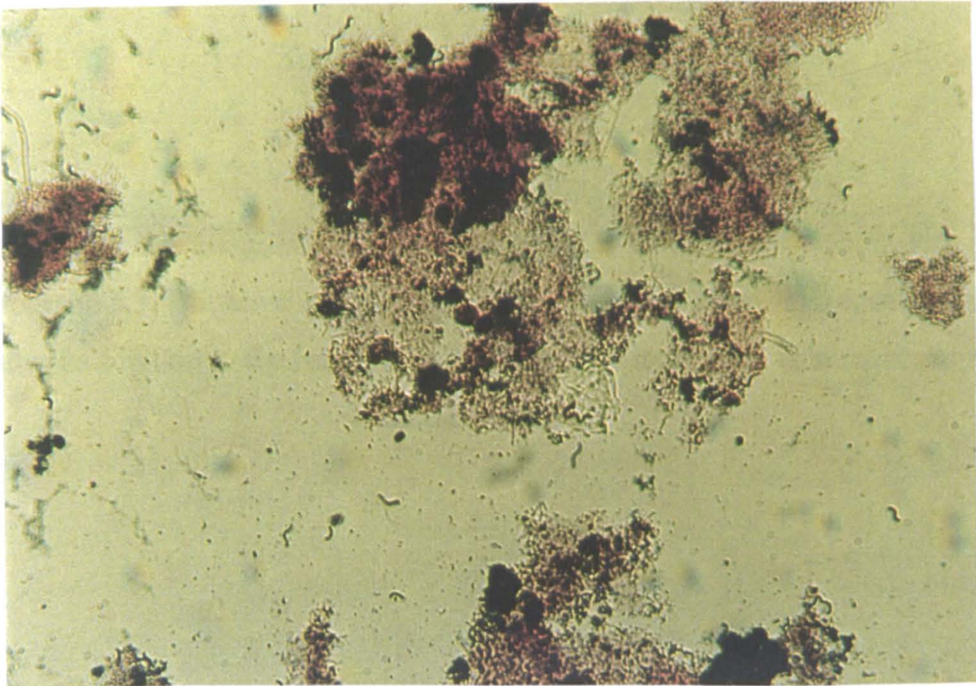
As with the storage experiments, the phosphorus release profile of the excess EBPR sludge was compared to the one obtained after thickening of conventional activated sludge. The phosphorus release rates of the conventional sludge were 0.04 mgP/l.h and the initial phase of no phosphorus release, observed in the EBPR sludge was absent (figure 5.14.).

<u>5.4.1.3. Comparison of phosphorus release phases observed in this study with the published literature</u>

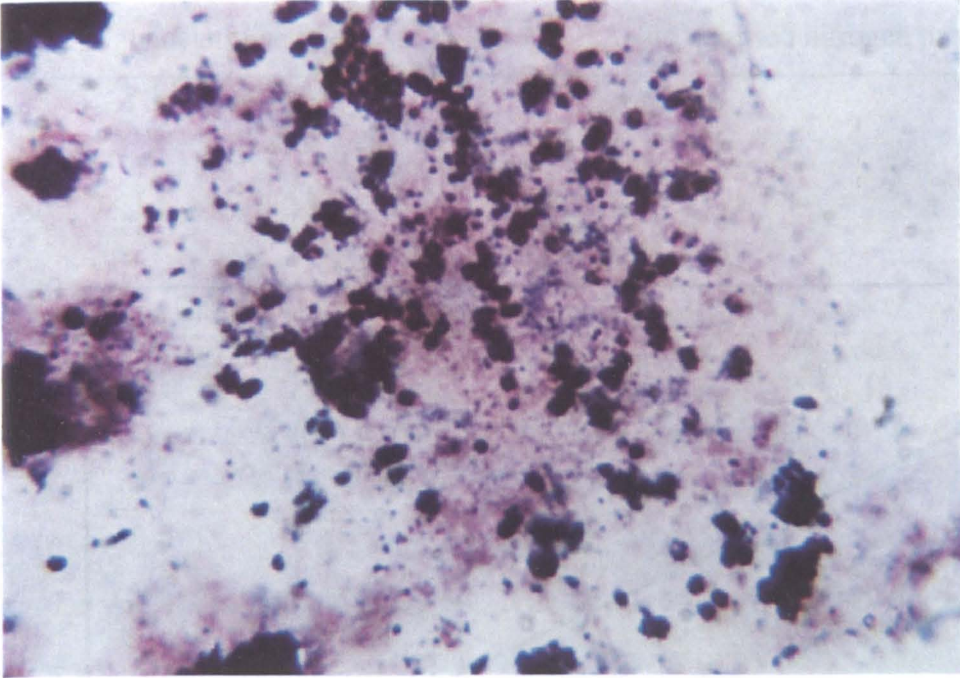
Comparisons of the results presented so far with published data are difficult as most researchers have investigated phosphorus release in the presence of carbon substrates and not during the endogenous decay phase.



Photograph 5.1. Neisser stained sludge one hour after its addition to the bench scale thickener (light microscopy, magnification x200).



Photograph 5.2. Neisser stained sludge seven days after its addition to the bench scale thickener (light microscopy, magnification x200).



Photograph 5.3. Dispersed cells among flocs of Neisser stained sludge, seven days after its addition to the bench scale thickener (Oil immersion light microscopy, magnification x1000).

TABLE 5.2. PHASES OBSERVED DURING GRAVITY THICKENING OF EXCESS EBPR SLUDGE

Phase	Duration	Phosphorus released (mgP/l·h)	Denitrification rate (mgN/l·h)
I	3 - 10 hours	0 - 0.5	0.8 - 10.5
II	7 days	0.6 - 4.2	0.02 - 0.4
III	undetermined	0 - 0.19	no oxidised nitrogen present

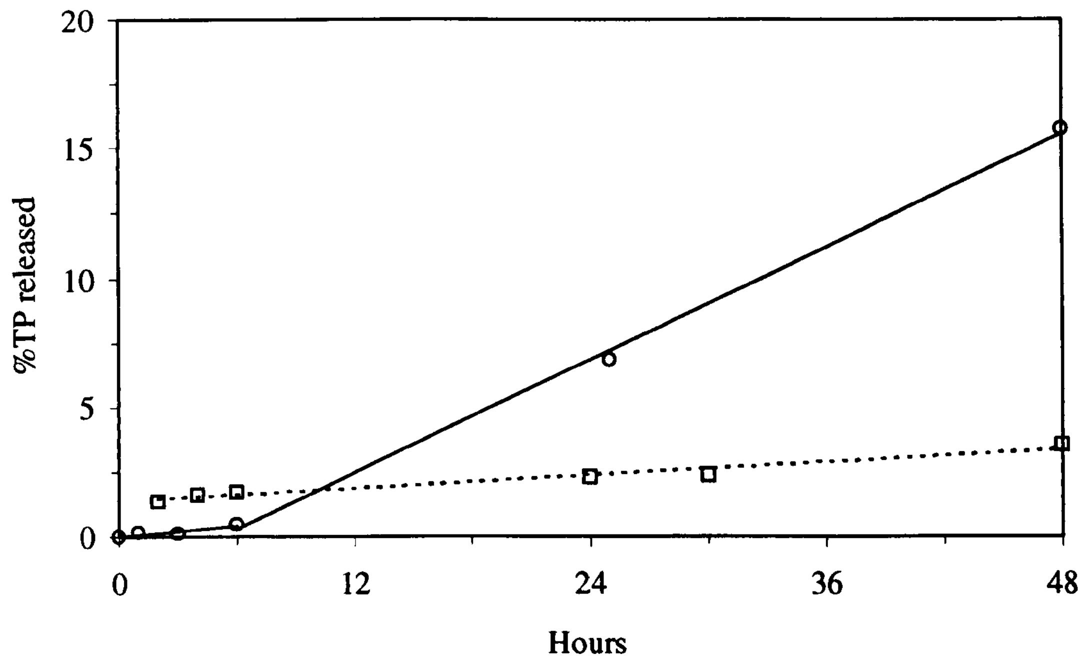


Fig. 5.14. Phosphorus release profile during gravity thickening of (O) EBPR sludge and (□) sludge from a conventional treatment plant.

TABLE 5.3. PHASES OBSERVED DURING TREATMENT OF EXCESS EBPR SLUDGE

Phase	Duration	Phosphorus released (mgP/l·h)	Description
I	3 - 20 (h)	0 - 0.5	No phosphorus release or cell lysis
II	5 -7 (d)	0.6 - 4.2	Degradation of intracellular polyphosphate stores and cell lysis
III	undetermined	0.0 - 0.19	cell lysis

Furthermore, the method used in this study to simulate gravity thickening conditions has, to the authors knowledge, not been used previously making direct comparisons difficult.

The sparse data available in literature has been derived from bench scale tests utilising either fully stirred methods of storage (Kroiss and Negm, 1994), or quiescent storage, with the sludge shaken before sampling (Rasmussen *et al.*, 1994).

In the absence of external substrate, as is the case during sludge treatment, two phases of phosphorus release were identified by Kroiss and Negm (1994). The first phase, lasting for approximately 24 hours, was characterised by a rapid release attributed to the phosphorus that had been previously taken up, to biomass hydrolysis and also phosphorus precipitation. The phosphorus release rate was of 0.8 mgP/lh. For the second phase, comprising the next 24 - 48 hours, the release was attributed to biomass hydrolysis and phosphorus precipitation and it was in the range of 0.017 mgP/lh. Bark *et al.*, (1992) has attributed phosphorus release during anaerobic sludge treatment solely to cell lysis as have many others.

The gap in the knowledge regarding phosphorus release during sludge handling is evident. Kroiss and Negm (1994), have given very general explanations of the observed phases and the cell lysis theory can not explain the release observed during the first few hours of storage, as EBPR sludges are subject to anaerobic conditions daily and have therefore been acclimatised to this type of stress.

In complete contrast to the aforementioned research, in this study, for both thickening and storage experiments, an initial phase of no or extremely slow phosphorus release and high denitrification rates was identified (table 5.3). The presence of nitrates did in general induce a retardation factor to the release of phosphorus (for more details on the effect of oxidised nitrogen on phosphorus release see section 5.4.3.3.), but it was by no means a prerequisite for the existence of this first phase. It appears that this initial phase corresponds to the maximum amount of time the bacteria are able to meet their maintenance requirements under anaerobic conditions. The fact that no increase in the bulk liquid COD was observed (figure 5.11.) indicates that no deflocculation due to bacterial death occurs.

This first phase has not been observed during storage experiments of EBPR sludge by either Kroiss & Negm (1994) or Rasmussen *et al.* (1994). In the case of Rasmussen *et al.* (1994) the sludge reached its maximum release capacity in only 4 hours (observed release rate 9μmol/gVS.h).

The possibility of phosphorus release with simultaneous precipitation was not investigated as an explanation for the low release observed during the first hours of sludge thickening in this study. This was due to the fact that the initial pH of all sludge batches was in the range of 7.8 - 7.4 and decreased as the experiments progressed. Furthermore, the sludge showed very low amounts of precipitated phosphates as discussed in chapter 4, section 4.3.3.6.

The presence of an initial period of extremely low phosphorus release rates is of crucial importance to operators of mechanical thickening systems such as centrifuges and flotation units which operate with short retention times. Gravity thickeners on the other hand, usually operated with retention times greater then half a day would encounter release rates in the range of the ones observed in stage two in this study.

The second phase during which intracellular phosphorus is progressively resolubilised, was characterised in this study by variable release rates in the range of 0.6 - 4.2 mgP/l.h. The explanation offered by Kroiss and Negm (1994) on the origin of the released phosphorus during what was their first (phosphorus previously taken up, biomass hydrolysis and phosphorus precipitation) may apply to the second phase of this study but a more detailed explanation of the phenomenon is proposed.

Initially the released phosphorus originates from the highly mobile, low molecular weight polyphosphates. Hydrolysis of the high molecular polyphosphates follows. The release observed during this second phase would therefore be a function of the LPP/HPP ratio as well as other sludge characteristics influenced by the plant operational parameters (see section 5.4.3.). The location of the released phosphorus during sludge treatment was not investigated in this study due to the difficulty and time needs of the phosphorus fractionation methods.

The length of this stage, defined as the time between initiation of phosphorus release at rates greater than 0.5 mgP/l.h until the time when the maximum releasable phosphorus concentration has been reached, was relatively constant in this study with

TABLE 5.4. MAXIMUM PHOSPHORUS RELEASED DURING ANAEROBIC TREATMENT OF EXCESS EBPR SLUDGE

Reference	Sludge Treatment Method	Time necessary for maximum P release
Rasmussen *et al.*, (1993)	Storage	4 (h)
Skalsky and Daigger, (1995)	Thick. fermented+EBPR WAS	6 (h)
Manning and Irvine, (1985)	Continous stirring	7 (h)
Kroiss and Negm, (1994)	Continous stirring	24 - 48 (h)
This study	Storage	5 (d)
This study	Thickening	7 (d)
Sekikawa *et al.*, (1967)	Continous stirring	10 (d)
Eikum *et al.*, (1975)	Storage	10-12 (d)

small variations around the 5 day value for the storage experiments and the 7 day value for the thickening ones. Published values vary considerably as shown in table 5.4.

On the other hand, the maximum releasable phosphorus fraction appears to be consistent irrespective of the sludge process method used. For example, 50% of the total sludge phosphorus was released during aerobic digestion (Sekikawa *et al.*, 1967) and a close 38% during the co-thickening of fermented and EBPR waste activated sludge (Skalsky and Daigger, 1995). In this study the average maximum releasable phosphorus was a comparable 40%.

5.4.2. Comparison of Quiescent Anaerobic Sludge Storage and Gravity Thickening

The release of phosphorus was investigated when identical batches of sludge, removed from the SBR at the same time, were put through the storage and thickening procedures.

As expected, the sludge showed distinctly different settling profiles between the two methods (figure 5.15.). The bench scale thickener reduced the sludge by 70% (volume), whereas simple storage achieved only a 55% volume reduction. The phosphorus release profiles between the two methods though, were for the first 2 days almost identical (figure 5.16.). The initial release rates were 0.58 mgP/l.h for the storage and 0.66 mgP/l.h for the thickening experiment (0.11 and 0.10 mgP/gVSS.h respectively). After the first 48 hours, the sludge in the slowly stirred reactor (thickener) showed an increase in its phosphorus release rate (1.15 mg/l.h). The stored sludge continued to release phosphorus at the rate of 0.58 mgP/l.h. After 5 days the stored sludge reached its maximum releasable concentration which was 10% of its total phosphorus content. On the other hand, the thickened sludge having by the same time released 15% of its total phosphorus, continued to release phosphorus.

The initial release phase, characterised by identical release profiles between storage and thickening, varied between half and 3 days of retention time for different sludge batches.

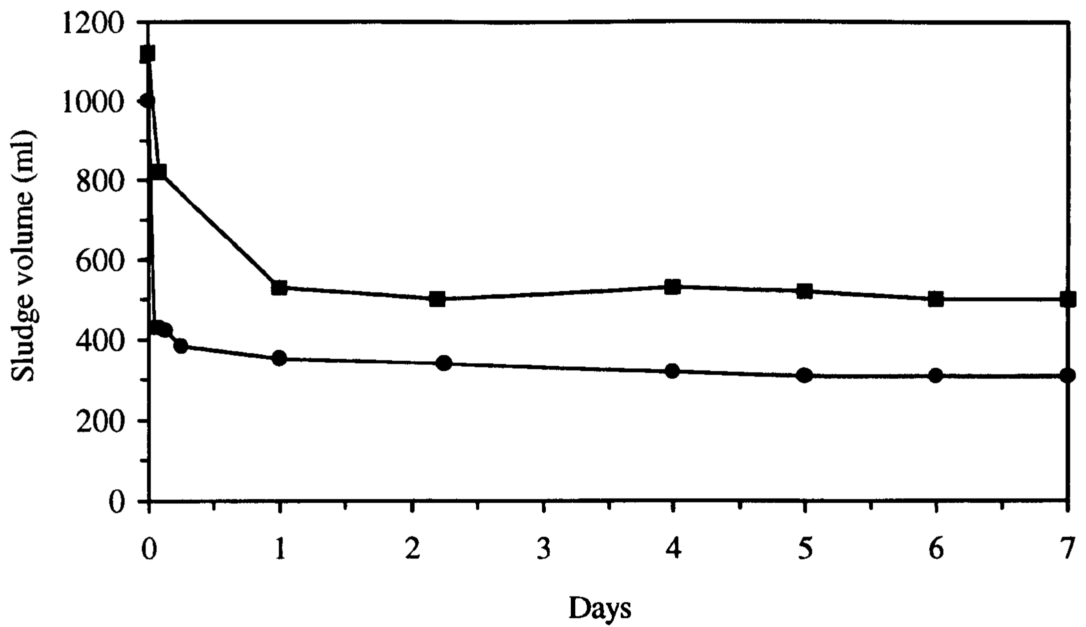


Fig. 5.15. Comparison of sludge settling profiles during anaerobic (■) quiescent storage and (●) gravity thickening.

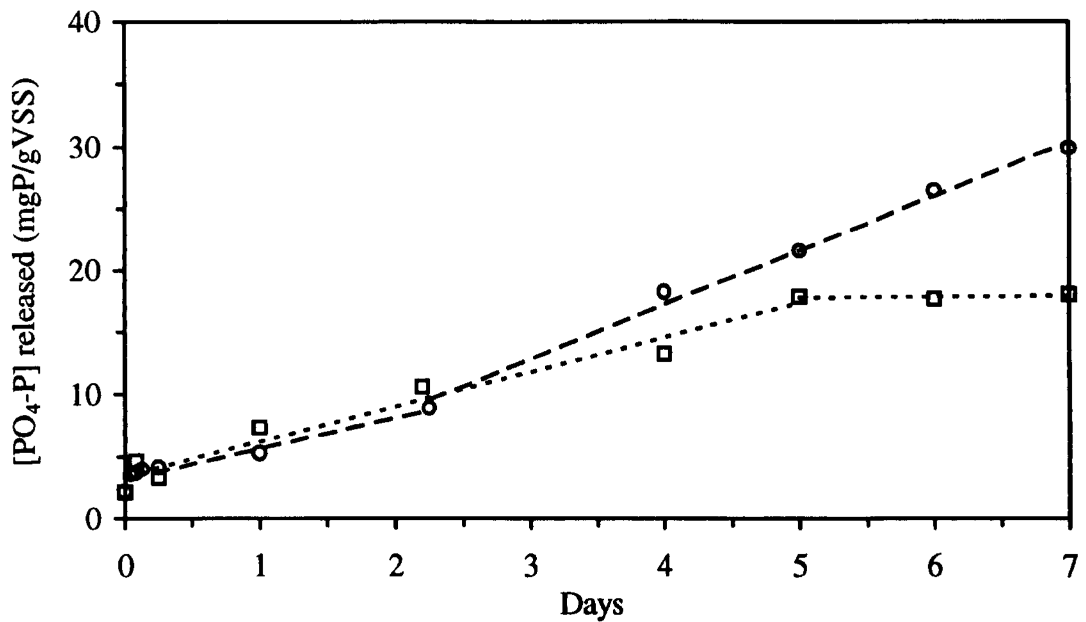


Fig. 5.16. Comparison of phosphorus release profiles during anaerobic (□) quiescent storage and (○) gravity thickening.

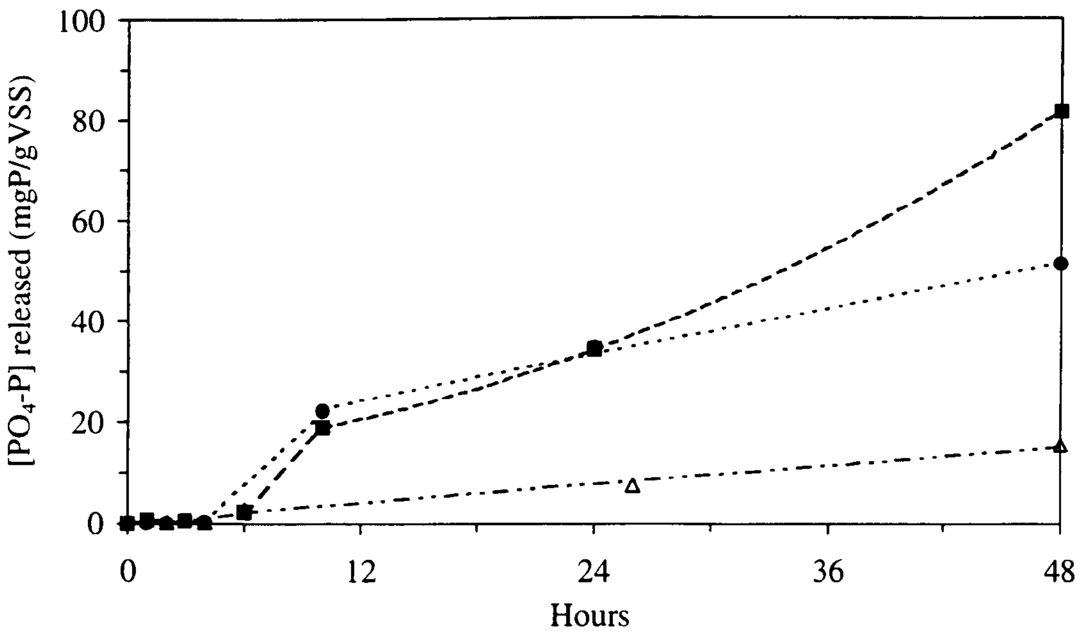


Fig. 5.17. Phosphorus release profiles during the first 48 hours of an anaerobic (■) gravity thickening, (●) quiescent storage and (Δ) completely stirred experiment.

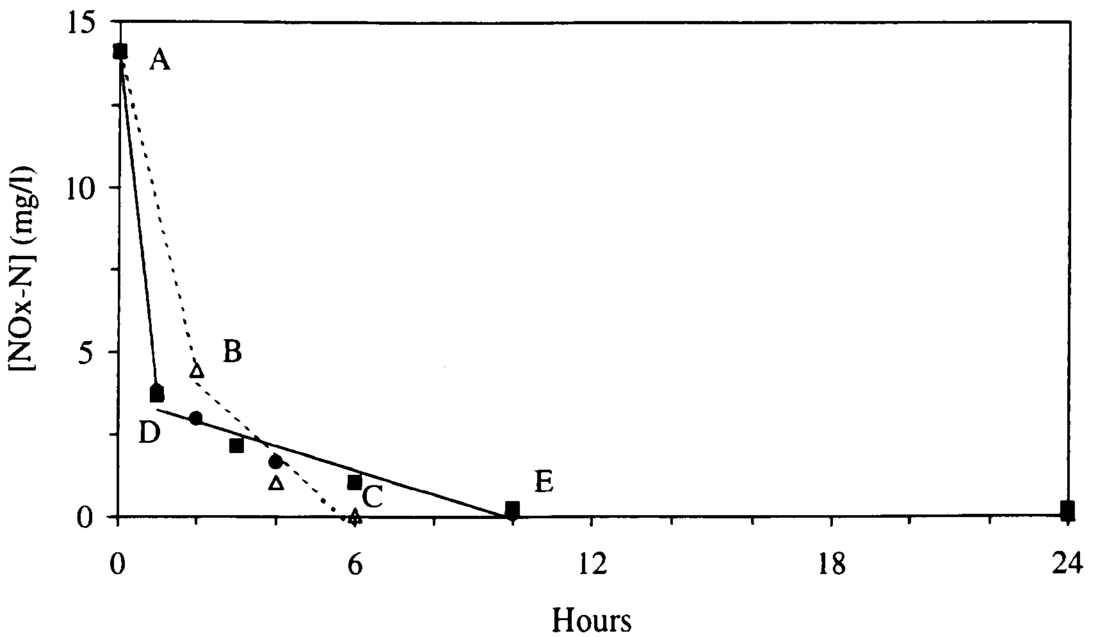


Fig. 5.18. Denitrification during the first 24 hours of an anaerobic (■) gravity thickening, (●) quiescent storage and (Δ) completely stirred experiment. De-N rates (slope of best fit line between sections): AB = 4.8 mgN/l·h, BC = 1.1 mgN/l·h, AD = 10.5 mgN/l·h and DE = 0.4 mgN/l·h.

After this time period the sludge in the thickener always exhibited higher release rates. The maximum amount of releasable phosphorus was also always 25 to 40% higher for the thickened sludge.

Apart from the thickening and quiescent storage experiments, fully stirred aerobic and anaerobic storage ones were performed, mainly because these types of procedures have been used in the literature when phosphorus release during sludge treatment is investigated.

Surprisingly, the completely stirred anaerobic experiments showed much lower phosphorus release when compared to both the thickening and quiescent storage ones. As shown in figure 5.17., the release for the first 48 hours of the completely stirred anaerobic experiment was 80% and 60% less than what was observed for the thickening and storage experiments respectively.

This finding does not agree with the results of Shapiro *et al.* (1967), who investigated the effect of stirring on anaerobic phosphorus release and found a 25% increase in the phosphorus released, after 7 hours, by the shaken sludge compared to the quiescently stored sludge. This may be explaned by the different method of stirring employed in their experiment. Another possible explanation may be that stirring facilitated the precipitation of the released phosphates with cations in this study. Nevertheless at the pH range that these experiments were performed (pH: 7.4 - 6.9), this seems unlikely.

The denitrification rates of the completely stirred anaerobic experiment were slower than the rates of the storage and thickening ones but only for the first two hours (section AB of dashed line: 4.8 mgN/l.h as opposed to section AD of solid line: 10.5 mgN/l.h). With increasing retention time the denitrification rate for the latter dropped to 0.4 mgN/l.h (section DE of solid line in figure 5.18.) whereas for the completely stirred experiment it proceeded with the higher rate of 1.1 mgN/l.h (section BC of dashed line in figure 5.18.). This eventually resulted in complete nitrate removal after 6 hours, almost half the time needed for the other two methods.

Figure 5.19. shows the phosphorus release patterns observed for a completely stirred aerobic experiment and a thickening one, performed with identical sludges. The

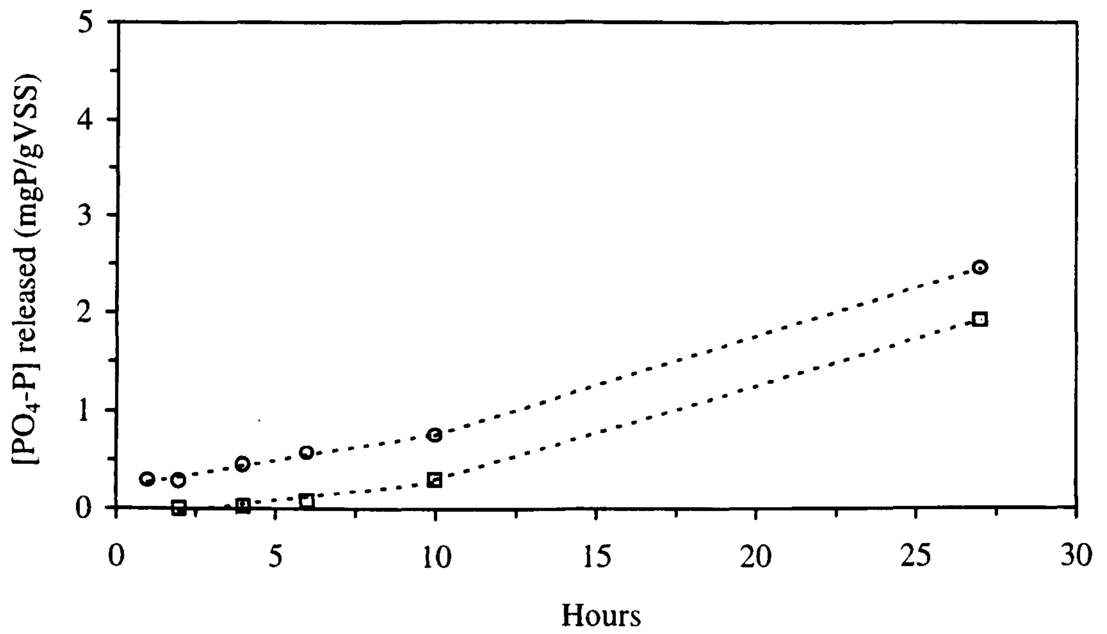


Fig. 5.19. Phosphorus release patterns during (O) gravity thickening and (□) aerobic storage.

release rates observed were similar, the main difference between the two methods being a delay in phosphorus release by 4 hours, exhibited by the aerobic experiment.

As shown by this research and also by Shapiro *et al.* (1967), the method of sludge treatment (quiescent, continuously or intermitently stirred etc.), affects the amount of phosphorus resolubilised. This variation must be taken into account for the correct interpretation of any published results as currently there is more than one way of defining storage and thickening in bench scale studies.

5.4.3. Effect of Reactor Operation on Phosphorus Release During Sludge Treatment

5.4.3.1. Effect of reactor influent phosphorus

The effect of influent phosphorus concentration on phosphorus release during sludge handling was investigated for influent phosphorus values in the range of 9 - 20 mgP/l.

Figure 5.20. shows the release profiles obtained over the first 24 hours, from two batch thickening experiments with influent phosphorus values of 9 and 12 mgP/l. The influent and operational parameters of the reactor run from which these sludges were derived are shown in table III.1 (appendix III).

As shown in figure 5.21, the two batches of sludge showed identical denitrification patterns, with the oxidised nitrogen disappearing during the first 6 hours of thickening. The highest denitrification rates, calculated as the slope of the best fit line, were obtained during the first two hours of the experiment and were 2.3 mgN/l·h for the run with the low influent phosphorus concentration (9 mgP/l) and 2.7 mgN/l·h for the run with the high influent phosphorus concentration (12 mgP/l).

The observed phosphorus release pattern on the other hand, was significantly different between the two runs, with higher release rates for the sludge with the highest influent phosphorus (figure 5.20).

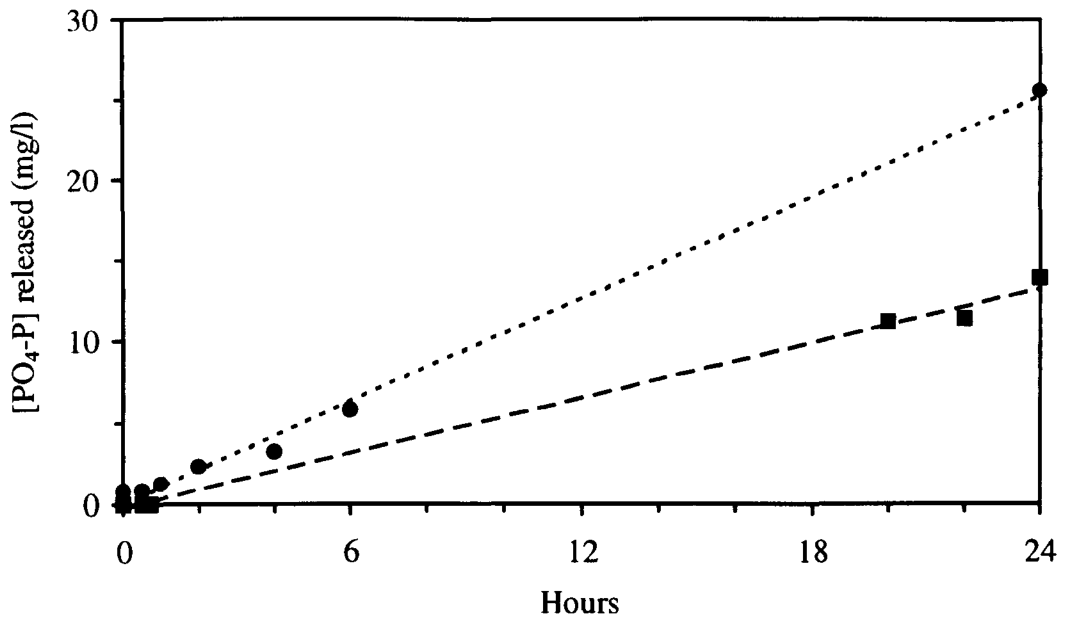


Fig. 5.20. Comparison of phosphorus release profiles during gravity thickening of sludge with (■) 9 mg/l influent phosphorus and (●) 12 mg/l.

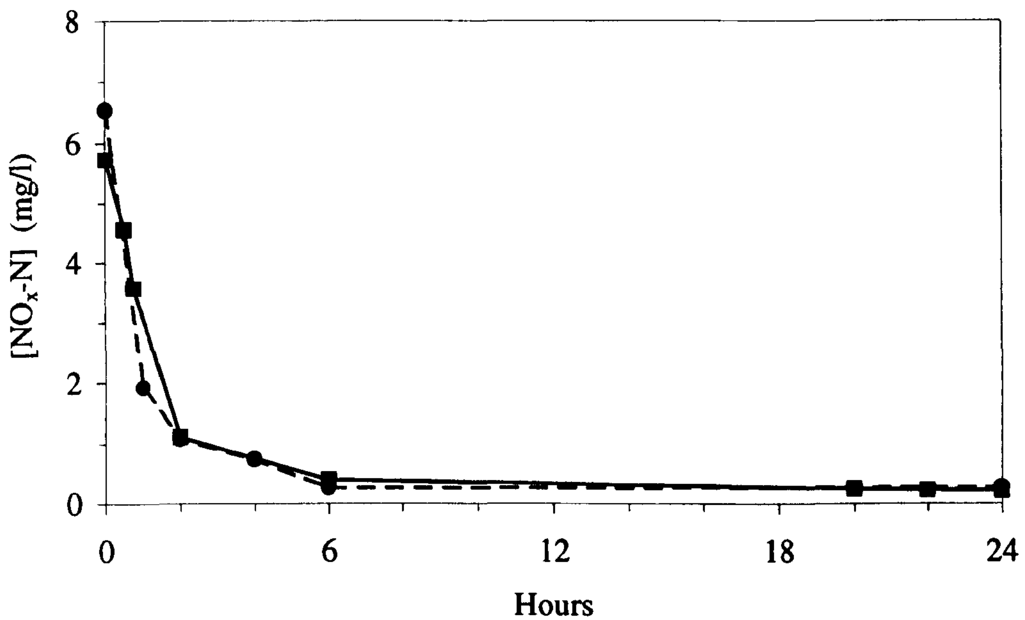


Fig. 5.21. Denitrification during the first 24 hours of gravity thickening of sludge with (■) 9 mg/l influent phosphorus and (●) 12 mg/l.

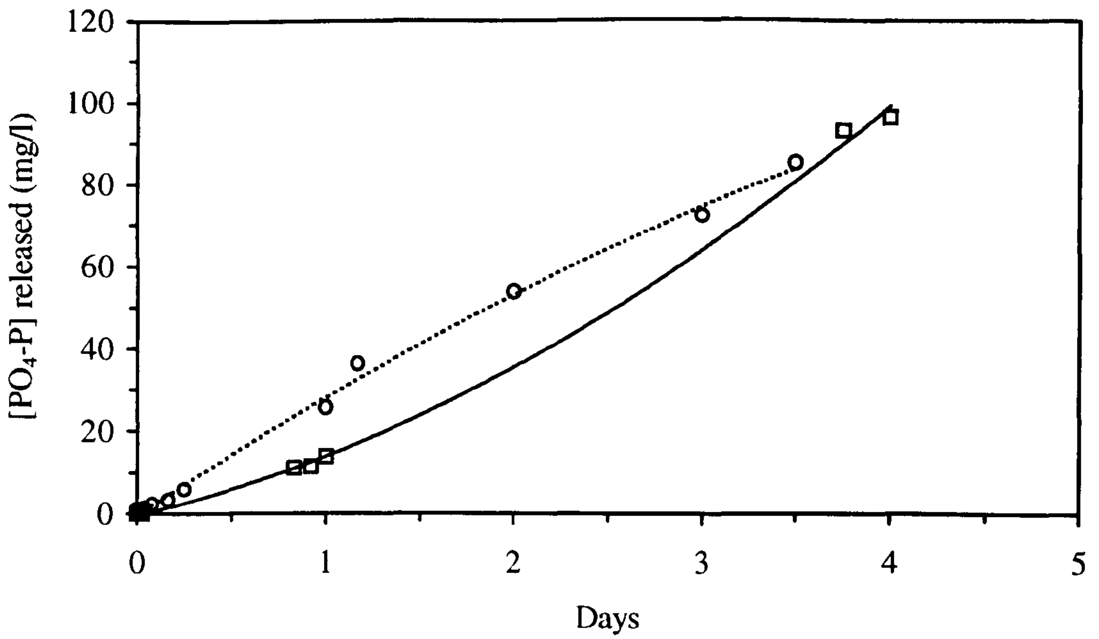


Fig. 5.22. Comparison of phosphorus release profiles during long term gravity thickening of sludge with (□) 9 mg/l influent phosphorus and (○) 12 mg/l.

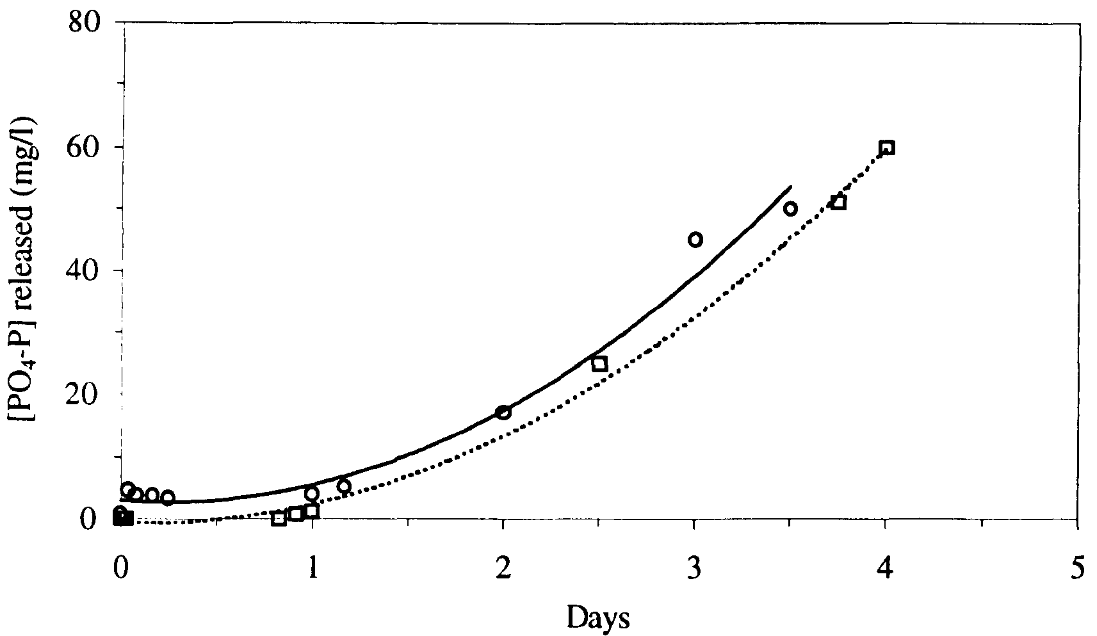


Fig. 5.23. Comparison of phosphorus release profiles during long term quiescent storage of sludge with (□) 9 mg/l influent phosphorus and (○) 12 mg/l.

Over the first 24 hours, the sludge with the high influent phosphorus showed release rates in the range of 1.06 mgP/l.h, twice as much as the observed release for the low influent phosphorus sludge (0.56 mgP/l.h). A 33% increase in the influent, resulted in a 84% increase in the amount of phosphorus released after 24 hours.

The effect of the influent phosphorus concentration though, disappeared for retention times in the thickener over 3 days. As figure 5.22 shows, after 3 days the concentration of dissolved orthophosphate in the supernatant of both thickened sludges was similar and remained so for the rest of the experiment.

Identical results were obtained for the same sludge batches when left in quiescent storage conditions (figure 5.23). The sludge with the highest influent phosphorus showed higher release for the first 24 hours of storage but after the first day, similar release was obtained for both sludges.

These results although performed for only a few influent values indicated that influent phosphorus concentrations have a short term effect on phosphorus release during sludge treatment and that higher release can be expected from sludges with higher influent phosphorus loads. The short term effect of the influent phosphorus concentration points to a variation in the cell LPP since it has been shown to be the polyphosphate fraction that is first degraded under anaerobic conditions (Appeldoorn *et al.*, 1992). This would indicate that transient phosphorus loads influence the low polymeric polyphosphate content of the cells.

No published data are avalaibale for comparison with these results.

5.4.3.2. Effect of reactor influent COD

As with the effect of influent phosphorus, the effect of influent COD on phosphorus release during sludge treatment, was investigated for sludges with comparable sludge phosphorus and oxidised nitrogen contents.

The range of influent COD values investigated was 300 to 740 mgCOD/l, resulting in an organic loading range of 0.4 - 0.66 mgCOD/gVSS d, in the SBR. The operational parameters of the run from which these sludges were produced are shown in table III.2 (Appendix III).

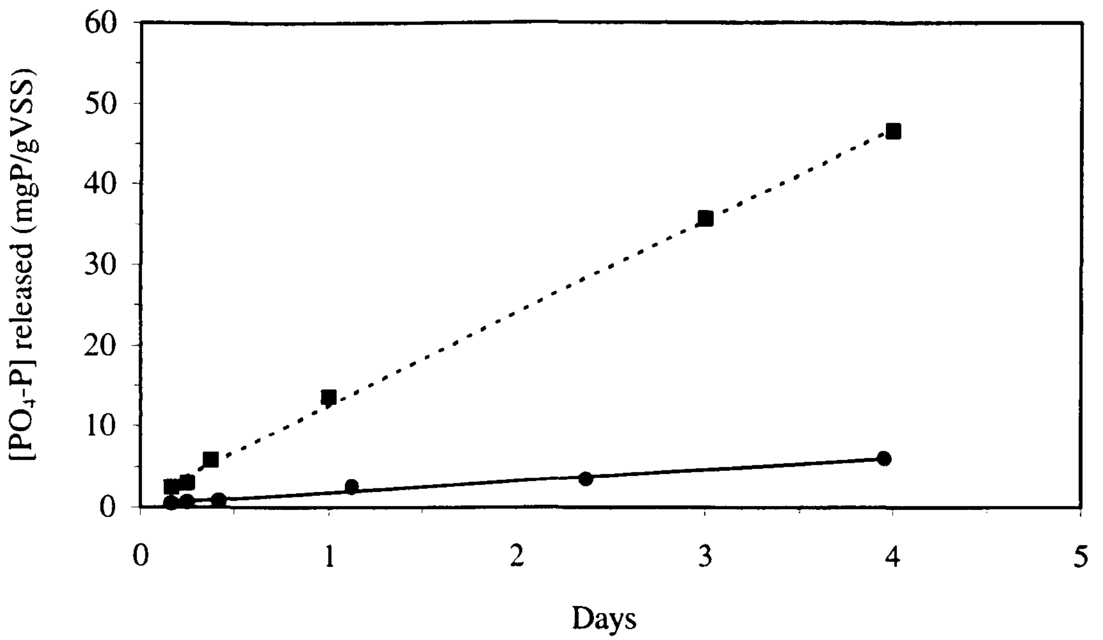


Fig. 5.24. Comparison of phosphorus release profiles during long term thickening of sludge with (■) 300 mg/l influent COD and (●) 740 mg/l.

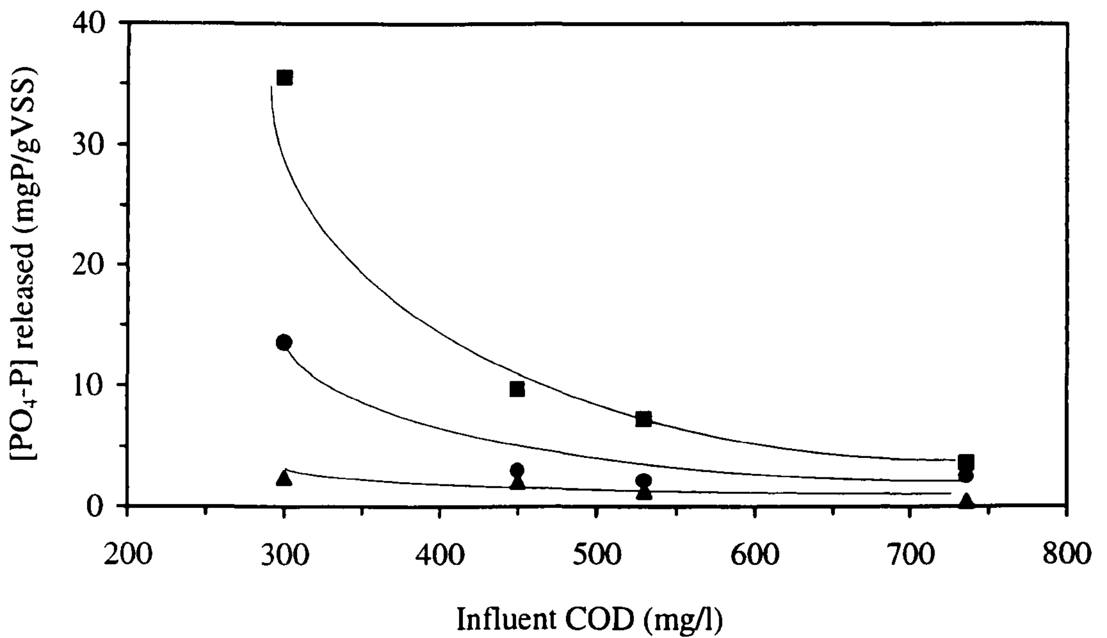


Fig. 5.25. Effect of increasing influent COD on phosphorus released after (▲) 4 hours, (●) 24 hours and (■) 72 hours of excess sludge thickening.

Figure 5.24., shows the phosphorus release profile over a period of four days of thickening, for the highest (740 mg/l) and lowest (300 mg/l) influent COD values investigated. The release rates observed for the sludge receiving 300 mgCOD/l influent were 1.57 mgP/l.h (0.47 mgP/gVSS.h) as opposed to only 0.25 mgP/l.h (0.06 mgP/gVSS.h), for the sludge receiving 740 mgCOD/l of feed.

The results obtained for a number of thickening experiments, showed that increasing the influent COD, decreased the phosphorus released during sludge treatment (figure 5.25.).

Unlike the effect of influent phosphorus, the influent COD values appear to have increasing effect with increasing retention time in the thickener, as shown by the increasing curvature of the best fit line for the plotted retention times of 4 hours and one and three days. The coefficient of the quadratic terms of the best fit curves were $1x10^{-6}$, $1x10^{-4}$, $3x10^{-4}$ respectively.

As expected the same results were obtained when the amount of phosphorus released was expressed as a percentage of the total sludge content as shown in figure 5.26.

A possible explanation for the effect the influent COD had on phosphorus release during sludge treatment, may lie in the fact that varying organic loadings result in varying amounts of cell growth before replication commences. It has been shown that storage is a function of the loading rate to which the organisms are acclimatised to (Speece *et al.*, 1973; Chudoba *et al.*, 1991). The lower the loading rate the longer the lag phase between substrate uptake and commencement of cell replication. In a system operated with a fixed aerobic time, the delay in cell replication may result in incomplete use of the stored PHB. This would leave higher amounts of stored carbohydrates in the cytoplasm which are available for polyP degradation during the anaerobic stress imposed while treating the sludge and hence higher phosphorus release rates.

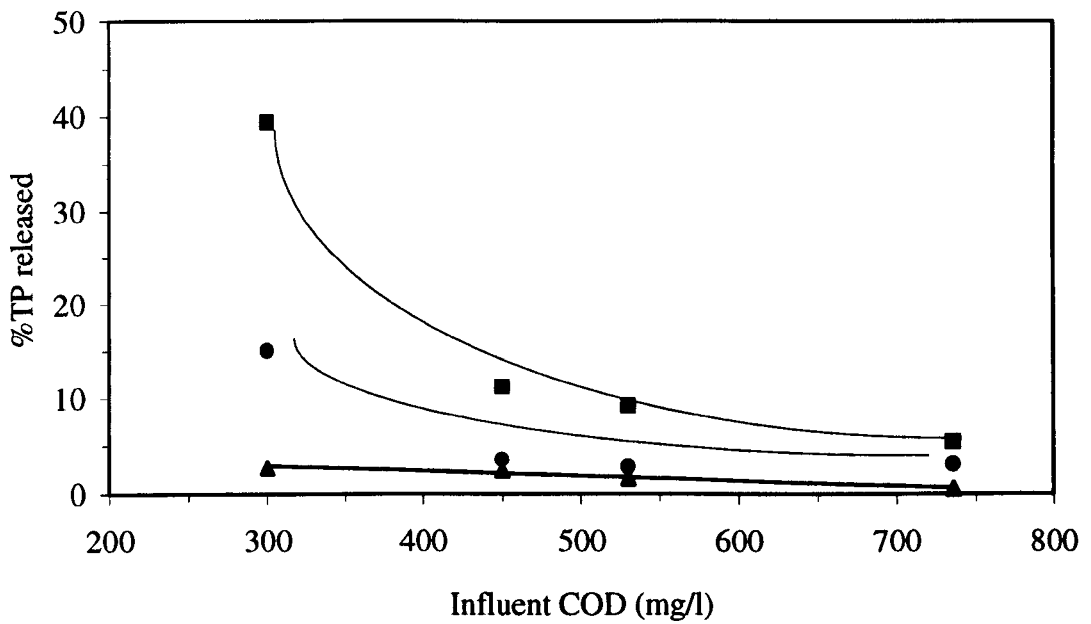


Fig. 5.26. Effect of increasing influent COD on phosphorus released after (▲) 4 hours, (●) 24 hours and (■) 72 hours of excess sludge thickening.

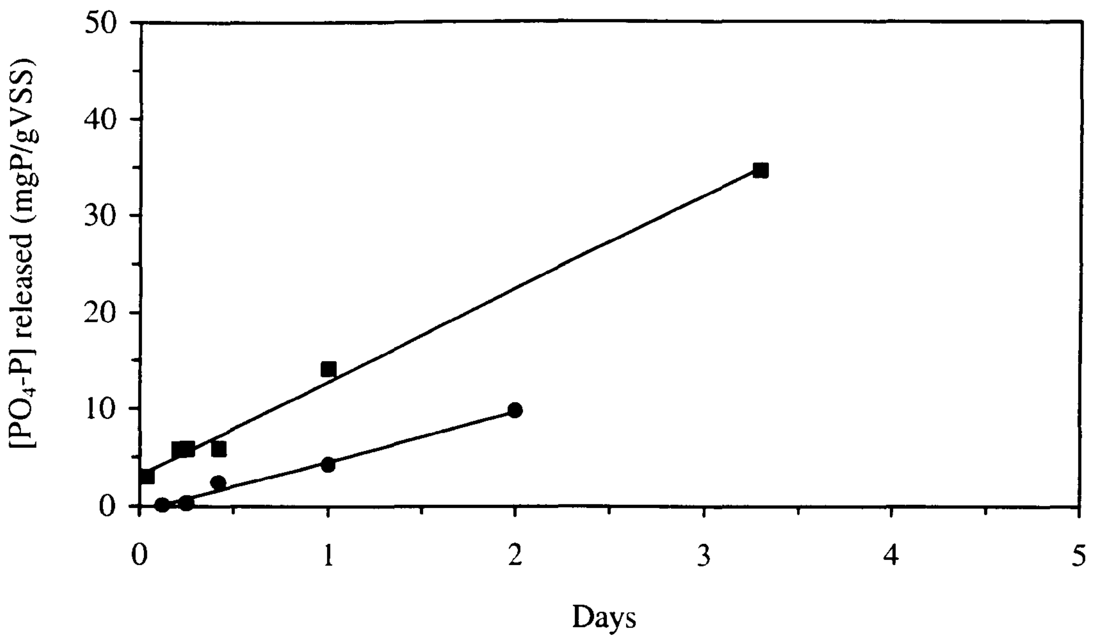


Fig. 5.27. Phosphorus release profile during thickening of excess sludge with an initial oxidised nitrogen concentration of (●) 15 mg/l and (■) 0.5 mg/l.

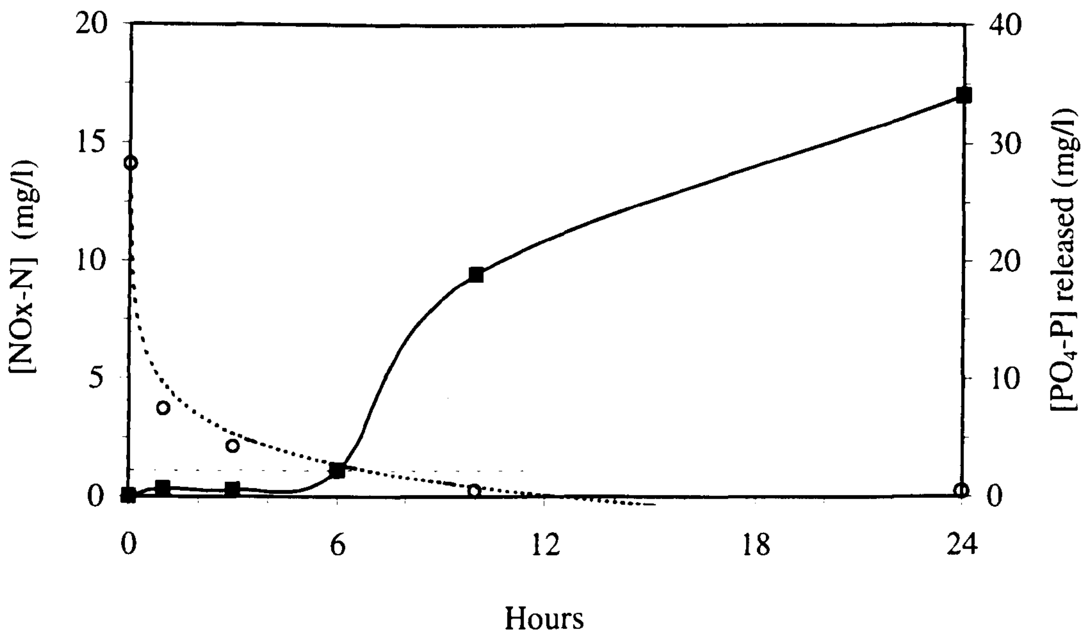


Fig. 5.28. Phosphorus release and oxidised nitrogen comsumption during thickening of excess sludge with an initial oxidised nitrogen concentration of 15 mg/l. (■) [PO_4-P] and (O) [NO_x-N].

5.4.3.3. Effect of sludge oxidised nitrogen content

Unlike the effect of influent phosphorus and carbon concentration on phosphorus release during sludge handling, the effect of oxidised nitrogen has been studied. The results obtained in this study verify the findings of other researchers, who have observed an inverse relationship between the rate of phosphorus released during batch thickening and the initial oxidised nitrogen concentration of the excess sludge (Kroiss and Negm, 1994).

As figure 5.27. shows, the release rate of a batch of sludge containing high amounts of nitrates and/or nitrites, 15 mg/l in this case, is almost half of that observed for a batch of sludge containing negligible amounts of oxidised nitrogen (0.5 mg/l). By the end of the first day, the [NO_x-N] free sludge has released three times more phosphorus (15 mgP/gVSS) than the nitrate containing sludge (5 mgP/gVSS).

In batch tests, it has been noted that the [NO_x-N] concentration has to drop to 0.1 mg/l for phosphorus to begin to be released (Malnou, 1984; Fukase *et al.*, 1984). In this study phosphorus release was observed even with oxidised nitrogen concentrations in the area of 2 mg/l. For most of the experiments though, the critical [NOx-N] concentration below which increased phosphorus release occurred was found to be 1 mg/l (figure 5.28).

The effect of the initial oxidised nitrogen concentration on phosphorus release during thickening was identical to the one observed for the storage experiments (figure 5.29.). The operational parameters of the reactor run from which these sludges were derived are shown in table III.3. (Appendix III).

The sludge with no or low concentration of oxidised nitrogen released phosphorus immediately and at a rate of 0.144 mgP/gVSS.h. In the presence of oxidised nitrogen, on the other hand phosphorus was released more slowly, at a rate of 0.09 mgP/gVSS.h.

Furthermore, for the storage experiments, the phosphorus release pattern showed a distinct variation between different batches of sludge, which wholly depended on the denitrification rate achieved at the start of the experiment.

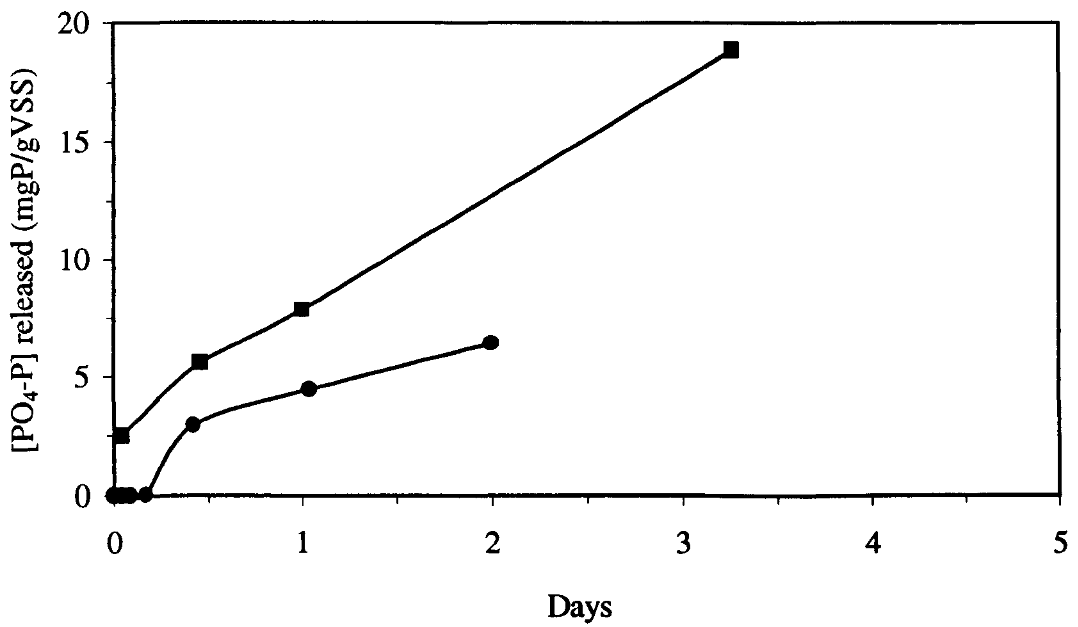


Fig. 5.29. Phosphorus release profile during storage of excess sludge with an initial oxidised nitrogen concentration of (●) 15 mg/l and (■) 0.5 mg/l.

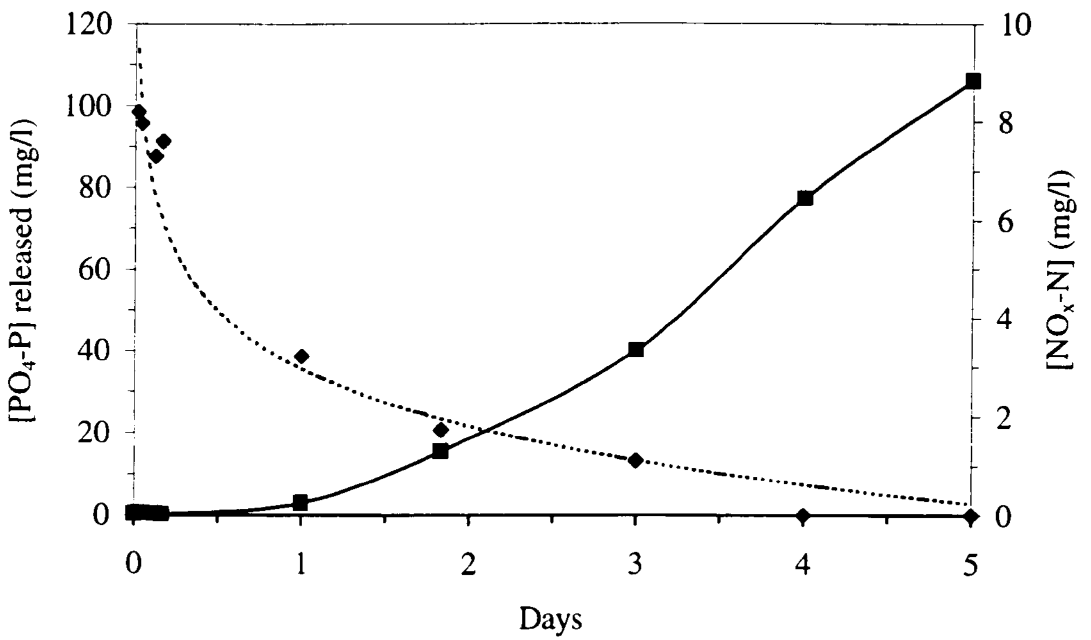


Fig. 5.30. Phosphorus release (■) and denitrification (◆) during storage of excess sludge.

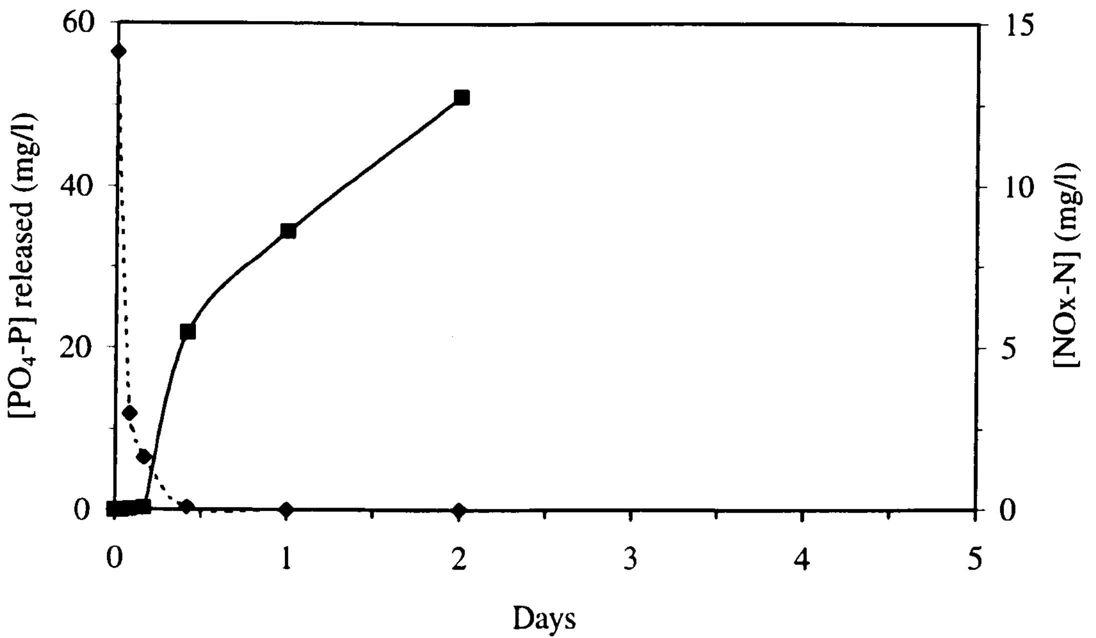


Fig. 5.31. Phosphorus release (■) and denitrification (◆) during storage of excess sludge.

If denitrification was not completed within the first 24 hours, phosphorus was released initially slowly at 0.7 - 0.9 mg/l.h and as soon as the level of oxidised nitrogen dropped below 1.5 mg/l, the release rate increased to 1.2-1.4 mgP/l.h (figure 5.30.). On the other hand, when nitrification was completed within the first few hours, phosphorus was initially released at rates around 1.1 to 3.6 mgP/l.h. followed by a slower 0.7 - 0.8 mgP/l.h (figure 5.31.).

The denitrification rates observed in both storage and thickening experiments in this study, were comparable to values published in the literature. The two phases with different phosphorus release rates and corresponding denitrification rates were also observed by Kroiss and Negm (1994). Phase I, as defined in tables 5.1 and 5.2. had a duration of 4 - 20 hours and an average denitrification rate of 5 mgN/l.h. The equivalent phase observed by Kroiss and Negm (1994) had a comparable duration of 12 - 18 hours and a denitrification rate of 3 mgN/l.h. Other authors have reported oxidised nitrogen consumption rates in the range of 4 mg/l.h for similar experimental conditions to the ones in this study (Skalsky and Daigger, 1995).

The denitrification rate observed in this study, for phase II (0.02 - 0.4 mgN/l.h) was also similar to the 0.45 mgN/l.h observed by Kroiss and Negm (1994), in the second phase of their experiments.

The effect of oxidised nitrogen on phosphorus release has been attributed to preferential use of the substrate created by cell lysis for denitrification rather that polyphosphate hydrolysis (Kroiss and Negm, 1994).

For the sludge used in this study the observed decrease in the phosphorus release rate for increasing oxidised nitrogen concentrations, may also have the implication that the organisms present in the sludge were able to denitrify. Since, microscopic investigations revealed that the sludge was dominated by polyP bacteria (90% of the floc surface stained Neisser positive), this observation would support the currently under discussion issue of the capability of polyP bateria to denitrify (Barker and Dold, 1996).

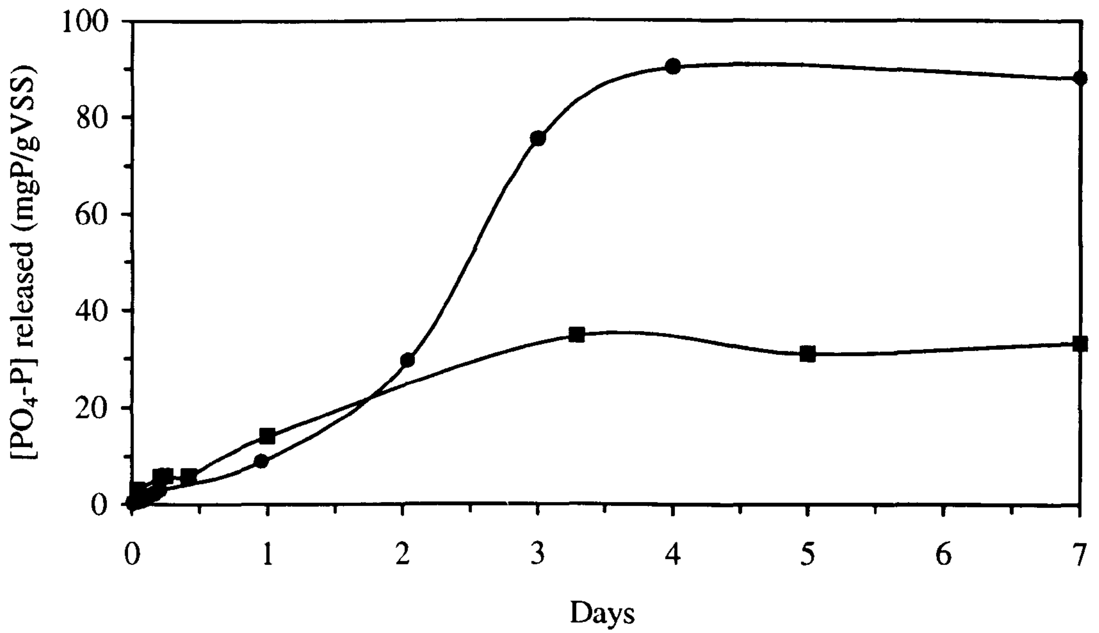


Fig. 5.32. Phosphorus release profile during long term thickening of excess sludge with an initial total phosphorus content of (●) 270 mgP/gVSS and (■) 57 mgP/gVSS.

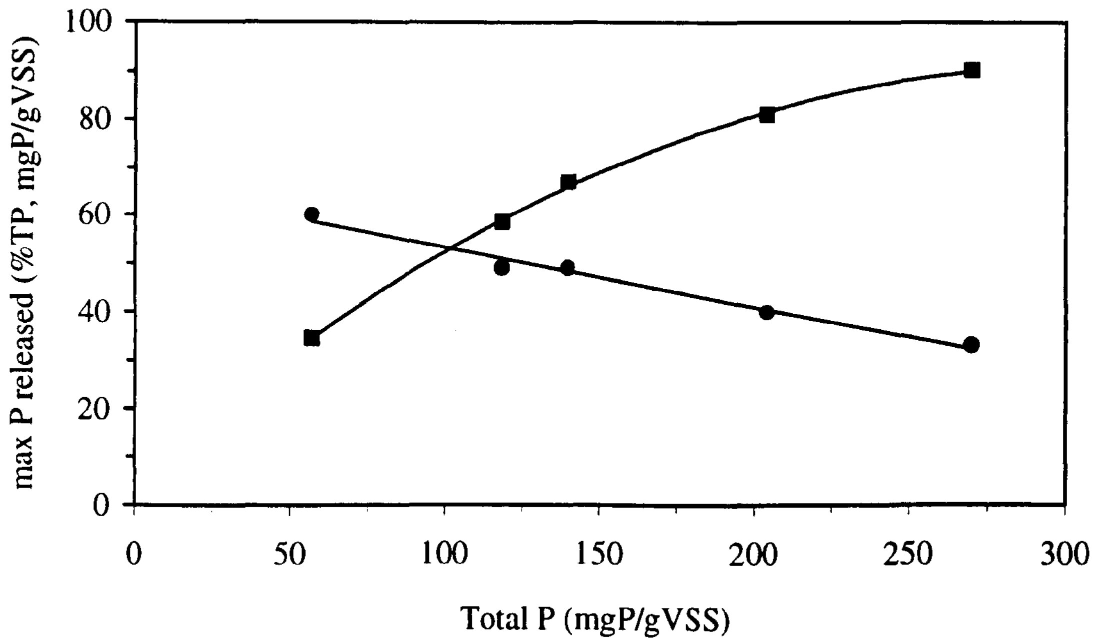


Fig. 5.33. Effect of total phosphorus sludge content on phosphorus released during long term thickening of excess sludge. Phosphorus released expressed as (●) %TP and (■) mgP/gVSS.

5.4.3.4. Effect of sludge phosphorus content

The amount of phosphorus released from the sludge was found to be directly related to the sludge phosphorus content. This was true though only for long term storage, as graph 5.32 shows. The two thickening experiments shown, have total phosphorus contents of 57 mgP/gVSS and 270 mgP/gVSS. By the end of the first 24 hours of thickening, both batches of sludge have released comparable amounts of phosphorus (10 mgP/gVSS and 14 mgP/gVSS), but after a day and a half, the sludge with the highest phosphorus content exhibits higher release rates (4.2 mgP/l.h as opposed to 1.42 mgP/l.h).

For the TP range investigated in this study (60 - 270 mgP/gVSS), the higher the total phosphorus of the sludge the more phosphorus was released during thickening (figure 5.33). Similar results have been obtained during anaerobic experiments performed with pure *Acinetobacter* cultures (Ohtake *et al.*, 1985) and with fill-and-draw system sludges (Appeldoorn *et al.*,1992).

When the phosphorus released was expressed as a percentage of the total phosphorus sludge content, opposite results were obtained. A smaller percentage of the total phosphorus leached in the supernatant from the sludges with the highest TP content.

This apparent controversy can be explained when the mechaninsm by which the sludge content increases is taken into account. Apart from the phosphorus loading of the sludge the phosphorus content has been shown to be a function of the amount of time the sludge had spent in the reactor before being removed for thickening (see figure 4.52 and discussion of section 4.3.3.6). This elapsed time can be defined as a "stabilisation" period.

Sludges that have been "stabilised" longer have a higher proportion of HPP which is degraded much later if at all (Mino *et al.*, 1985). In this study, the batches of sludge that had the lowest sludge phosphorus content were the ones that had remained for a shorter period of time in the reactor and therefore possibly had most of their polyphosphate in the LPP form.

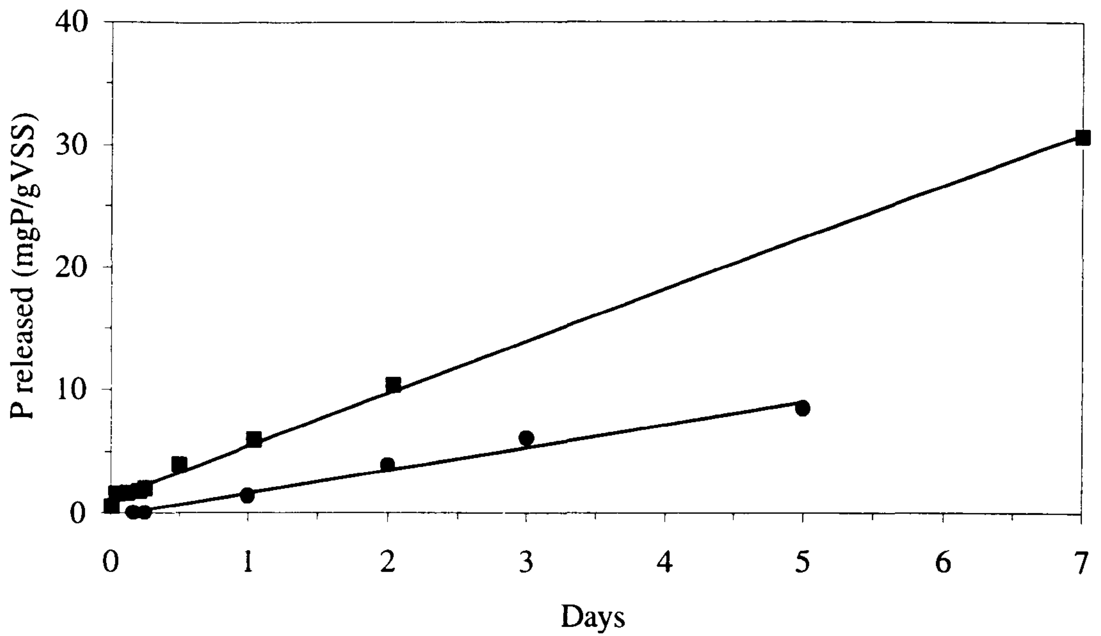


Fig. 5.34. Comparison of phosphorus release profile during thickening of excess sludge when wasted from the aeration tank (●) and the final sedimentation tank (■).

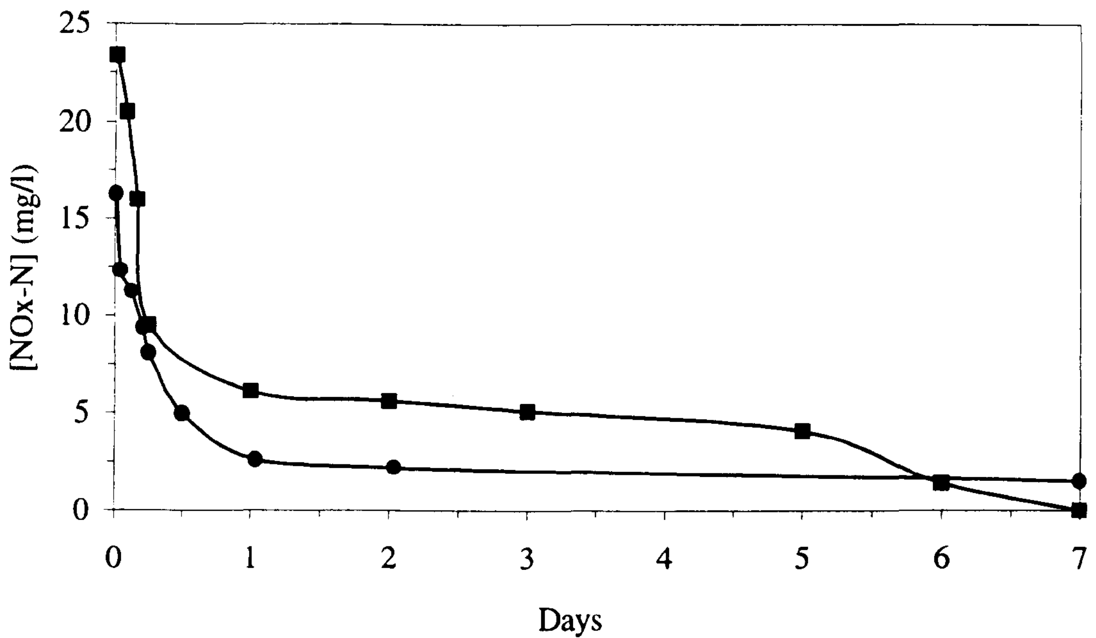


Fig. 5.35. Comparison of oxidised nitrogen uptake profile during thickening of excess sludge when wasted from the aeration tank (●) and the final sedimentation tank (■).

The operational parameters of the reactor run from which these sludges were derived are shown in table III.4 (Appendix III).

5.4.3.5. Effect of sludge waste line

One of the most widely quoted solutions to the problem of phosphorus release during sludge handling, is wasting the excess sludge from the aeration tank as opposed to wasting from the final sedimentation tank (see Literature Review, section 2.7.). This was tested in this study by removing the sludge either at the end of the aeration stage - aerobic tank wasting regime- or after the completion of the settling period, usually two hours later - final sedimentation wasting regime.

As expected the extra amount of time under anaerobic conditions imposed to the sludge sample through wasting at the end of settling, resulted in higher release rates compared to the sludge removed at the end of the aeration period (figure 5.34.). The detrimental effect of final sedimentation tank wasting regime is due to the combined effect of the longer anaerobic retention time and the lower nitrate concentration present in the sludge, as a portion of the nitrates has been denitrified during settling (figure 5.35.). The difference in the release rates observed was 0.175 mgP/gVSS for the final sedimentation tank wasting regime and 0.076 mgP/gVSS for the aeration tank wasting regime. The operational parameters of the reactor run from which these sludges were derived are shown in table III.5 (Appendix III).

5.4.3.6. Effect of reactor operation under D.O. limitation

As described in chapter 4, section 4.3.3.4., the SBR was operated for a period of one month under oxygen limiting conditions (D.O. < 1 mg/l) during the aerobic stage. During this period of operation, thickening experiments were performed and the release patterns obtained were compared to the ones obtained from similar sludges produced with no oxygen limitation. A typical example is shown in figure 5.36.

For any set of two sludges with comparable properties, the amount of phosphorus released was practically identical, irrespective of the amount of dissolved oxygen in the reactor.

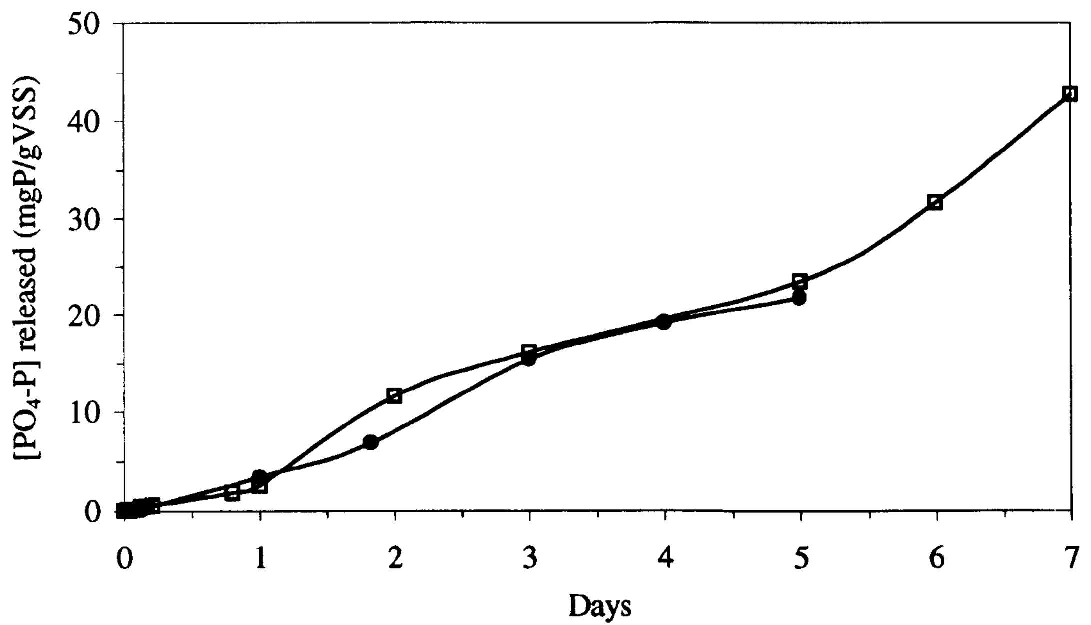


Fig. 5.36. Comparison of phosphate release profile during thickening of excess sludge derived from (●) a low D.O. run and (□) from a run with normal D.O. levels.

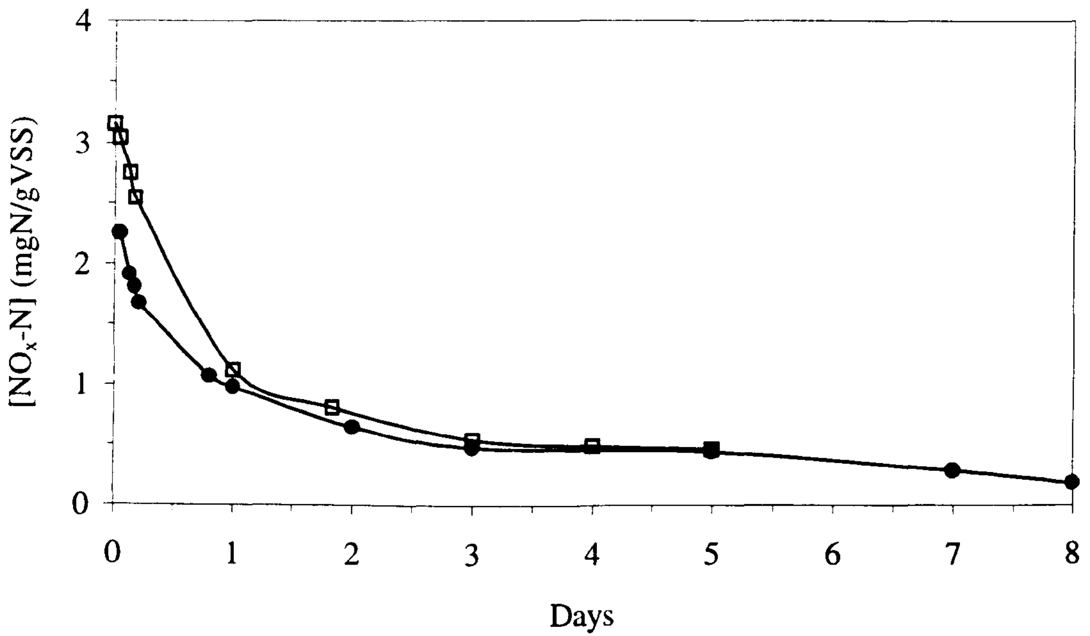


Fig. 5.37. Comparison of nitrate uptake profile during thickening of excess sludge derived from (●) a low D.O. run and from (□) a run with normal D.O. levels.

As seen in chapter 4, the effect of the low dissolved oxygen was limited to nitrification, resulting in incomplete ammonia removal under D.O. imitation. This effect was evident in the sludge treatment experiments as well, with the low D.O. sludges containing lower amounts of nitrates. In the example shown in figure 5.37., the difference was only 1 mg/l and this was not significant enough to affect the phosphorus release patterns.

The operational parameters of the reactor run from which these sludges were derived are shown in table III.6 (Appendix III).

5.4.3.7. Sludge settling characteristics

For all operational parameters used, the sludge settling characteristics, as described by the SSVI, were in the range of 72 - 140 ml/mg. On only one occasion did the sludge have a SSVI of 200 ml/mg, but even this value did not result in sludge washout as the sludge blanket did not exceed the reactor wasting port.

5.4.4. Correlation of Anaerobic Phosphorus Release During the Daily Run and During Subsequent Sludge Treatment

As mentioned in chapter 4, section 4.3.1., the ultimate objective of the second experimental phase was to determine any link between the parameters found to influence the phosphorus release in the anaerobic zone of a EBPR treatment plant with those affecting the release observed during excess sludge treatment.

Figure 5.38. shows the phosphorus release and nitrification/denitrification profile for a full cycle of the SBR followed by thickening of the excess sludge. It is immediately obvious that for the sludge to release during thickening, the same amount of phosphorus as it does within the two hours of anaerobic conditions it is necessary for it to remain in the thickener for almost 10 days. The main difference between the two situations is the presence of exogenous carbon substrates during the SBR anaerobic phase and their absence during thickening.

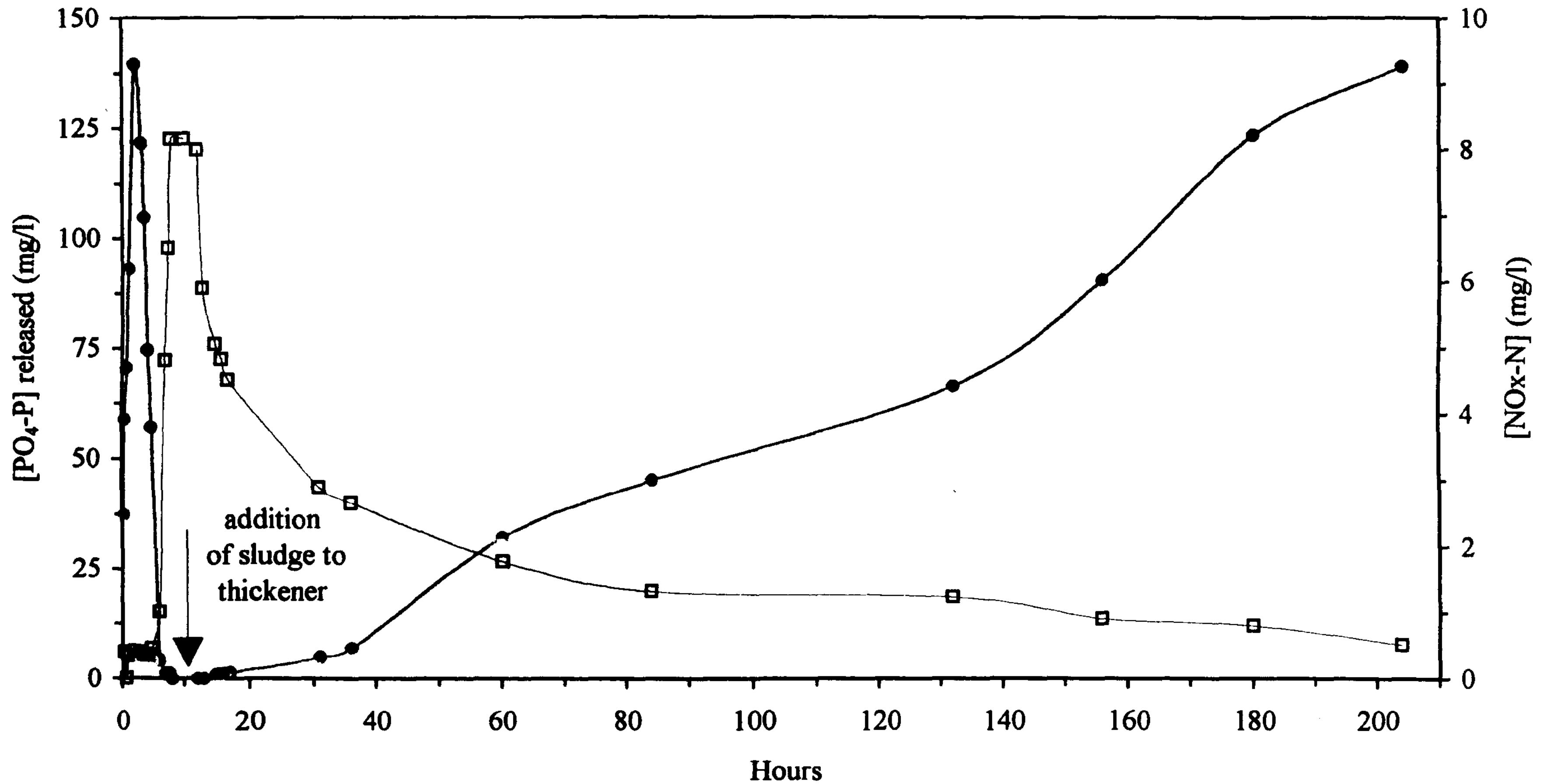


Fig. 5.38. Phosphorus release and oxidised nitrogen metabolism during a full SBR cycle followed by excess sludge thickening. (●) [PO_4-P] and (□) [NOx-N].

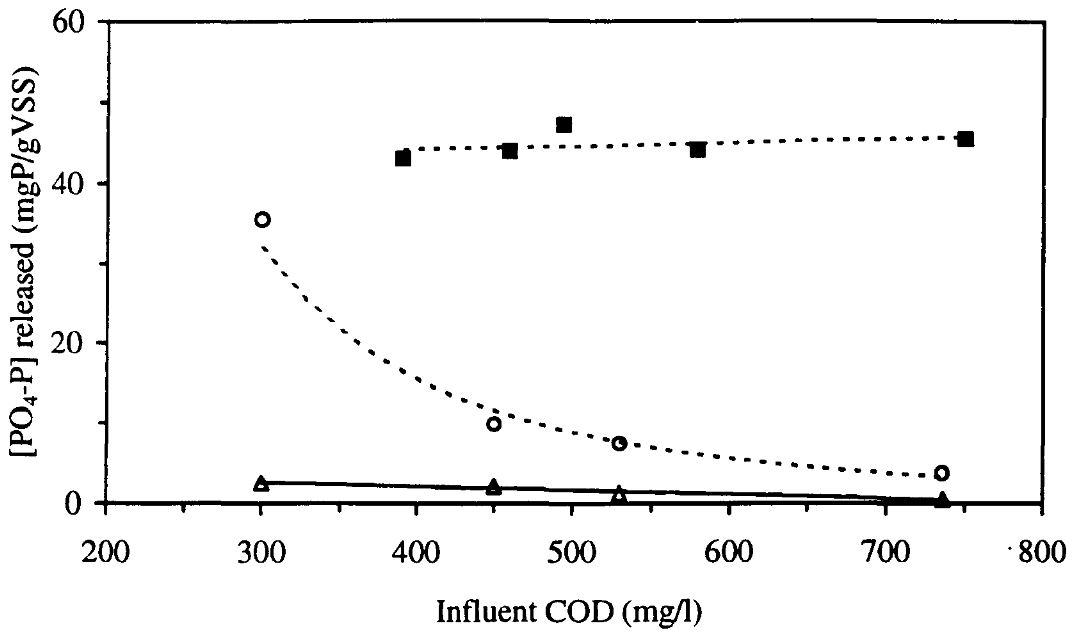


Fig. 5.39. Comparison of the effect of increasing influent COD concentration on phosphorus released in the anaerobic zone of the SBR (■) and after 4 (Δ) and 72 (O) hours of thickening of excess sludge.

A comparison of the effect of influent phosphorus and carbon concentration and low D.O. operation on anaerobic phosphorus release to the effect of the same parameters on release during sludge treatment follows.

5.4.4.1. Effect of influent COD

In chapter 4, the effect of varying the influent COD and subsequently the organic loading of the sludge, was investigated in the range of 100 mg/l - 700 mg/l. It was found that altering the COD in that range had no effect on any of the characteristics of EBPR (% influent P removed, anaerobic P release), for P/M values of 3 - 4.5 mgP/gVSS.

For a comparable P/M range (3.5 - 4.5 mgP/gVSS), operating the SBR with varying influent COD values did effect the amount of phosphorus released during the sludge treatment experiments but only for long retention times. As shown in figure 5.39., after 4 hours of thickening similar amounts of phosphorus had been released for all influent COD values. After 72 hours though, as described in section 5.4.3.2., increasing amounts of influent COD resulted in decreasing amounts of phosphorus in the thickener supernatant.

These results would point to the conclusion that changes in the sludge do take place when the influent COD changes which were not made evident in the anaerobic zone of the SBR, or which could not be expressed in the parameters investigated i.e. sludge phosphorus removal and release rates.

Finally, for comparable P/M values, the amount of phosphorus released after 2 hours in the anaerobic zone of the SBR was approximately 20 times higher than the release observed during the thickening of excess sludge. The obvious difference between the two anaerobic conditions was the presence of exogenous substrate in the former.

5.4.4.2. Effect of influent phosphorus

The effect of influent phosphorus concentration on the amount released in the anaerobic zone was investigated as discussed in chapter 4, section 4.3.3.2, in the

range of 4 to 30 mgP/l. This increase in the influent resulted in increased anaerobic phosphate release in the anaerobic zone.

The effect of influent phosphorus concentration on phosphorus release during sludge handling was investigated for influent phosphorus values in the range of 9 - 20 mgP/l. As with the anaerobic zone results, the observed phosphorus release was higher for the sludge with the highest influent phosphorus.

A 33% increase in the influent, resulted in a 95% increase in the amount of phosphorus released after two hours of thickening per unit biomass. This effect progressively decreased as already discussed in section 5.4.3.1. A similar increase in the influent (33%) had a less dramatic effect on the amount of phosphate released in the anaerobic zone. The increase observed after two hours of anaerobic conditions in the SBR, was 13%.

5.4.4.3. Effect of reactor operation with low dissolved oxygen during the aerobic phase

As shown in chapter 4, section 4.3.3.4., operating the reactor with low D.O. levels (maximum aerobic D.O. 0.5 - 8 mg/l), over a period of 30 days, had no effect on the anaerobic phosphorus release and removal properties of the sludge, although a progressive deterioration in the ammonia removal rates was observed.

For any set of two sludges with comparable properties, the amount of phosphorus released during sludge treatment was also found to be practically identical, irrespective of the amount of dissolved oxygen in the reactor.

5.4.4.4. The behaviour of metals in EBPR

Both in the anaerobic stage of the SBR operating cycle and the sludge storage experiments, the release of phosphorus was associated with the release of cations, with potassium and magnesium showing the closest correlation to the phosphorus release profile.

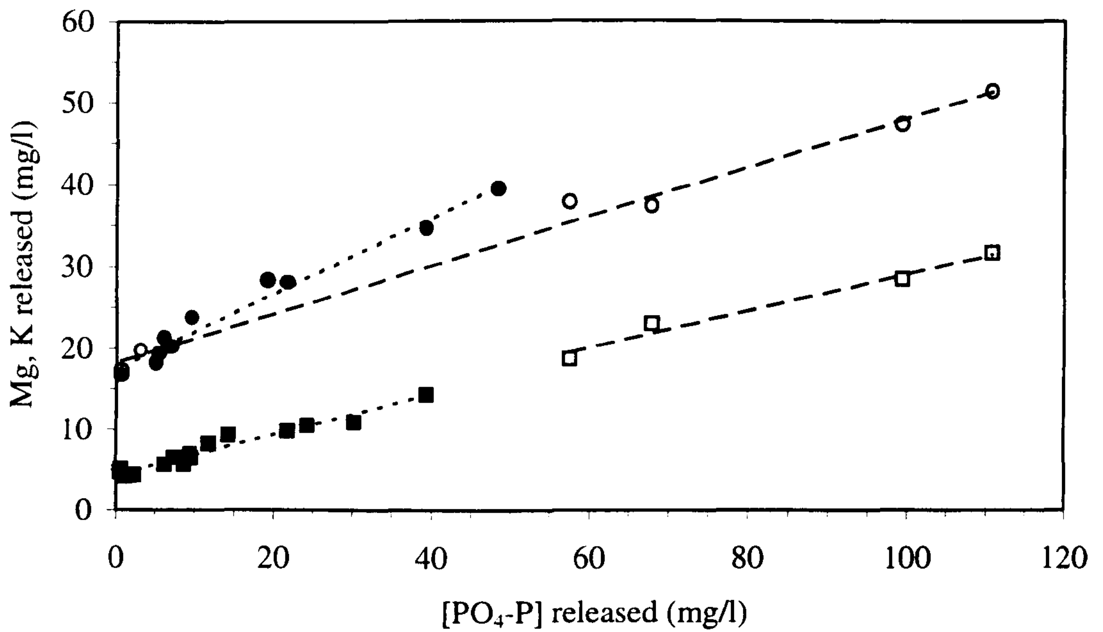


Fig 5.40. Release of cations as a function of phosphorus release in the anaerobic zone of the SBR and during thickening of excess EBPR sludge. (■) Mg^{2+}/P released during thickening, (□) Mg^{2+}/P released in the anaerobic zone of the SBR, (●) K^{+}/P released during thickening and (○) K^{+}/P released in the anaerobic zone of the SBR.

As shown in figure 5.40. the observed average molar ratio of Mg/P released, was very similar for both types of anaerobic release (0.23 $mgMg^{2+}/mgP$ in the SBR anaerobic zone and 0.25 $mgMg^{2+}/mgP$ during thickening).

The molar ratio of potassium to phosphorus released on the other hand showed a slight variation between the two with 0.46 mgK^{+}/mgP released during thickening and 0.3 mgK^{+}/mgP released in the SBR anaerobic zone.

5.5. OVERVIEW

The main objective of this chapter was to investigate the process of phosphorus resolubilisation during the treatment of excess EBPR sludge.

For the investigation of the primary objective, three targets were set. The first was to determine the different phases describing phosphorus release during short term (0-24 hours) and long term (2-7 days) batch storage and gravity thickening.

Three distinct phases were observed. Phase one was characterised by no or extremely slow phosphorus release and high denitrification rates. The presence of this initial period of extremely low phosphorus release rates is of crucial importance to operators of mechanical thickening systems such as centrifuges and flotation units which operate with short retention times.

The second phase during which intracellular phosphorus was progressively resolubilised, was characterised in this study by variable release rates in the range of 0.6 - 4.2 mgP/l.h.

The third phase was again characterised by little phosphorus release which was attributed to cell lysis. Since this phase was reached after 5 to 7 days retention in the thickener, it was considered to be of little relevance to full scale treatment facilities and was investigated no further.

A second target set was to determine any difference in the release observed between the two sludge handling methods employed, namely quiescent anaerobic storage and gravity thickening.

Although the sludge showed distinctly different settling profiles between the two methods the phosphorus release profiles, were for the first 2 days almost identical. After this time period the sludge in the thickener always exhibited higher release rates. The maximum amount of releasable phosphorus was also always 25 to 40% higher for the thickened sludge. Variations in the phosphorus release profiles were also observed between the thickening, storage and completely stirred experiments performed.

These observations indicate that when comparing data between different studies, it is important to take into account the method the authors used to simulate the excess sludge treatment procedure.

The third and final target was to determine any difference in the release patterns observed between sludges that had been derived from runs with different operational parameters. The ultimate goal was to determine weather a set of operational parameters exists, which produces excess sludge with minimal release during the handling of EBPR sludges.

The first parameter to be tested was the influent phosphorus concentration. Its effect on phosphorus release during sludge handling was investigated for influent phosphorus values in the range of 9 - 20 mgP/l. The results obtained indicated that influent phosphorus concentrations have a short term effect on phosphorus release during sludge treatment and that higher release can be expected from sludges with higher influent phosphorus loads.

Following the influent phosphorus concentration, the effect of the influent COD on phosphorus release during sludge treatment was investigated for sludges with comparable sludge phosphorus and oxidised nitrogen contents. For the range of influent COD values investigated (300 to 740 mgCOD/l), it was found that increasing the influent COD, decreased the phosphorus released during sludge treatment. Unlike the effect of influent phosphorus, the influent COD values appear to have increasing effect with increasing retention time in the thickener.

The effect of oxidised nitrogen was also investigated and the results obtained in this study verified the findings of other researchers, who have observed an inverse

relationship between the rate of phosphorus released during batch thickening and the initial oxidised nitrogen concentration of the excess sludge (Krois and Negm, 1994).

The amount of phosphorus released from the sludge was found to be directly related to the sludge phosphorus content for long term thickening and storage. For the TP range investigated in this study (60 - 270 mgP/gVSS), the higher the total phosphorus of the sludge the more phosphorus was released during thickening. On the other hand, when the phosphorus released was expressed as a percentage of the total phosphorus sludge content, a smaller percentage of the total phosphorus leached in the supernatant from the sludges with the highest TP.

Wasting the excess sludge from the aeration tank as opposed to wasting from the final sedimentation tank, resulted in lower release rates.

Finally, for sludges with comparable properties, the amount of phosphorus released was identical, irrespective of the amount of dissolved oxygen in the reactor.

In an attempt to investigate a link between the parameters found to influence the phosphorus release in the anaerobic zone of a EBPR treatment plant with those affecting the release observed during excess sludge treatment, the effect that similar parameters had in the two situations was compared.

For a comparable P/M range (3.5 - 4.5 mgP/gVSS), operating the SBR with varying influent COD values, affected the amount of phosphorus released during the sludge treatment experiments and resulted in changes in the sludge which were not made evident in the anaerobic zone of the SBR.

The effect of influent phosphorus concentration on phosphorus release was similar in the anaerobic zone and during sludge treatment. The observed phosphorus release was higher for the sludge with the highest influent phosphorus but a similar increase in the influent phosphorus, resulted a less dramatic increase in the amount of phosphate released in the anaerobic zone.

CHAPTER 6. CONCLUSIONS AND FUTURE WORK

6.1. MAJOR CONCLUSIONS

The research conducted in this study, resulted in a number of significant findings which are summarised in the following paragraphs.

1. The addition of a pure culture of *Acinetobacter lwoffi* to an activated sludge without phosphorus removal capabilities, resulted in the development of EBPR in 14 days. This method of EBPR development, produced the shortest published start-up time.
2. When the VFA/P ratio, as opposed to the more general COD/P ratio, is used to describe the influent characteristics of a treatment plant, values as low as 15 mgCOD/mgP result in high phosphorus removal. The much higher quoted values in the literature, using the COD/P ratio are therefore not representative of the true potential for EBPR of the particular wastewater.
3. During anaerobic storage and batch gravity thickening of the bioaugmented sludge, the presence of an initial phase characterised by no or extremely slow phosphorus release, with a maximum duration of 20 hours, was observed (at a temperature of 20 °C). The presence of this initial period of extremely low phosphorus release rates, is of crucial importance to operators of mechanical thickening operating with short retention times.
4. For retention times in sludge treatment units in the range of 1 to 48 hours the following parameters were found to decrease the amount of phosphorus resolubilisation:

- Decreasing influent phosphorus concentration at the head of the works
- Increasing oxidised nitrogen content of the excess sludge
- Wasting the excess sludge from the aeration tank as opposed to the final sedimentation tank

5. For retention times in sludge treatment units longer than 48 hours the following parameters were found to decrease the amount of phosphorus released:
   - Increasing influent COD
   - Lower total phosphorus sludge content
   - Higher sludge "stabilisation" rates as defined by the amount of time the sludge has spent in the reactor
   - Quiescent conditions of storage as opposed to slow stirring as practised in gravity thickening.

6.2. OTHER CONCLUSIONS

A number of other conclusions were also reached regarding both the EBPR process and the treatment of EBPR sludge:

For SRT_E in the range of 5 - 13 days, EBPR functioned independently of the sludge retention time.

For the sludge produced in this study the a P/M ratio of 5.5 mgP/gVSS, seemed to be the threshold value above which the influent COD/P ratio was crucial to EBPR. Above this value increasing the influent COD increased the phosphorus removal in the SBR.

For P/M values below 5.5 mgP/gVSS, increasing the influent COD or the influent phosphorus concentration had no effect on the phosphorus removal efficiency of the reactor. Increasing the influent phosphorus did result in increased phosphate release in the anaerobic zone of the SBR.

Operating the reactor with low D.O. levels, over a period of 30 days, had no effect on the phosphorus removal properties of the sludge and on the amount of

phosphorus released in the anaerobic zone and during sludge treatment. This indicated that when full nitrification is not necessary, an EBPR treatment plant can operate satisfactorily with minimal aeration requirements.

6.3. RECOMMENDATIONS FOR FUTURE WORK

The results collected in this study, point to certain routes of investigation which would further explore the possibility of reducing the amount of phosphorus resolubilised during anaerobic treatment of excess EBPR sludge.

As research in the phosphorus resolubilisation area during sludge treatment has just started, the first objective to be completed should be an investigation into the different phases of phosphorus release, as defined in this study, for various types of sludges from full and lab scale treatment plants. A mathematical description of the release observed, including constants describing the effect of influent phosphorus, COD etc. would result in a predictive tool for plant operators.

A promising area of research appears to be the investigation of the effect of the extent of "stabilisation" of EBPR sludge and the implication this has on the LPP and HPP content of the biomass. Determination of the sludge phosphorus fractions during progressive sludge treatment would indicate the location of the resolubilised phosphorus and point to the less mobile fraction. This knowledge could be exploited in conjunction with the knowledge of the operational parameters which result in maximising this particular fraction to result in the production of sludges with low mobile phosphorus.

Finally, the investigation of other operational parameters such as pH, anaerobic retention time and varying sludge cation content, which were not investigated in this study due to lack of time should be pursued and their effect on phosphorus release during sludge treatment determined.

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APPENDICES

APPENDIX I. REACTOR OPERATION AND AVERAGE INFLUENT AND OPERATIONAL VALUES OF RUNS INCLUDED IN THIS STUDY

Key for run definitions used in the appendices:

- A, B: reactor type used
- 1,2,....: arabic numbers indicate reactor start-up with new, unacclimatised batch of sludge
- I, II, : capital latin numbers indicate reactor operating strategy as defined in chapter 3
- i, ii,....: small latin numbers indicate reactor start-up with EBPR sludge inoculation
- a: the small letter (a) after an arabic number indicates a start-up phase of run were *A.lwoffi* bioaugmentation took place and (b) steady state following start-up
- Phase 1,2.....: runs were divided into phases according to the operational parameters employed

TABLE I.1. REACTOR OPERATION DURING THE EXPERIMENTAL RUNS INCLUDED IN THIS STUDY

Run	Descriptioı	Op. Str.	Phase	COD (mg/l)	[PO_4-P] (mg/l)	[NH_4-N] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	ESS (mg/l)	EVSS (mg/l)	SRT (d)	SRT_E (d)
A 1 a	Control	I	start-up	600	14	34	1734	1128	45	45	21	9
A 1 i		I	1	500	12	43	3400	2000	45	45	21	9
A 1 i		I	2	400	5	36	3340	2338	45	45	25	10
A 1 i		I	3	330	5	25	3200	2240	45	45	37	16
A 1 ii		I, III, IV	1	400	6	35	2615	2092	80	80	26	11
A 1 ii		I, III, IV	2	400	5	35	2970	2376	80	80	31	13
A 2 a	Bio/gm t=25	I	start-up	500	8	27	2283	2054	80	60	17	7
A 2 b		I	1	450	7	29	2303	2100	40	40	21	9
A 2 b		I	2	450	11	27	2462	2020	37	37	22	9
A 3 a	Bio/gm t=5	I	start-up	450	15	29	3120	2660	76	53	24	8
A 3 b		I		450	15	52	3827	2688	57	57	19	6
A 3 i	EBPR in.	I	1	450	18	56	3155	2425	30	30	24	8
A 3 i		I	2	450	17.5	56	2621	2241	30	30	18	6
A 3 ii	EBPR in.	I	1	450	20	54	4418	2280	12	10	16	5
A 3 ii		I	2	450	20	54	4503	2592	12	10	33	11
A 3 iii	EBPR in.	I	1	500	22	52	3951	2394	7	7	22	7
A 3 iv	EBPR in.	I	1	500	22	55	3883	2331	8	8	25	8
A 3 iv		I	2	500	22	55	4177	2476	8	8	35	11
A 3 v	EBPR in.	I	1	450	25	54	4300	2600	12	10	24	8
A 3 v		I	2	450	30	54	3719	2224	12	10	26	9

TABLE I.1.(cont.). REACTOR OPERATION DURING THE EXPERIMENTAL RUNS INCLUDED IN THE STUDY

Run	Descriptioı	Op. Str.	Phase	COD (mg/l)	[PO_4-P] (mg/l)	[NH_4-N] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	ESS (mg/l)	EVSS (mg/l)	SRT (d)	SRT_E (d)
A 4 a	ɜioaugm t=3	I	start-up	400	20	30	2238	1907	19	19	30	10
A 4 b		I		400	20	52	2824	1985	19	19	21	9
A 4 i	EBPR in.	I, III, IV	1	450	19	52	3875	2724	19	15	40	13
A 4 i		I, III, IV	2	450	19	52	3648	2390	19	15	22	7
A 4 ii	EBPR in.	I, III, IV	1	450	19	52	3900	2600	19	12	40	13
A 4 iii	EBPR in.	I, III, IV	1	460	19	52	3688	2506	12	8	19	8
A 4 iv	EBPR in.	I, III, IV	1	470	17	52	3485	2474	16	11	24	10
A 4 v	EBPR in.	I, III, IV	1	470	18	52	3500	2350	10	9	23	10
A 4 vi	EBPR in.	I, II, IV	1	470	18	52	4020	2444	10	10	23	10
A 5 a	ɜioaugm t=2	I	start-up	500	8	30	2689	2380	80	80	17	8
A 5 b		I	1	400	6	25	2705	2380	80	80	18	8
A 5 b		I	2	550	7	29	2334	2086	80	80	13	5
A 5 i	EBPR in.	I	1	470	11	27	3357	2468	25	25	26	11
B 1 a	ɜioaugm. t=(	I, II	start-up	550	14	31	1713	1541	22	18	41	9
B 2	Bioaugm t=(	I	1	550	7	30	2249	2060	80	60	21	9
B 4	Bioaugm t=(	I	1	400	19	30	2350	1952	20	19	24	10
B 5	Bioaugm t=(	I	1	500	8	30	2700	2426	80	40	21	9
B 6	EBPR in.	I	1	460	19	52	2773	1942	19	13	20	8
B 6		I	2	460	19	52	2773	1942	10	10	21	9
B 6 i	EBPR in.	I	1	460	19	52	2391	1990	8	6	27	11

APPENDIX II. REACTOR INFLUENT AND OPERATIONAL VALUES OF EXPERIMENTS USED IN CHAPTER 4

TABLE II.1. INFLUENT VALUES AND SOLIDS CONCENTRATION OF THE RUNS USED FOR THE DETERMINATION OF THE OPTIMUM START-UP METHOD

RUN	COD (mg/l)	[PO_4-P] (mg/l)	[NH_4-N] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	ESS (mg/l)
Control A1a	600	14	34	1734	1128	45
Bioaugmentation B1a	550	14	30	1713	1541	22
EBPR inoculum B6	460	19	50	2773	1942	19

TABLE II.2. INFLUENT VALUES AND SOLIDS CONCENTRATION OF THE RUNS USED FOR THE EFFECT OF THE L VALUE DURING START-UP.

Bioaugmentation at t=0						
RUN	COD (mg/l)	[PO_4-P] (mg/l)	[NH_4-N] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	ESS (mg/l)
B 1	550	14	30	1713	1541	22
B 4	400	19	30	2350	1952	20
B 5	500	8	30	2700	2426	80
B 2	550	7	30	2249	2060	80

TABLE II.3. INFLUENT VALUES AND SOLIDS CONCENTRATION OF THE RUNS USED FOR THE DETERMINATION OF THE EFFECT OF THE TIME OF ADDITION OF THE PURE CULTURE DURING START-UP.

BIOAUG/TION (days)	**RUN**	**COD** (mg/l)	**[PO_4-P]** (mg/l)	**[NH_4-N]** (mg/l)	**MLSS** (mg/l)	**MLVSS** (mg/l)	**ESS** (mg/l)
t = 0	B 1a	550	14	30	1713	1541	22
t = 25	A 5a	500	8	30	2689	2380	80
t = 30	A 4a	400	20	30	3238	1907	19

TABLE II.4. EFFECT OF SLUDGE WASTING RATE ON REACTOR $MCRT_E$

RUN	**STR_E** (d)	**Waste** (mg/d)
A4i - Phase 1	13.2	339
A4ii - Phase 1	13.0	324
B6i - Phase 1	11.2	372
A3ii - Phase 3	10.9	392
A3v - Phase 3	8.7	423
B6b - Phase 1	8.7	468
A3iv - Phase 2	8.2	467
A3v - Phase 1	7.9	544
A3i - Phase 1	7.8	513
A3iii - Phase 1	7.4	537
A4i - Phase 2	7.3	540
A3i - Phase 2	6	619
A3ii - Phase 2	5.3	715

TABLE II.5. EFFECT OF SLUDGE WASTING RATE ON REACTOR VSS CONCENTRATION

RUN	STR_E (d)	VSS (mg/l)
A 3i - Phase 1	7.8	2425
A 3i - Phase 2	6	2241
A 4i - Phase 2	7.3	2390
A 3ii - Phase 2	10.9	2592
A 3ii - Phase 1	5.3	2280
A 3iii - Phase 1	7.4	2394
A 4i - Phase 1	13.2	2724

TABLE II.6. EFFECT OF $MCRT_E$ ON PHOSPHORUS REMOVAL FOR RUNS WITH CONSTANT P/M AND L

P/M=0.65 mgP/gVSS - L=0.24 mgCOD/gVSS·d					
Run	**SRT** (d)	**SRT_E** (d)	**MLSS** (mg/l)	**VSS** (mg/l)	**Prem** (mgP/gVSS)
A 1i - Phase 2	25	10.5	3340	2338	0.51
A 1ii - Phase 1	31	13.0	2970	2376	0.59
A 1i - Phase 3	37	15.6	3200	2240	0.60
P/M=3.0 mgP/gVSS - L=0.4 mgCOD/gVSS·d					
A 4i - Phase 1	40	13.2	3648	2724	3.16
A 4ii - Phase 1	40	13.0	3900	2600	2.92
A 3ii - Phase 2	33	10.9	4503	2592	3.01
A 4iv - Phase 1	24	10.0	3485	2474	3.14
A 4vi - Phase 1	23	9.6	4020	2444	3.28
A 4iii - Phase 1	19	8.0	3688	2506	3.23
A 4i - Phase 2	22	7.3	3648	2390	3.50
A 3i - Phase 2	18	6	2621	2241	3.01

TABLE II.7. EFFECT OF $MCRT_E$ ON ANAEROBIC PHOSPHORUS RELEASE FOR RUNS WITH CONSTANT P/M AND L

P/M=4.0 mgP/gVSS - L=0.55 mgCOD/gVSS·d					
Run	**SRT** (d)	**SRT_E** (d)	**MLSS** (mg/l)	**VSS** (mg/l)	**P released** (mgP/gVSS)
A3ii - Phase 1	16	5.3	4418	2280	45.46
A3iii - Phase 1	22	7.4	3951	2394	47.19
A3iv - Phase 1	25	8.2	3883	2331	43.88
A4iv - Phase 1	24	10	3485	2474	44.13

TABLE II.8. EFFECT OF INCREASING INFLUENT COD ON PHOSPHORUS REMOVAL FOR RUNS WITH CONSTANT P/M

RUN	DAY	COD (mg/l)	L (gCOD/gVS.d)	COD/P (gCOD/gP)	VSS (mg/l)	P rem (mgP/gVS)
P/M = 3.2 (mgP/gVSS)						
A 4iv - Phase 1	18	579	0.52	34	2372	3.14
A 4iv - Phase 1	19	526	0.45	30	2474	3.19
A 4vi - Phase 1	11	470	0.41	27	2444	3.12
A 3i - Phase 1	4	450	0.51	26	2425	3.15
A 4i - Phase 1	6	432	0.43	22	2724	3.16
A 4vi - Phase 1	22	421	0.37	23	2444	3.28
A 4i - Phase 2	9	387	0.43	22	2412	3.19
A 4i - Phase 1	2	288	0.29	14	2724	3.26
P/M = 4.5 (mgP/gVSS)						
B 6i - Phase 1	40	736	0.94	44	2100	4.46
B 6i - Phase 1	38	579	0.74	27	2110	4.50
B 6i - Phase 1	39	526	0.66	23	2139	4.67
A 3ii - Phase 3	47	500	0.52	22	2620	4.32
A 3iv - Phase 1	7	500	0.58	34	2331	4.20
A 4vi - Phase 1	7	470	0.41	14	2444	4.41
A 3iv - Phase 1	1	460	0.54	22	2314	4.25
A 3iv - Phase 1	11	459	0.50	30	2498	4.32
B 6i - Phase 1	36	421	0.57	26	1990	4.61
P/M = 6.0 (mgP/gVSS)						
A 3v - Phase 3	45	150	0.18	5	2224	3.23
A 3v - Phase 3	49	250	0.31	8	2224	4.12
A 3v - Phase 3	44	438	0.53	14	2263	5.35
B 6 - Phase 1	5	460	0.64	24	1942	5.52
B 6 - Phase 1	10	494	0.68	25	1942	5.76

TABLE II.9. EFFECT OF INCREASING INFLUENT COD ON ANAEROBIC PHOSPHORUS RELEASE FOR RUNS WITH CONSTANT P/M.

P/M = 0.6 (mgP/gVSS)						
RUN	**DAY**	**COD** (mg/l)	**L** (gCOD/gVS.d)	**COD/P** (gCOD/gP)	**VSS** (mg/l)	**P released** (mgP/gVS)
A 1ii - Phase 2	9	400	0.25	89	2375	19.62
A 1ii - Phase 2	11	350	0.24	87	2193	19.24
A 1i - Phase 3	33	330	0.21	73	2240	19.41
P/M = 4.0 (mgP/gVSS)						
A 3ii - Phase 2	42	750	0.9	36.6	2280	45.46
A 3iv - Phase 1	11	459	0.59	19.13	2498	43.88
A 3iii - Phase 2	10	494	0.57	21.3	2402	47.20
A 4i - Phase 2	12	390	0.44	20.82	2390	42.90
A 4iv - Phase 1	18	579	0.52	34.4	2372	44.14

TABLE II.10. EFFECT OF INCREASING INFLUENT P ON PHOSPHORUS REMOVAL FOR RUNS WITH CONSTANT L.

RUN	DAY	P (mg/l)	COD/P (mgCOd/gP)	P/M (mgP/gVSS)	P removed (mgP/gVSS)
L = 0.20 (mgCOD/gVSS.d)					
A 1i - Phase 3	32	3.9	77	0.52	0.45
A 1i - Phase 2	29	4.2	77	0.54	0.51
A 1i - Phase 2	25	4.6	59	0.59	0.59
A 1i - Phase 3	33	4.5	73	0.60	0.60
A 1i - Phase 2	23	5.0	60	0.64	0.48
A 1i - Phase 2	28	5.5	49	0.70	0.64
A 1ii - Phase 2	12	4.8	80	0.62	0.62
A 1ii - Phase 2	9	4.5	89	0.58	0.59
A 1ii - Phase 2	11	3.9	102	0.51	0.51
A 1ii - Phase 1	2	3.6	96	0.55	0.55
3 Av - Phase 3	45	32.0	5	6.48	3.24
L = 0.30 (mgCOD/gVSS.d)					
A 1ii - Phase 1	4	5.3	71	0.75	0.75
A 1ii - Phase 1	5	6.7	67	0.95	0.95
A 1ii - Phase 1	8	5.1	82	0.72	0.72
A 4i - Phase 1	2	20.0	14	3.25	3.25
A 4i - Phase 1	3	17.8	18	2.92	2.92
A 5i - Phase 1	176	13.1	32	2.21	2.21
A 2b - Phase 1	124	9.0	50	1.28	1.24
A 1i - Phase 1	7	11.2	36	1.67	1.67
A 1i - Phase 1	8	7.1	56	1.06	1.06

TABLE II.10. (cont.) EFFECT OF INCREASING INFLUENT P ON PHOSPHORUS REMOVAL FOR RUNS WITH CONSTANT L.

L = 0.40 (mgCOD/gVSS.d)					
RUN	**DAY**	**P** (mg/l)	**COD/P** (mgCOd/gP)	**P/M** (mgP/gVSS)	**P removed** (mgP/gVSS)
A 5b - Phase 2	12	6.6	68	1.25	1.16
A 2b - Phase 1	121	7.5	60	1.57	1.51
A 5i - Phase 1	130	8.8	54	1.48	1.48
A 5i - Phase 1	132	9.5	49	1.60	1.60
A 5i - Phase 1	124	10.4	45	1.66	1.59
A 5i - Phase 1	177	11.8	39	1.99	1.99
A 2b - Phase 1	117	13.3	30	2.94	2.54
A 5i - Phase 1	117	14.2	40	2.38	2.31
A 4iv - Phase 1	17	14.6	32	2.60	2.51
A 4iv - Phase 1	16	16.3	26	2.85	2.82
A 4iii - Phase 1	7	16.5	28	2.99	2.93
A 4i - Phase 2	9	17.2	22	3.19	3.19
A 4vi - Phase 1	11	17.3	27	3.16	3.12
A 4vi - Phase 1	22	18.0	23	3.28	3.28
A 4i - Phase 2	11	18.0	19	3.36	3.36
A 4i - Phase 2	10	18.3	21	3.45	3.37
A 4i - Phase 1	1	18.9	21	3.09	3.09
A 4iii - Phase 1	4	19.3	24	3.30	3.23
A 4i - Phase 1	6	19.3	22	3.16	3.16
A 4iii - Phase 1	5	19.8	25	3.53	2.86
A 5i - Phase 1	189	19.8	27	3.33	3.33
A 3ii - Phase 1	41	20.5	37	3.40	3.36
A 4i - Phase 2	13	20.6	19	3.85	3.85
A 4vi - Phase 1	7	24.7	19	4.51	4.41
A 3ii - Phase 2	48	28.7	15	4.86	4.82

TABLE II.10. (cont.) EFFECT OF INCREASING INFLUENT P ON PHOSPHORUS REMOVAL FOR RUNS WITH CONSTANT L.

L = 0.50 (mgCOD/gVSS.d)					
RUN	**DAY**	**P** (mg/l)	**COD/P** (mgCOd/gP)	**P/M** (mgP/gVSS)	**P removed** (mgP/gVSS)
A 5b - Phase 2	13	3.4	162	0.69	0.69
5A b - Phase 2	16	7.0	79	1.43	1.43
A 2b - Phase 1	132	9.5	47	2.10	2.02
A 5i - Phase 1	115	9.6	57	1.75	1.67
A 3i - Phase 1	7	11.0	41	2.04	2.04
A 2b - Phase 1	135	11.9	38	2.51	2.35
A 1i - Phase 1	3	13.12	31	3.43	2.04
A 4iv - Phase 1	18	16.8	34	3.17	3.14
A 3i - Phase 1	3	17.0	26	3.15	3.06
A 4iv - Phase 1	19	17.7	30	3.19	3.19
A 3ii - Phase 3	50	18.5	27.	3.13	3.01
A 3i - Phase 1	8	19.0	24	3.53	3.53
A 3iv - Phase 1	1	22.2	21	4.31	4.25
A 3iii - Phase 2	10	23.2	21	4.35	4.35
A 3iv - Phase 1	11	24.0	19	4.32	4.32
A 3ii - Phase 2	47	25.6	20	4.39	4.32
A 3v - Phase 3	41	27.4	16	5.41	3.73
A 3v - Phase 1	1	27.8	16	4.80	4.75
A 3v - Phase 3	42	28.0	15	5.86	3.36
A 3v - Phase 3	50	31.0	14	6.23	4.44
A 3v - Phase 3	49	31.0	14	6.16	5.35

TABLE II.10. (cont.) EFFECT OF INCREASING INFLUENT P ON PHOSPHORUS REMOVAL FOR RUNS WITH CONSTANT L.

L = 0.60 (mgCOD/gVSS.d)					
RUN	**DAY**	**P** (mg/l)	**COD/P** (mgCOd/gP)	**P/M** (mgP/gVSS)	**P removed** (mgP/gVSS)
A 5b - Phase 2	17	6.9	87	1.48	1.48
A 5b - Phase 2	19	8.6	64	1.88	1.94
A 3iii - Phase 2	8	22.0	23	4.14	4.13
A 3iii - Phase 2	7	22.2	23	4.28	4.24
A 3iv - Phase 1	7	22.2	23	4.28	4.20
A 3v - Phase 3	48	33.2	14	6.74	3.49
L = 0.70 (mgCOD/gVSS.d)					
A5b - Phase 2	18	7.8	87	1.70	1.76
A 3ii - Phase 3	49	22.5	27	4.15	4.11
L = 0.90 (mgCOD/gVSS.d)					
A 3ii - Phase 3	53	31.7	19	7.36	7.23
A 3i - Phase 1	14	15.0	25	3.01	3.01
A 3ii - Phase 2	42	20.5	37	4.05	4.00

TABLE II.11. EFFECT OF INCREASING INFLUENT P ON PHOSPHORUS RELEASE FOR RUNS WITH CONSTANT L.

RUN	DAY	P (mg/l)	COD/P (mgCOD/gP)	P/M (mgP/gVS)	P released (mgP/gVS)	P uptaken (mgP/gVS)
			L = 0.20 (mgCOD/gVSS.d)			
A 1i - Phase 3	33	4.5	73.33	0.60	19.41	25.03
A 1ii - Phase 2	11	4.0	87.50	0.56	19.24	19.80
			L = 0.30 (mgCOD/gVSS.d)			
A 1ii - Phase 2	9	4.5	88.89	0.58	19.62	20.21
A 1ii - Phase 1	2	3.6	95.77	0.55	12.13	12.67
A 1ii - Phase 1	4	5.3	70.51	0.75	20.89	21.64
A 1ii - Phase 1	5	6.7	67.46	0.95	28.44	29.39
			L = 0.40 (mgCOD/gVSS.d)			
A 4i - Phase 2	12	18.7	20.82	3.50	42.90	46.31
			L = 0.50 (mgCOD/gVSS.d)			
A 3iv - Phase 1	11	24.0	19.13	4.32	43.88	-
A 4iv - Phase 1	18	16.8	34.36	3.17	44.14	47.42
A 3v - Phase 3	49	31.0	14.10	6.16	52.10	60.36
			L = 0.60 (mgCOD/gVSS.d)			
A 3iii - Phase 2	10	23.2	21.27	4.35	47.19	50.67
A 5b - Phase 2	18	7.8	86.54	1.70	24.65	26.12
			L = 0.90 (mgCOD/gVSS.d)			
A 3ii - Phase 2	42	20.5	36.59	4.05	45.46	49.35
A 3ii - Phase 3	50	18.5	27.1	3.13	35.39	38.30

APPENDIX III. REACTOR INFLUENT AND OPERATIONAL VALUES OF EXPERIMENTS USED IN CHAPTER 5

TABLE III.1. INFLUENT VALUES AND OPERATIONAL PARAMETERS OF THE RUNS EMPLOYED FOR THE DETERMINATION OF THE EFFECT OF INFLUENT PHOSPHORUS ON PHOSPHORUS RELEASE DURING SLUDGE STORAGE AND THICKENING

Experiment No	Run	Waste Line	COD (mg/l)	[PO_4-P] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	COD/P	L (mgCOD/gVSS.d)	TP (mgP/gVSS)	No (mgN/gVSS)
Thick/Sto 1	2b-Phase 2	FST	450	9	5630	4617	50	0.47	27.8	1.24
Thick/Sto 2	2b-Phase 2	FST	450	12	5274	4325	38	0.47	28.7	1.5

TABLE III.2. INFLUENT VALUES AND OPERATIONAL PARAMETERS OF THE RUNS EMPLOYED FOR THE DETERMINATION OF THE EFFECT OF INFLUENT COD ON PHOSPHORUS RELEASE DURING SLUDGE THICKENING

Experiment No	Run	Waste Line	COD (mg/l)	[PO_4-P] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	COD/P	L (mgCOD/gVSS.d)	TP (mgP/gVSS)	No (mgN/gVSS)
Thick 3	5i	FST	530	19.8	10562	7116	27	0.41	79.1	0.03
Thick 13	4b	FST	300	17	3959	2821	18	0.43	92.3	0.78
Thick 16	6i	FST	450	18	4773	3361	25	0.58	86.82	0.55
Thick 15	4iv	FST	736	17	4849	3393	43	0.66	81.7	2.19

TABLE III.3. INFLUENT VALUES AND OPERATIONAL PARAMETERS OF THE RUNS EMPLOYED FOR THE DETERMINATION OF THE EFFECT OF SLUDGE OXIDISED NITROGEN CONTENT ON PHOSPHORUS RELEASE DURING SLUDGE THICKENING

Experiment No	Run	Waste Line	No (mgN/l)	COD (mg/l)	[PO_4-P] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	COD/P	L (mgCOD/gVSS.d)	TP (mgP/gVSS)
Thick 12	4a	FST	0.45	480	19	4023	3236	25	0.45	56.9
Thick 17	4vi	FST	14.1	420	18	10792	7214	23	0.41	61.22

TABLE III.4. INFLUENT VALUES AND OPERATIONAL PARAMETERS OF THE RUNS EMPLOYED FOR THE DETERMINATION OF THE EFFECT OF TOTAL PHOSPHORUS SLUDGE CONTENT ON PHOSPHORUS RELEASE DURING SLUDGE THICKENING

Experiment No	Run	Waste Line	TP (mgP/gVSS)	COD (mg/l)	[PO_4-P] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	COD/P	L (mgCOD/gVSS.d)	No (mgN/gVSS)
Thick 4	3a	FST	270	445	12	3708	2364	30	0.46	0.33
Thick 5	3b	FST	118.8	420	17	4154	3128	30	0.46	0.03
Thick 7	3ii-2	FST	204	600	22	4552	2533	25	0.53	0.5
Thick 8	3iii-1	FST	140	460	22	3810	2314	23	0.57	10.09
Thick 12	4a	FST	56.9	480	19	4023	3236	20	0.45	0.14

TABLE III.5. INFLUENT VALUES AND OPERATIONAL PARAMETERS OF THE RUNS EMPLOYED FOR THE DETERMINATION OF THE EFFECT OF THE SLUDGE WASTE LINE ON PHOSPHORUS RELEASE DURING SLUDGE THICKENING

Experiment No	Run	Waste Line	COD (mg/l)	[PO_4-P] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	COD/P	L (mgCOD/gVSS.d)	TP (mgP/gVSS)	No (mgN/gVSS)
Thick 14	4i-2	FST	387	19	5043	3312	20	0.45	113.5	4.91
Thick 6	3i-1	AET	430	19	3146	2451	23	0.48	105.4	9.53

TABLE III.6. INFLUENT VALUES AND OPERATIONAL PARAMETERS OF THE RUNS EMPLOYED FOR THE DETERMINATION OF THE EFFECT OF REACTOR D.O. ON PHOSPHORUS RELEASE DURING SLUDGE THICKENING

Experiment No	Run	Waste Line	COD (mg/l)	[PO_4-P] (mg/l)	MLSS (mg/l)	MLVSS (mg/l)	COD/P	L (mgCOD/gVSS.d)	TP (mgP/gVSS)	No (mgN/gVSS)
Thick 9	3iv-2	AET LOW	533	23	4191	2625	23	0.55	135.4	2.25
Thick 10	3iv-3	AET	670	27.8	4300	2600	23	0.57	149	3.15