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6

Enhanced biological phosphorus removal

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6.1 INTRODUCTION

Phosphorus is the key element that needs to be removed from aquatic environments in order to limit the growth of aquatic plants and algae, and thus, to control eutrophication. Unlike nitrogen, which can be fixed from the atmosphere and which contains approximately 80% nitrogen gas, phosphorus can only come from upstream of aquatic systems (neglecting atmospheric deposition). Diffuse sources of phosphorus, *e.g.* from agricultural fields, are best controlled by fertilisation plans, while point sources of phosphorus, *e.g.* from wastewater treatment plants, can be removed by chemical or biological processes. Considering the benefit to aquatic environments, stricter regulations are now being applied for phosphorus removal from wastewater.

The enhanced biological phosphorus removal (EBPR) phenomenon, insofar as it pertains to P removal in activated sludge systems, was noted first in the late 1950s. In the decades since, the understanding, conceptualization and application of the phenomenon have grown from initial incidental observations into well-structured biochemical and mathematical descriptions that are applied in the design and control of major full-scale works. However, the impetus for these developments did not stem from a pure scientific interest, but almost wholly from the recognition, albeit slowly, in the 1960s of the pivotal role that P plays in eutrophication of aquatic environments. This recognition, together with the massive increase in phosphorus loads on the aquatic environment since 1950, gave rise to an urgent need to develop effective countermeasures to limit the discharge of P. One such countermeasure is EBPR.

The expression enhanced biological phosphorus removal (EBPR) is used in this chapter to describe what is also referred to in the literature as biological enhanced phosphorus removal or biological excess phosphorus removal (BEPR), or sometimes biological phosphorus removal (BPR), where a wastewater treatment biomass removes phosphorus beyond its anabolic requirements by accumulating intracellular polyphosphate reserves. In addition to P removal for cell synthesis, further phosphorus (P) removal may also take place by chemical

precipitation either with chemicals present in the wastewater or added to the treatment system.

Achieving low concentrations of total phosphorus in effluents can be achieved by combining various processes as indicated in Table 6.1. For example, two combinations of processes can be used to reach 0.5 mgP/l, EBPR with sand filtration, without (combination D) or with chemical coagulation (combination E). Biological phosphorus removal combined with a limited supply of chemicals can achieve effluent values below 0.1 mgP/l, with coagulation and filtration being mainly used to remove the phosphate bound in the effluent suspended solids.

In this chapter, the intention is to present the mechanisms of biological P removal, to trace the development of practical systems for biological P removal, and to set out guidelines for the design of biological P removal systems. To facilitate the development of design guidelines for this textbook, the concepts are presented for strictly aerobic phosphorus-accumulating organisms (aerobic PAOs) which can use only oxygen as the electron acceptor for energy production. Considering that some denitrifying PAOs (DPAOs) exist and may have a significant impact on the performance of the process, their influence is discussed where appropriate.

Considering the potential benefits of removing phosphorus biologically rather than chemically, along with organic matter and nitrogen, EBPR has stimulated much interest in the study of biochemical mechanisms, the microbiology of the systems, the process engineering and optimization of plants, and in mathematical modelling. Reviews of the development of EBPR have been regularly published over the years (Marais *et al.,* 1983; Arvin, 1985; Wentzel *et al.,* 1991; Jenkins and Tandoi, 1991; Van Loosdrecht *et al.,* 1997; Mino *et al.,* 1998; Blackall *et al.,* 2002; Seviour *et al.,* 2003; Oehmen *et al.,* 2007; Gebremariam *et al.,* 2011; Yuan *et al.,* 2012; Zheng *et al.,* 2014; Guo *et al.,* 2019; Liu *et al.,* 2019; Nielsen *et al.,* 2019).

6.2 PRINCIPLES OF ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL (EBPR)

Enhanced biological phosphorus removal (EBPR) is the biological uptake and removal by activated sludge systems in excess of the amount that is removed by conventional, completely aerobic activated sludge systems. This is in excess of the 'normal' P requirements for growth of activated sludge. In the completely aerobic activated sludge system, the amount of P typically incorporated in the sludge mass is approximately 0.02 mgP/mgVSS (0.015 mgP/mgTSS). By the daily wastage of surplus sludge, phosphorus is thus effectively removed (Figure 6.1).

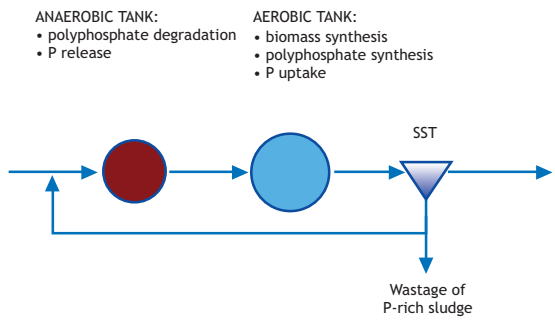


Figure 6.1 Observations of the behaviour of PAOs in an EBPR system (adapted from Tchobanoglous *et al.*, 2003).

This can give a P removal rate of approximately 15-25% in many municipal wastewaters. In an EBPR activated sludge system, the amount of P incorporated in the sludge mass is increased from the normal value of 0.02 mgP/mgVSS to values around 0.06-0.15 mgP/mgVSS (0.05-0.10 mgP/mgTSS). This is achieved by system design or operational modifications that stimulate, in addition to the 'ordinary' heterotrophic organisms present in activated sludge, the growth of organisms that can take up large quantities of P and store them internally in long chains called polyphosphates (sometimes known as poly P); generically these organisms are called phosphorus-accumulating organisms (PAOs).

Table 6.1 Combinations of processes required to achieve given effluent total phosphorus concentration for municipal effluents (adapted from Barnard and Steichen, 2007).

Treatment processes	P limit to achieve (mgP/l)						
	< 1			< 0.5	< 0.1	< 0.05	< 0.01
Combination	A	B	C	D	E	F	G
Chemical coagulation	●		●		●		
EBPR[1)]		●	●	●	●	●	●
Post-coagulation						●	●
Sand filtration				●	●	●	
Adsorption							●
Membrane filtration							●

[1)] with efficient final settling >99.9%.

PAOs (sometimes also called polyphosphate-accumulating organisms) can incorporate up to 0.38 mgP/mgVSS (0.17 mgP/mgTSS). In the biological P removal system both the ordinary heterotrophic organisms (OHOs, which do not remove P in excess) and the PAOs coexist; the larger the proportion of PAOs that can be stimulated to grow in the system, the greater the percentage phosphorus content of the activated sludge and, accordingly, the larger the amount of P that can be removed from the influent. Thus, the challenge in design is to increase the amount of the PAOs relative to the OHOs present in the activated sludge as this will increase the capacity for P accumulation and thereby the phosphorus removal efficiency. The relative proportion of the two organism groups depends, to a large degree, on the fraction of the influent wastewater biodegradable COD that each organism group obtains. The greater the proportion of influent biodegradable COD the PAOs obtain, the greater will be the fraction of PAO in the mixed liquor, the greater the %P content of the activated sludge and the greater the EBPR. This is shown graphically in Figure 6.2.

Design and operational procedures are oriented towards maximizing the growth of PAOs. In an appropriately designed EBPR system, the PAOs can make up approximately 11% of the TSS (or 15% of the VSS), and this system can usually remove approximately 10-12 mgP/l per 500 mg influent COD/l.

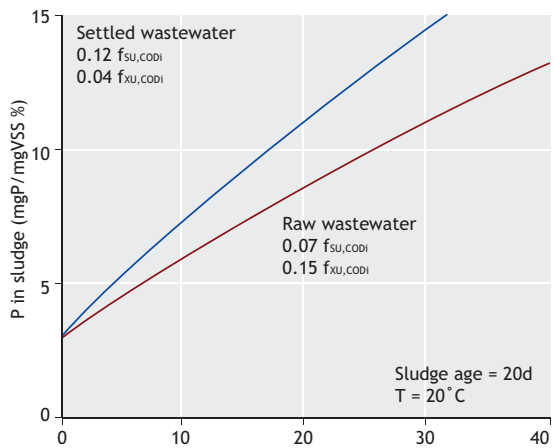


Figure 6.2 Percentage P in VSS mass *versus* the proportion of biodegradable COD mass (as %) obtained by PAOs.

However, from the first publications reporting enhanced P removal in some activated sludge systems, there has been some controversy as to whether the mechanism is a precipitation of inorganic compounds, albeit perhaps biologically mediated, or biological through formation and accumulation of P compounds in the organisms. The objective of this chapter is not to discuss the evidence that supports the biological nature of enhanced P removal, but to briefly describe the theory of biological P removal as understood by the authors and to demonstrate how this theory can be used as an aid for the design of biological P removal

activated sludge systems. This does not imply that precipitation of P due to chemical changes resulting from biological action, *e.g.* alkalinity, pH, does not take place. Such inorganic precipitation can certainly take place, but it would appear that in the treatment of municipal wastewaters by an appropriately designed activated sludge system, within the normal ranges of pH, alkalinity and calcium concentrations in the influent, enhanced P removal is principally caused by a biological mechanism (Maurer *et al.,* 1999; De Haas *et al.,* 2000).

6.3 EBPR MICROBIOLOGY

Polyphosphates can be accumulated by a wide range of bacteria. In general, they are accumulated as a phosphate reserve in relatively low amounts intracellularly. The general definition of what constitutes a PAO has changed in recent years, but is currently most frequently defined as an organism that metabolises and stores organic carbon sources under anaerobic conditions, usually with polyphosphate hydrolysis serving as the primary energy source for this process, and then taking up phosphorus and storing it as polyphosphate under aerobic or anoxic conditions.

The classical PAO definition that existed for many years principally involved the hydrolysis of polyphosphates to accumulate volatile fatty acids (VFAs) as the main carbon source that can be used by PAOs, which sequester VFAs as poly-ß-hydroxyalkanoates (PHAs) under anaerobic conditions (in the absence of an external electron acceptor such as oxygen or nitrate). The PHAs are then oxidised in the presence of an electron acceptor (*i.e.* oxygen, nitrate or nitrite) for cell growth and polyphosphate storage. This definition has since been expanded to include other PAO phenotypes, particularly those involving the anaerobic/aerobic (and/or anoxic) metabolism of polyphosphate-storing organisms based on other carbon sources, such as sugars and amino acids. These mechanisms do not necessarily imply the PHA cycling associated with VFA-driven EBPR.

Linking the microbiological identity of bacteria with their function in EBPR systems has been a challenge for many years, but in recent times it has been facilitated by the development and application of advanced microbiological methods. In the original research on EBPR microbiology conducted with cultivation studies, it was incorrectly considered that PAOs were of the genus *Acinetobacter* (Fuhs and Chen, 1975; Buchan, 1983; Wentzel *et al.,* 1986), *Microlunatus phosphovorus* (Nakamura *et al.,* 1995) or *Lampropedia* (Stante *et al.,* 1997). However, since then culture-independent methods have shown the importance of other organisms, including *Candidatus* Accumulibacter phosphatis (or *Accumulibacter*). A member of the genus *Rhodocyclus* within the *Betaproteobacteria*, *Accumulibacter* is widely regarded to be one of the most important PAOs corresponding to the classical PAO definition described previously. Techniques such as fluorescence *in-situ* hybridisation probes (FISH) combined with chemical staining to detect polyphosphate cycling have shown this organism to correspond to the PAO phenotype and to be an important PAO in full-scale EBPR processes worldwide, as well as lab-scale enrichments fed with VFA (Wagner *et al.,* 1994; Hesselmann *et al.,* 1999; Crocetti *et al.,* 2000; Martin *et al.,* 2006; Oehmen *et al.,* 2007). *Accumulibacter* is the most understood PAO in terms of its microbiological and biochemical characteristics, based on many years of study. *Accumulibacter* clades or sub-groups have also been identified (He *et al.,* 2007; Camejo *et al.,* 2016) and correlations have been attempted to relate the specific identity with observed metabolic behaviour (Flowers *et al.,* 2009; Oehmen *et al.,* 2010; Acevedo *et al.,* 2012; Camejo *et al.,* 2016, 2019; Rubio-Rincon *et al.,* 2017, 2019). It has also been suggested that other organisms, such as *Accumulimonas* and *Dechloromonas*, behave similarly to the classical PAO phenotype and are relevant in at least some EBPR plants (Stokholm-Bjerregaard *et al.,* 2017; Wu *et al.,* 2019), though further investigation is needed to determine the importance of these and other PAOs.

In recent years, another group of PAOs has been found to be of importance in EBPR systems, which belong to the *Tetrasphaera* (Stokholm-Bjerregaard *et al.,* 2017; Liu *et al.,* 2019; Nielsen *et al.,* 2019). *Tetrasphaera* PAOs have been found to be of high abundance in numerous full-scale EBPR systems, particularly under certain configurations, such as sidestream EBPR processes (see Section 6.6.11). *Tetrasphaera* are believed to metabolise mainly sugars and amino acids within EBPR anaerobic zones, though some VFA uptake has also been observed (Nguyen *et al.,* 2011). Thus far, studies have suggested that most *Tetrasphaera* are unlikely to be capable of PHA storage and degradation, quite unlike *Accumulibacter* (Kristiansen *et al.,* 2013). *Tetrasphaera* probably obtain most of their energy for anaerobic organic carbon uptake from fermentation of these carbon sources, with storage of sugars (as *e.g.* glycogen) and certain amino acids suggested to contribute to aerobic P uptake, instead of PHA oxidation (Kristiansen *et al.,* 2013; Nguyen *et al.,* 2015). While anaerobic P release is typically also observed by *Tetrasphaera*, suggesting that polyphosphate is an additional source of energy under anaerobic conditions, results have also suggested that fermentation of certain substrates can yield sufficient energy to contribute towards anaerobic P uptake (Marques *et al.,* 2017). Denitrifying P removal by *Tetrasphaera* is currently believed to be of lesser significance for most members of this group of organisms as compared to aerobic P uptake, which is also unlike previous findings for *Accumulibacter* (Marques *et al.,* 2018). Due to the uncertainty surrounding numerous mechanistic aspects related to *Tetrasphaera* PAOs, the classical PAO phenotype and characteristics, and how they impact EBPR design and operation, will encompass the main focus throughout the remainder of this chapter.

6.4 EBPR MECHANISMS

6.4.1 Background

The basic requirement for EBPR is the presence in the activated sludge system of microorganisms which can accumulate P in excess of normal metabolic requirements, in the form of polyphosphate stored in granules called volutins. In the design procedures presented in this chapter, all the organisms in the activated sludge system accumulating polyphosphate in this fashion and exhibiting the classical observed EBPR behaviour - anaerobic P release, aerobic P uptake and associated PHA production and consumption processes - are grouped together and represented by a generic PAO group. It remains unclear if or how the activity of other PAOs (such as *Tetrasphaera*) can or should be incorporated into EBPR design, thus their implications require further study.

Historically, several research groups have made a number of important contributions towards elucidating the mechanisms of enhanced biological phosphorus removal (EBPR), including Fuhs and Chen (1975), Nicholls and Osborn (1979), Rensink (1981), Marais *et al.* (1983), Comeau *et al.* (1986), Wentzel *et al.* (1986, 1991), Van Loosdrecht *et al.* (1997), Mino *et al.* (1987, 1994, 1998), Kuba *et al.* (1993), Smolders *et al.* (1994a, b, 1995), Maurer *et al.* (1997), Seviour *et al.* (2003), Martin *et al.* (2006), Oehmen *et al.* (2007), Lopez-Vazquez *et al.* (2009b), Oyserman *et al.* (2016), Fernando *et al.* (2019), Rubio-Rincon *et al.* (2019), and Nielsen *et al.* (2019). In this section, an explanation of the basic concepts underlying the more sophisticated mechanistic models for the biological P removal phenomenon is presented. For detailed description of the mechanisms, the reader is referred to the references above.

6.4.2 Prerequisites

As noted earlier, to achieve EBPR in activated sludge systems, the growth of organisms that accumulate polyphosphate (PAOs) has to be stimulated. To accomplish this, two conditions are essential, namely: (*i*) an anaerobic then aerobic (or anoxic) sequence of reactors/conditions, and (*ii*) the addition or formation of VFAs in the anaerobic reactor.

6.4.3 Observations

With the prerequisites for EBPR present, the following observations have been made at full-, pilot- and laboratory-scale (Figure 6.3).

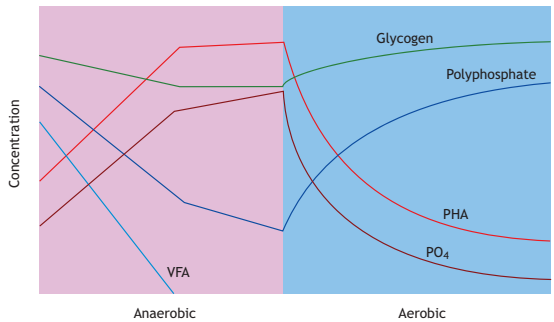


Figure 6.3 Schematic diagram showing the changes as a function of time in concentrations of volatile fatty acids (VFA), phosphate (PO_4), polyphosphate, poly-ß-hydroxyalkanoate (PHA) and glycogen through the anaerobic aerobic sequence of reactors in an EBPR system.

Under anaerobic conditions, bulk solution VFAs and intracellular polyphosphate decrease, soluble phosphate, Mg^{2+}, K^{+} and intracellular PHA increase (Rensink, 1981; Hart and Melmed, 1982; Fukase *et al.,* 1982; Watanabe *et al.,* 1984; Arvin, 1985; Hascoet *et al.,* 1985; Wentzel *et al.,* 1985, 1988; Comeau *et al.,* 1986, 1987; Murphy and Lötter, 1986; Gerber *et al.,* 1987; Satoh *et al.,* 1992; Smolders *et al.,* 1994a; Maurer *et al.,* 1997). In many cases, glycogen also decreases, though this is not a strict requirement (see Section 6.4.4).

Under aerobic conditions; intracellular polyphosphate increases; soluble phosphate, Mg^{2+}, K^{+} and intracellular PHA decrease (Fukase *et al.,* 1982; Arvin, 1985; Hascoet *et al.,* 1985; Comeau *et al.,* 1986; Murphy and Lötter, 1986; Gerber *et al.,* 1987; Wentzel *et al.,* 1988a; Satoh *et al.,* 1992; Smolders *et al.,* 1994b; Maurer *et al.,* 1997). Glycogen is also produced in most cases.

6.4.4 Biological P-removal mechanism

In describing the mechanisms of EBPR, a clear distinction is made between the PAOs and the organisms not able to accumulate polyphosphate, termed ordinary heterotrophic organisms (OHOs). In the anaerobic/aerobic sequence of reactors, it is considered that VFAs are present in the influent waste stream entering the anaerobic reactor or produced in the anaerobic reactor by fermenting bacteria.

6.4.4.1 In the anaerobic reactor

The reactions taking place in PAOs under anaerobic conditions are illustrated in a simplified biochemical model (Figure 6.4), in a biochemical model showing more explicitly the sources and uses of energy and carbon (Figure 6.5) and in a quantitative model obtained from an enriched culture grown on acetate as sole carbon source at an SRT of 8 days and grown at 20 ºC (Figure 6.6).

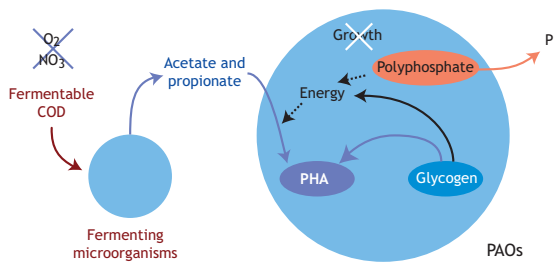


Figure 6.4 Simplified biochemical model for PAOs under anaerobic conditions. Anaerobic uptake of volatile fatty acids (VFAs), originating from the influent or from fermentation in the anaerobic reactor, and storage of polyhydroxyalkanoates (PHAs) by the PAOs take place with associated P release.

The OHOs cannot utilize the VFAs due to the absence of an external electron acceptor, oxygen or nitrate. The PAOs, however, can take up the VFAs from the bulk liquid and store them internally by linking the VFAs together to form complex long-chain carbon molecules of poly-ß-hydroxyalkanoates (PHAs). The two common PHAs are poly-

ß-hydroxybutyrate (PHB: a 4-carbon compound synthesized from two acetate molecules) and polyhydroxyvalerate (PHV: a 5-C from Ac + Prop) but poly-ß-hydromethylbutyrate (PH_2MB: 5-C from Ac + Prop) and poly-ß-hydroxymethylvalerate (PH_2MV: 6-C from two Prop) can also be present as minor constituents.

Forming PHAs from the VFAs requires energy for three functions: active transport of VFAs across the cell membrane, energisation of VFAs into coenzyme A compounds (*e.g.* acetylCoA), and reducing power (NADH) for PHA formation. Polyphosphate degradation is associated with formation of ADP from AMP, and with the phosphokinase enzyme 2 ADP are converted into ATP and AMP (Van Groenestijn *et al.,* 1987). When ATP is used orthophosphates are released and accumulate in the cell interior together with the counter-ions of polyphosphate (potassium and magnesium). The efflux of these compounds might be related to building a proton motive force, which either can help in the uptake of acetate or in the generation of a small amount of extra ATP. It is observed (Smolders *et al.* 1994a) that the energy requirements for acetate uptake increase with increasing pH. This can be associated with the fact that the energy needed for acetate transport increases with pH. ATP is used, notably, for the energisation of acetate and propionate into acetyl-CoA and propionyl-CoA. When glycogen degradation occurs, this also results in ATP formation, NADH production and intermediates that are transformed into acetyl-CoA (or propionyl-CoA). However, the ATP and NADH generated through glycogen degradation can be replaced by additional polyphosphate degradation and the activity of the TCA cycle anaerobically, respectively, as observed in certain situations (Zhou *et al.,* 2010; Majed *et al.,* 2012; Lanham *et al.,* 2013). Finally, acetyl-CoA and propionyl-CoA are stored as PHA (Comeau *et al.,* 1986; Wentzel *et al.,* 1986; Mino *et al.,* 1998; Smolders *et al.,* 1994a; Martin *et al.,* 2006; Oehmen *et al.,* 2007; Saunders, 2007).

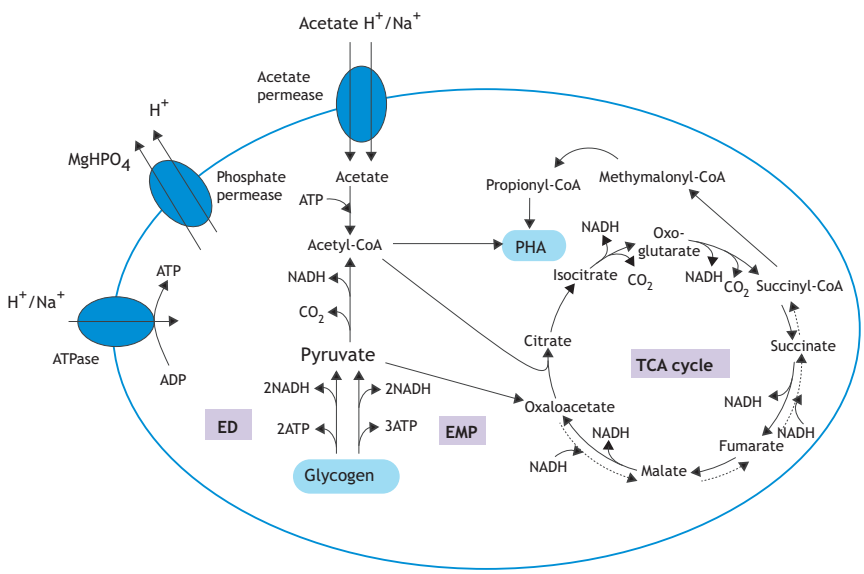


Figure 6.5 Anaerobic metabolic pathways and substrate uptake mechanisms in *Accumulibacter*. Acetate is taken up across the cell membrane, where the energy necessary for transport of acetate is generated by proton (H^+) or sodium (Na^+) efflux, which is promoted by different enzymes depending on the organism. In addition to acetate transport, the occurring ATP and NADH production (and consumption) processes are shown, including P release, glycolysis, and the oxidative and reductive TCA cycle pathways performed by certain PAOs (adapted from Oehman *et al.*, 2010).

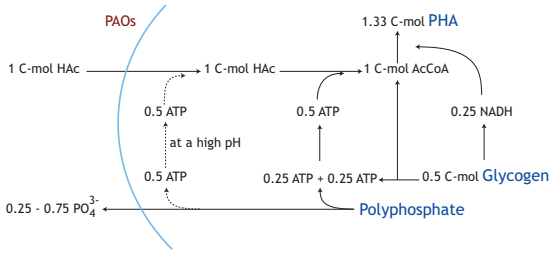


Figure 6.6 Quantitative biochemical model for PAOs under anaerobic conditions (adapted from Smolders *et al.*, 1994a). Values were obtained from an enriched culture grown on acetate as the sole carbon source at 20 °C at an SRT of 8 days.

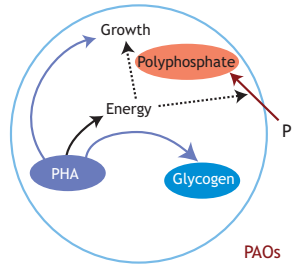


Figure 6.7 Simplified biochemical model for PAOs under aerobic (or anoxic) conditions.

Thus, the PAOs in the anaerobic reactor have taken up the VFAs for their exclusive use under conditions (anaerobic) where ordinary heterotrophic organisms are unable to use this COD. To accomplish this, some of the stored polyphosphate has been consumed and P released to the bulk solution. To stabilize the negative charges on the polyphosphate, the cations Mg^{2+}, K^{+} and sometimes Ca^{2+} are complexed. When polyphosphates are consumed and P is released, mainly Mg^{2+} and K^{+} cations are released in the approximate molar ratio $P:Mg^{2+}:K^{+}$ of 1:0.33:0.33 (Comeau *et al.,* 1987; Brdjanovic *et al.,* 1996; Pattarkine and Randall, 1999).

6.4.4.2 In the subsequent aerobic reactor

In the presence of oxygen (or of nitrate under anoxic conditions) as an external electron acceptor, the PAOs utilize the stored PHA as a carbon and energy source for energy generation, growth of new cells and typically for regenerating the glycogen consumed in the anaerobic period (figures 6.7 and 6.8).

The stored PHA is also used as an energy source to take up P from the bulk solution to regenerate the polyphosphate used in the anaerobic reactor, and to synthesize polyphosphate in the new cells that are generated.

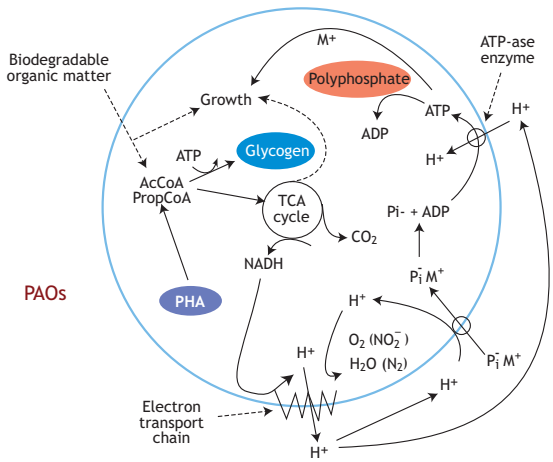


Figure 6.8 Biochemical model for PAOs under aerobic (or anoxic) conditions (adapted from Comeau *et al.* 1986). For design purposes, it is considered that only PHA reserves are used, via acetylCoa and propionylCoaA for PAO growth and not external biodegradable organic matter. The TCA is used to produce carbon intermediates for growth, energy (such as ATP) and reducing power (such as NADH). NADH, in the presence of an electron acceptor such as oxygen or nitrate, is then used to expel protons through the electron transport chain, creating a proton motive force that is used for phosphate (Pi^{-}) transport with metallic cations (M^{+}) and ATP synthesis that serves for PAO growth and polyphosphate storage.

The uptake of P to synthesize polyphosphate in the new cells generated means that more P is taken up than is released in the anaerobic reactor, giving a net removal of P from the liquid phase in the

activated sludge system. Accompanying the P uptake, the cations Mg^{2+} and K^{+} are also taken as countercharge for the negatively charged polyphosphate polymer, in the approximate molar ratio $P:Mg^{2+}:K^{+}$ of 1:0.33:0.33. The PAOs, with stored polyphosphate, are removed from the aerobic reactor of the system (where the internally stored polyphosphate concentration in the PAOs is the highest in the system) via the waste sludge stream (wastage from the underflow recycle stream is possible, but not desirable for hydraulic control of sludge age, see Chapter 4). At steady state the mass of PAOs wasted per day (with stored polyphosphate) equals the mass of new PAOs generated per day (with stored polyphosphate). Thus, for a fixed sludge age, loading and system operation, the mass of PAOs in the biological reactors remains constant, so that in the activated sludge system at steady state there is neither a build-up nor a loss of PAOs, and the P/VSS ratio stays approximately constant. The mass of new PAOs formed depends on the mass of stored substrate (PHA) available to the PAOs. Accordingly, the enhanced P removal attained will depend on the mass of PHA stored in the anaerobic reactor.

6.4.4.3 Quantitative anaerobic-aerobic PAO model

A quantitative model for PAOs subjected to anaerobic and aerobic conditions is shown in Figure 6.9. This model was determined using an enriched PAO culture system operated at an SRT of 8 days, pH of 7.0 and with acetate as the sole carbon source (Smolders *et al.,* 1994a, b). Under anaerobic conditions, influent acetate is taken up by PAOs with energy coming from polyphosphate and glycogen degradation that result in PHB (or PHA) formation and some CO_2 production. Under aerobic conditions, oxygen is consumed for the synthesis of polyphosphate, glycogen and biomass, and for cell maintenance. These aerobic processes result in PHB formation and CO_2 production. With biomass wastage to maintain the SRT, each 1 C-mol of acetate results in 0.04 mol of P removed in the form of polyphosphate.

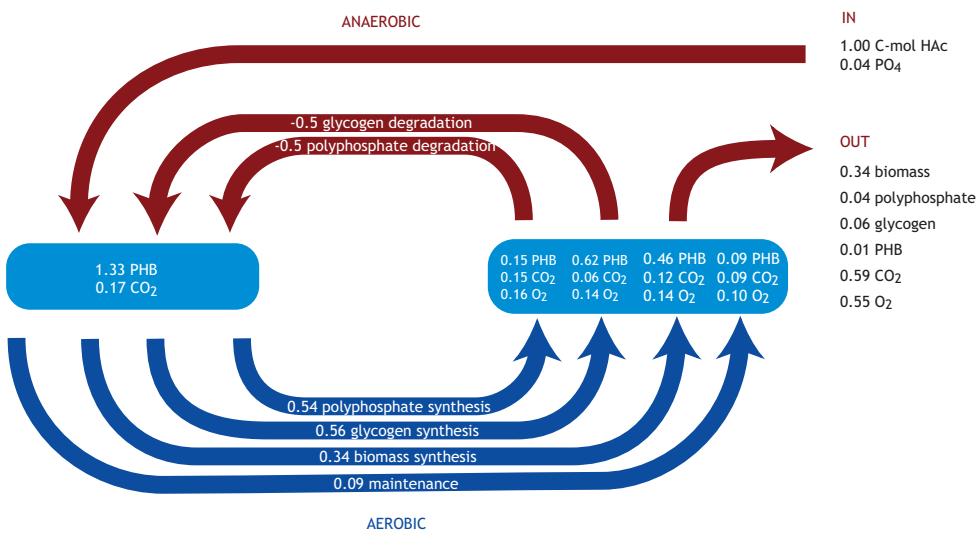


Figure 6.9 Quantitative model for PAOs subjected to anaerobic and aerobic conditions (adapted from Smolders *et al.,* 1994b). Each C-mol acetate corresponds to 0.5 mol of acetate. For acetate, 1 C-mol thus corresponds to 32 gCOD. All other carbon compound concentrations are expressed in C-mol units.

6.4.5 Fermentable COD and slowly biodegradable COD

As indicated above, under anaerobic conditions, PAOs can only store VFAs (S_{VFA}). Some wastewater which contains very few VFAs, however, exhibits significant EBPR which is related to the rapidly biodegradable COD (S_S) which is composed of both S_{VFA} and fermentable COD (S_F) (Siebritz *et al.,* 1982; Wentzel *et al.,* 1985; Nicholls *et al.,* 1985; Pitman *et al.,* 1988; Wentzel *et al.,* 1990; Randall *et al.,* 1994). Thus, it is considered that VFAs coming from the influent and those fermented from S_F are available for anaerobic storage by PAOs.

Slowly biodegradable COD (X_S), even though it can be hydrolyzed into rapidly biodegradable COD under anaerobic conditions, has been shown not to be linked to anaerobic phosphate release. This aspect is of crucial importance as it will influence both the design and operation of BNR systems, such as sizing and determining the number of anaerobic reactors, inclusion of primary sedimentation and maximum EBPR achievable. For the purpose of this design chapter, the experimental evidence linking EBPR to S_S is accepted, and hence a significant conversion of X_S to VFAs is considered unlikely. Accordingly, where VFA production does occur, this will be essentially from the rapidly biodegradable COD. One exception to this consideration is when primary sludge fermentation is provided upstream of the anaerobic reactor, which favours the hydrolysis of some X_S into S_S and VFAs.

6.4.6 Functions of the anaerobic zone

From the description of the mechanisms above, with typical domestic wastewater as influent, the anaerobic zone/reactor serves two functions:

(i) It stimulates conversion of fermentable COD into VFAs by heterotrophic organisms, *i.e.* facultative acidogenic fermentation.
(ii) It enables the PAOs to sequester the VFAs by taking them up and storing them as PHA. In effect this process enables the PAOs to take up and store some of the substrate under conditions (no external electron acceptor, anaerobic) where it is not available to the OHOs. The PAOs then do not have to compete for substrate when an external electron acceptor becomes available (anoxic/aerobic).

Of the above two processes, the former is the slower and determines the size of the anaerobic reactor. Should primary sludge fermentation be implemented at the treatment plant, the first process would not be needed as much and the size of the anaerobic reactor could be decreased.

6.4.7 Influence of recycling oxygen and nitrate on the anaerobic reactor

As noted by numerous investigators (*e.g.* Barnard, 1976a, 1976b; Venter *et al.,* 1978; Rabinowitz and Marais, 1980; Hascoet and Florentz, 1985), recycling of oxygen and/or nitrate to the anaerobic reactor causes a corresponding decrease in EBPR. In terms of the mechanisms described above, if oxygen and/or nitrate is recycled to the anaerobic reactor, the OHOs are able to utilize the fermentable COD for energy and growth using the oxygen or nitrate as external electron acceptor. For every 1 mg O_2 recycled to the anaerobic reactor, 3 mg COD of fermentable COD are consumed and for every 1 mg N of nitrate recycled, 8.6 mg COD of fermentable COD are consumed by the OHOs. The ratio of 3 mg S_F per mg O_2 consumed comes from considering a net yield of 0.67 mg VSS-COD produced per mg COD consumed with the rest, 0.33 mg per mg, serving for energy production using oxygen. Thus, for every mg of O_2 consumed, 3 times as much S_F is consumed. Similarly, considering that 1 mg of nitrate is the equivalent of 2.86 mg of oxygen, a ratio of 8.6 mg COD consumed by mg NO_3-N reduced is obtained.

The fermentable COD consumed is no longer available for conversion by OHOs to VFAs and, therefore, the amount of VFAs generated and released to the solution is reduced, by the amount of RBCOD consumed by the OHOs. Consequently, the mass of VFAs available to the PAOs for storage is

reduced, and correspondingly so is the P release, P uptake and net P removal.

Should the influent RBCOD already consist of VFAs and oxygen and/or nitrate be recycled, then the PAOs and OHOs will compete for the VFAs, the PAOs to sequester the VFAs and the OHOs to metabolize it. Accordingly, even in this situation, recycling of oxygen and/or nitrate will reduce the EBPR.

Thus, preventing the recycling of oxygen and nitrate to the anaerobic reactor is one of the primary considerations in the design and operation strategy for EBPR systems (Siebritz *et al.,* 1980).

6.4.8 Denitrification by PAOs

The extent of denitrification with associated anoxic P uptake by the PAOs appears to be very variable (Ekama and Wentzel, 1999), from near-zero anoxic P uptake (*e.g.* Clayton *et al.,* 1991) to anoxic P uptake dominant over aerobic P uptake (*e.g.* Sorm *et al.,* 1996). Experimental evidence tends to suggest that the magnitude of anoxic P uptake is influenced by the anoxic mass fraction and the mass of nitrate loaded on the anoxic reactor relative to its denitrification potential (Hu *et al.,* 2001, 2002). Only some PAOs are actually capable of complete denitrification, reducing nitrate all the way to nitrogen gas (Camejo *et al.,* 2016, 2019). Most appear capable of reducing nitrite to nitrogen gas, where the conversion of nitrate to nitrite can be carried out by flanking organisms (Rubio-Rincon *et al.,* 2017, 2019).

For the purpose of design it will be considered that anoxic P uptake is not significant. Anoxic P uptake decreases the magnitude of P removal in the system (Ekama and Wentzel, 1999), and from a design point of view in which maximising P removal is a priority, anoxic P uptake should be avoided in the system. Hence, in this design chapter anoxic P uptake will not be considered. It must be emphasized, however, that due to the anaerobic storage of RBCOD by PAOs, the kinetics of denitrification do change when an anaerobic reactor is included in the system.

6.4.9 Relationship between influent COD components and sludge components

The relationship described above between influent COD components and the various sludge organic masses (active, endogenous and inert) is shown in Figure 6.10.

6.5 FACTORS IMPACTING EBPR PROCESS PERFORMANCE

6.5.1 Total influent COD (COD_i)

As discussed previously, the total influent COD has a direct influence on the EBPR process since it contains the main substrates required by PAO to perform such a process. To illustrate the effect of the total influent COD on the EBPR process, the stroichiometric steady-state model presented in Section 6.8 is used. Using this model, figures 6.11 and 6.12 plot the effect of a total influent COD concentration of 500 mgCOD/l (in Figure 6.11) and 1,000 mgCOD/l (in Figure 6.12) on the P-removal process at different sludge ages.

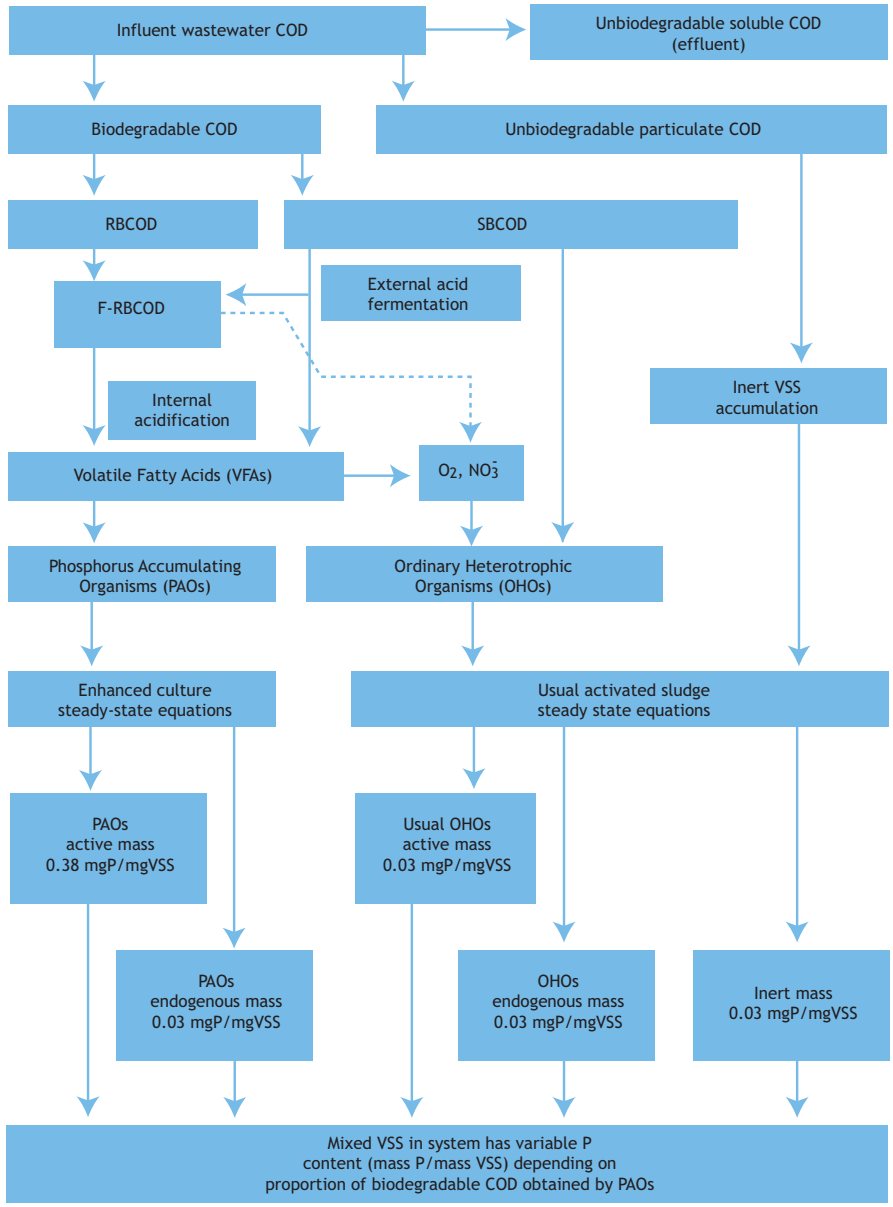


Figure 6.10 Schematic diagram showing the fate of various influent COD fractions in relation to the various active, endogenous and inert masses of the sludge.

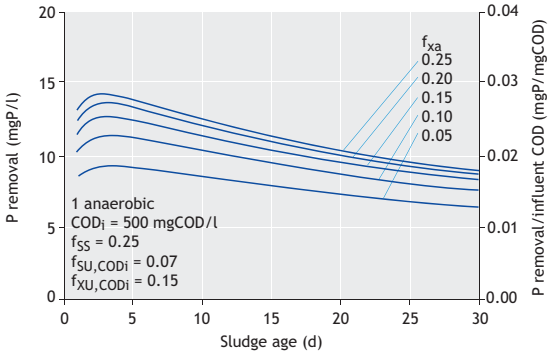


Figure 6.11 Predicted P removal *versus* sludge age for various anaerobic mass fractions (f_{xa}), for a single anaerobic reactor system treating unsettled wastewater with a total COD of 500 mgCOD/l, with characteristics as shown.

To assist a comparison of the effects of the different influent COD concentrations, the right axis is given as P removal/COD_i. It appears that with an increase in COD_i, the P removal efficiency (*i.e.* P removal/COD_i) increases. This is due to the increased magnitude of fermentable COD concentration (influent RBCOD fraction constant at f_{SS} = 0.25), and conversion with increased COD_i as a result of the higher OHO biomass.

6.5.2 Raw or settled sewage

The effect that settling the wastewater has on P removal is illustrated in Figure 6.13 where P removal is plotted against the sludge age for various anaerobic mass fractions f_{xa}, given an influent COD_i of 1,000 mgCOD/l and subject to primary sedimentation (resulting in a settled wastewater with a strength of 600 mgCOD/l). Comparing the P removal for the original unsettled wastewater (Figure 6.12) with that for the settled wastewater (Figure 6.13), it is evident that settling will reduce the P removal of the system. This reduction is due to the decrease in the mass of biodegradable COD entering the activated sludge system which causes a reduction in the fermentable COD converted and in the mass of OHOs generated. However, P removal per influent COD entering the biological reactor is higher for the settled than for the unsettled wastewater. This is apparent from figures 6.12 and 6.13, by comparing the P removal/COD_i on the right-hand axes. This arises because the ratio $S_{S,i}/COD_i$ is higher for settled (f_{SS} = 0.38) than for unsettled wastewater (f_{SS} = 0.25, it should be noted that it is assumed no $S_{S,i}$ is removed in settling. Although this will not be strictly correct the $S_{S,i}$ removal in settling appears to be minimal).

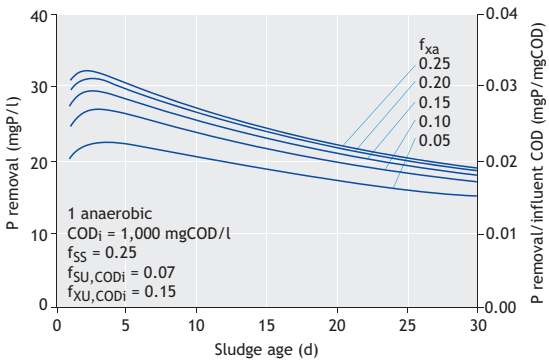


Figure 6.12 Predicted P removal *versus* sludge age for various anaerobic mass fractions (f_{xa}), for a single anaerobic reactor system treating unsettled wastewater with a total COD of 1,000 mgCOD/l, with characteristics as shown.

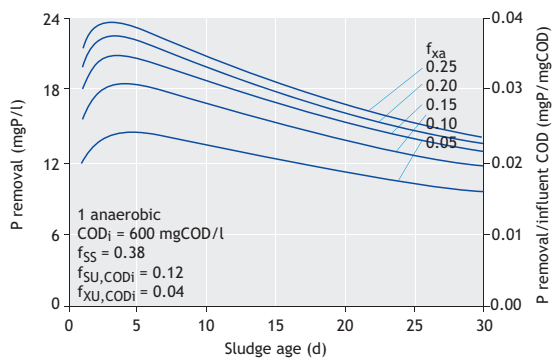


Figure 6.13 Predicted P removal *versus* sludge age for various anaerobic mass fractions (f_{xa}), for a single anaerobic reactor system treating settled wastewater with a total COD of 600 mgCOD/l, with characteristics as shown (c.f. Figure 6.12 for the original unsettled wastewater).

6.5.3 Influence of influent RBCOD fraction

Assuming a zero discharge of nitrate into the anaerobic reactor and considering that VFA ($S_{VFA,i}$) and other fermentable organics ($S_{F,i}$) compose the RBCOD ($S_{S,i}$) present in an influent wastewater, the effect of the influent RBCOD fraction with respect to biodegradable COD ($f_{SS} = S_{S,i}/COD_{b,i}$) is illustrated in Figure 6.14. In this figure, the theoretical P removals are plotted *versus* f_{SS} for a system with two-in-series anaerobic reactors, an SRT of 20 days and anaerobic mass fraction f_{xa} (with regard to the total mass; $f_{xa} = X_A V_A / X_{TSS} V_R$) and wastewater characteristics as shown. It appears that for a selected f_{xa}, as the RBCOD fraction (f_{SS}) increases, the P removal also increases. If needed and as previously discussed, one option to improve the P removal efficiency can be to increase the availability of RBCOD through, for example, acid fermentation of the primary sludge or the addition of external carbon sources (Pitman *et al.* 1983; Barnard 1984; Osborn *et al.* 1989; Vollertsen *et al.,* 2006; Barnard *et al.,* 2017).

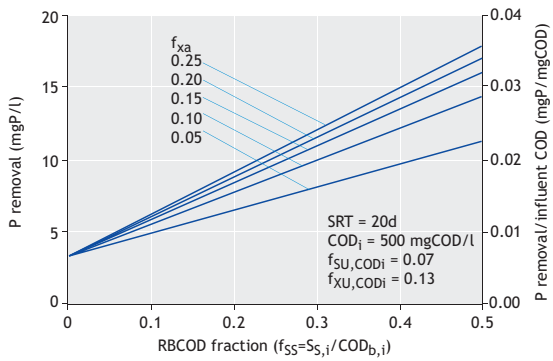


Figure 6.14 Predicted P removal *versus* readily biodegradable COD (RBCOD, $S_{S,i}$) as a fraction of the biodegradable ($COD_{b,i}$) COD ($f_{SS} = S_{S,i}/COD_{b,i}$) for various anaerobic mass fractions (f_{xa}) for a two-in-series anaerobic reactor system at 20 d sludge age, treating unsettled wastewater of 500 mgCOD/l, with characteristics as shown.

6.5.4 Influence of recycling nitrate and oxygen on the anaerobic reactor

The influence of nitrate recycled to the anaerobic reactor is illustrated in Figure 6.15 using the stoichiometric EBPR model where theoretical P removals are plotted *versus* nitrate concentration recycled for a system with two-in-series anaerobic reactors, recycle ratio 1:1, SRT of 20 days and f_{xa} and wastewater characteristics as shown. It appears that recycling of nitrate has a markedly deleterious influence on the magnitude of P removal (in agreement with numerous experimental observations). As the nitrate concentration recycled to the anaerobic reactor increases, the P removal decreases, as explained below.

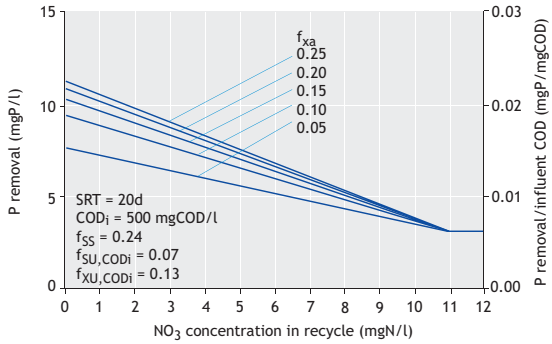


Figure 6.15 Predicted P removal *versus* nitrate concentration in recycle to anaerobic (recycle 1:1) for various anaerobic mass fractions (f_{xa}), for a two-in-series anaerobic reactor system at 20 d sludge age, treating unsettled wastewater of 500 mgCOD/l, with characteristics as shown.

If oxygen and/or nitrate is recycled to the anaerobic reactor, the OHOs no longer transform fermentable COD into VFAs but are able to utilize it for energy and growth using the oxygen or nitrate as an external electron acceptor. For every 1 mgO_2 and 1 $mgNO_3$-N recycled to the anaerobic reactor, 3.0 and 8.6 mgCOD, respectively, are utilized. Consequently, allowing oxygen and/or nitrate to enter the anaerobic reactor reduces the mass of VFAs available to the PAOs for storage, and

correspondingly reduces the P release, P uptake and P removal.

From Figure 6.15, when the nitrate concentration in the recycle exceeds approximately 11 mgN/l, the P removal remains constant at approximately 3 mgP/l; for this condition all the influent RBCOD for this wastewater is denitrified by OHOs with the result that no VFAs are released, no COD is available to the PAOs and EBPR no longer takes place; the P removal obtained is due to wastage of sludge with normal metabolic P content (0.03 mgP/mgVSS). If the influent RBCOD concentration increases or decreases, the mass of recycled nitrate that completely consumes the RBCOD will increase or decrease correspondingly below 11 mgN/l (provided the recycle ratio remains unchanged).

From the above it is clear that one of the principal orientations in any design for EBPR is to minimize oxygen entrainment and recycling of nitrate to the anaerobic reactor. In conditions where nitrification is obligatory, a number of different system configurations have been developed specifically to prevent this by incorporating complete denitrification, or passing the underflow recycle through anoxic zones before discharge to the anaerobic reactor.

6.5.5 The effects of the SRT

Using the characteristics of a typical unsettled municipal wastewater with a total influent COD of 250 mgCOD/l, and assuming that no nitrate enters the anaerobic reactor and that a recycle ratio to the anaerobic of 1:1 is present, P removal *versus* sludge age is shown in Figure 6.16 for a single anaerobic reactor with f_{xa} of 0.05; 0.10; 0.15; 0.20 and 0.25. On the same plots P removal/COD_i is also shown.

The plots indicate that the effect of SRT on P removal is complex. For SRT < 3 days, the P removal increases with an increase in the SRT. However, for SRT > 3 days, P removal decreases with an increase in SRT. The reason for this is that an increase in SRT causes an increase in the system OHO mass, which in turn causes an increase in fermentable COD conversion and, therefore, an increase in P release and P uptake. However, the increased SRT also causes a decrease in P uptake due to the lower PAO active mass (and its associated P content) wasted per day. At SRT < 3d, the former effect dominates the P removal, while at SRT > 3d the latter dominates, giving rise to the shape of the curve. The latter effect, that is the decrease in both PAO and OHO active masses with increase in SRT, would be crucially affected by the specific endogenous mass loss rate of the PAOs. However, should the endogenous mass loss rate of the PAOs (0.04 d^{-1}) have been the same as that of the OHOs (0.24 d^{-1}), virtually no EBPR would have been obtained.

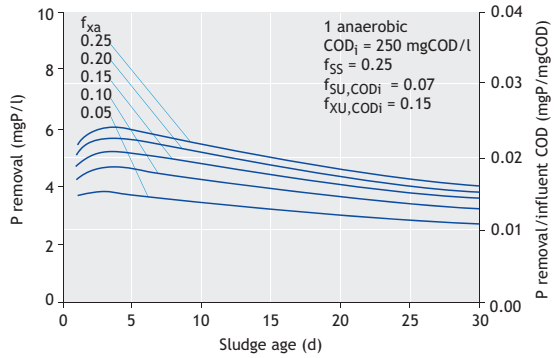


Figure 6.16 Predicted P removal *versus* sludge age for various anaerobic mass fractions (f_{xa}), for a single anaerobic reactor system treating unsettled wastewater with a total COD of 250 mgCOD/l, with characteristics as shown.

In EBPR systems, the aerobic SRT plays an important role due to the aerobic conversion processes that help to replenish the intracellular polymers. In particular, this is because the behaviour of the three storage pools in the cells (PHA, polyphosphate and glycogen) is highly dynamic and is determined by their conversion during the anaerobic and aerobic (or anoxic) phase. The PHA content of the biomass depends on the biomass concentration in the reactor. The biomass

concentration can be easily controlled by the manipulation of the substrate loading and SRT. While the anaerobic PHA production depends on the substrate loading to the system, the aerobic PHA consumption depends on the PHA level inside the biomass and on the kinetics of four PHA utilizing processes. The PHA formed under anaerobic conditions must be consumed during the aerobic phase. Otherwise, the PHA level in the cells will increase until a maximum level is reached. From that moment on, no substrate uptake will occur under anaerobic conditions, leading to the deterioration of the EBPR process.

In activated sludge systems designed for the removal of organic matter and nitrogen the SRT is directly linked to the growth rate of the microorganisms; the minimum required SRT corresponds to the maximum growth rate ($SRT_{min}= 1/\mu_{max}$). However, in the EBPR systems where storage materials play an important role in bacterial metabolism, the determination of the total SRT_{min} (defined as a sum of the minimum required anaerobic and aerobic SRT: $SRT_{min,total} = SRT_{min,AN} + SRT_{min,OX}$) depends on the process kinetic rates and on a number of process conditions, notably the time needed for anaerobic RBCOD conversion to PHA, the time required for PHA consumption under aerobic or anoxic conditions, the biomass specific substrate loading rate, the temperature, the operation of the system, and the cell maximum PHA content. Since growth only occurs under aerobic conditions, only the aerobic EBPR process (and therefore only the $SRT_{min,OX}$) is considered here. Clearly there does exist a minimum aerobic oxidation time below which the anaerobically produced PHA cannot be further oxidised. The model for the prediction of the minimum required aerobic SRT as a function of process parameters was developed and compared with experimental data used to evaluate several operational aspects of EBPR in a SBR system (Brdjanovic *et al.,* 1998b). The model was proved as capable of describing them satisfactorily (Figure 6.17).

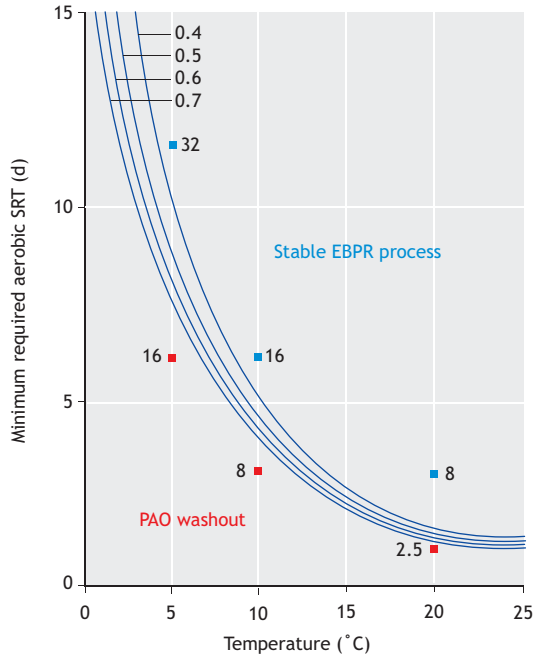


Figure 6.17 Minimum required aerobic SRT as a function of maximum PHA storage capacity of enriched culture biomass (0.4 - 0.7 gCOD-PHA/gCOD-active biomass) and temperature. The symbols indicate the aerobic SRT of several laboratory-scale SBR systems (Smolders *et al.*, 1994c, Brdjanovic *et al.*, 1998b). The numbers next to square symbols indicate the total SRT of the systems. A good EBPR was achieved at the SRTs marked as blue, while the EBPR failed at SRTs marked as red due to too short SRT.

6.5.6 Influence of the anaerobic stage

6.5.6.1 Effect of the anaerobic mass fraction (f_{xa})

The effect of f_{xa} on P removal is also shown in Figure 6.16. For a selected SRT, an increase in f_{xa} gives rise to an increase in P removal. This is due to the increased conversion of fermentable COD with larger anaerobic mass fractions. The improvement in P removal, however, diminishes with each step increase in f_{xa}, due to the first-order nature of the conversion kinetics. From the plot, with a single anaerobic reactor one should select $f_{xa} > 0.15$ because the modest increase in P removal for $f_{xa} > 0.20$ does not seem warranted.

6.5.6.2 Effect of the number of anaerobic reactors (n)

The effect of subdividing the anaerobic reactor is shown in Figure 6.18. The plot is similar to Figure 6.11, but with the anaerobic zone subdivided into two equal reactors. Comparing the P removal behaviour in Figures 6.11 and 6.18, the series operation of the anaerobic zone significantly improves the P removal. This improvement is due to the increased fermentable COD conversion with the in-series anaerobic reactor operation as a result of the first-order nature of the conversion kinetics. A comparison (not shown) between single, two-in-series and four-in-series anaerobic reactors indicates that the main improvement is from single to two-in-series reactors. For design, at least two equally sized in-series anaerobic reactors should be used.

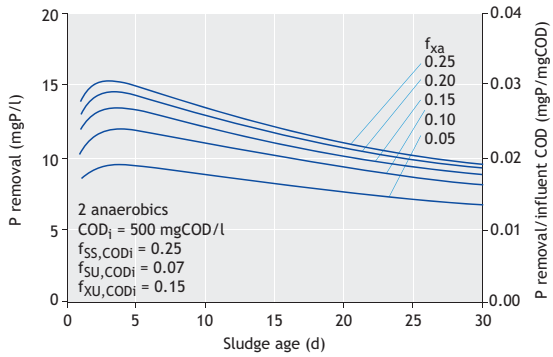


Figure 6.18 Predicted P removal *versus* sludge age for various anaerobic mass fractions (f_{xa}), for a two-in-series anaerobic reactor system treating unsettled wastewater with a total COD of 500 mgCOD/l, with characteristics as shown (c.f. Figure 6.11 for the single reactor system).

6.5.7 Presence of GAOs

Glycogen Accumulating Organisms (GAOs) have a metabolism similar to that of PAOs. However, unlike PAOs, GAOs only rely on the glycolysis of their intracellular glycogen pool as their energy and carbon source for the anaerobic storage of VFAs as PHA (Filipe *et al.,* 2001a; Zeng *et al.,* 2003). Thus, GAOs do not exhibit the typical anaerobic P release and subsequent aerobic P uptake. Therefore, from an EBPR process perspective, GAOs are undesirable microorganisms since they are able to take up VFAs under anaerobic conditions, competing with PAOs for the same carbon source, without contributing to phosphorus removal. So far, two of the best known GAO are *Competibacter* and *Defluviicoccus* (Oehmen *et al.,* 2006a, 2006b; Burow *et al.,* 2007; Lanham *et al.,* 2008). These two GAO are able to compete against PAO under certain environmental and operating conditions as discussed in this and the following subsections.

Different operating and environmental conditions have been identified as important factors to understand the PAO-GAO competition: type of carbon source (acetate and/or propionate), pH, temperature, and influent P/COD ratio. The type of carbon source plays an important role in the competition between PAOs and GAOs because the different PAO and GAO strains identified so far have different preferences, affinities and substrate uptake rates. However, overall a mix of different VFAs (*e.g.* 75 % HAc and 25 % HPr) tend to favour PAO over GAO (Lopez-Vazquez *et al.,* 2009b; Carvalheira *et al.,* 2014). Such observations have been confirmed in full-scale systems with much broader mixtures of carbon sources and amino acids being able to support a satisfactory EBPR process even at high temperatures (Qiu *et al.,* 2019).

pH has a major influence on the anaerobic metabolism of PAOs and GAOs. At higher pH levels (> 7.0), the energy required (ATP) for the transportation of substrate through the cell membrane increases (Smolders *et al.,* 1995; Filipe *et al.,* 2001a). This results in a higher degree of utilization of the intracellular storage fractions of polyphosphate and glycogen. Different reports have described the dominance of PAOs and, thus, stability of the EBPR process performance at high pH levels (pH > 7.25) and the dominance of GAOs at lower pH (pH < 7.25) (Filipe *et al.,* 2001a, 2001b; Schuler and Jenkins, 2002; Oehmen *et al.,* 2005a). These observations suggest that at higher pH either the hydrolysis of glycogen is the limiting metabolic process in GAO metabolism or that PAOs have metabolic advantages

over GAOs because they not only rely on the glycolytic pathway but also on the hydrolysis of polyphosphate (Filipe *et al.,* 2001a).

Temperature has a major impact on the competition and occurrence of PAOs and GAOs in activated sludge systems. At moderate and lower temperature (<20 ºC) PAOs tend to be the dominant microorganisms and have considerable metabolic advantages over GAOs whereas the opposite occurs at higher temperature (>20 ºC). This can be explained by considering that at temperatures lower than 20 ºC, PAOs have higher biomass growth rates than GAOs (Lopez-Vazquez *et al.,* 2008b, 2009a) and lower anaerobic maintenance requirements (Lopez-Vazquez *et al.,* 2007) potentially limiting the occurrence of GAOs in wastewater treatment systems operated at lower temperature (Lopez-Vazquez *et al.,* 2008a). At temperatures higher than 20 ºC, however, GAOs have higher substrate uptake rates than PAOs (Whang and Park, 2006; Lopez-Vazquez *et al.,* 2007; 2009a) favouring their occurrence when warm wastewaters (>20 ºC) are treated. Nevertheless, the applicability of a high pH level and a broader mixture of carbon sources and amino acids appears to give competitive advantages to PAO despite high activated-sludge temperatures (Whang *et al.,* 2007; Lopez-Vazquez *et al.,* 2009b; Qiu *et al.,* 2019).

6.5.9 Carbon sources

Aiming at obtaining highly enriched PAO cultures, several carbon sources have been used for the operation of lab-scale bioreactors (Jeon and Park, 2000; Jeon *et al.,* 2001; Oehmen *et al.,* 2004, 2007; Puig *et al.,* 2008), with a strong focus on the use of acetate and propionate as sole carbon sources due to their abundance in influent wastewaters (Oehmen *et al.,* 2004, 2007). However, the sole use of either acetate or propionate as a single carbon source has proven to lead to EBPR process instability because of the growth and proliferation of GAO (such as *Competibacter* or *Defluviicoccus*) (Oehmen *et al.,* 2006a, 2006b; Burow *et al.,* 2007; Lanham *et al.,* 2008). In view of the capabilities of PAO to take up either acetate or propionate at a relatively similar kinetic rate, Oehmen *et al.* (2006a) and Lu *et al.* (2006) proposed to alternate these two carbon sources periodically to outcompete GAO and obtain a highly enriched PAO culture. By applying a metabolic model, Lopez-Vazquez *et al.* (2009) forecasted that an influent containing 75% acetate and 25% propionate could be sufficient to obtain an enriched PAO culture (*Accumulibacter*). Following the outcome of Lopez-Vazquez *et al.* (2009), Carvalheira *et al.* (2014) conducted a long-term cultivation study that confirmed that a ratio of 75% acetate to 25% propionate can indeed lead to a highly enriched PAO culture (>80% based on FISH analysis).

In addition to the use of acetate and propionate, other carbon sources such as glucose, lactate and alcohols have also been tested (Jeon and Park, 2000; Jeon *et al.,* 2001; Puig *et al.,* 2008). Regarding the sole supply of glucose, the results are somewhat contradictory. While some studies indicate that glucose can lead to the deterioration of the EBPR process due to the proliferation of other organisms that can outcompete PAO by taking up glucose and storing it intracellularly as glycogen (Griffiths *et al.,* 2002; Xie *et al.,* 2017), other studies suggest that glucose can be fermented and produce VFA in the anaerobic stage of EBPR process configurations, resulting in a stable EBPR process performance. Similarly, the supply of lactate or alcohols seems to be associated with a stable EBPR performance whenever the anaerobic stage is long enough to favour their fermentation (Jeon and Park, 2000; Jeon *et al.,* 2001; Puig *et al.,* 2008). In addition, Tu and Schuler (2013) documented that PAO have a higher affinity for C uptake than GAO, outcompeting GAO at low C-source concentrations. Then, in an anaerobic stage, if the fermentation products are formed within the flocs, the higher affinity of PAO can help them to take the C sources as soon as they are generated, to the detriment of GAO. These observations underline the need to ensure that the anaerobic stage volume must be large enough to facilitate the fermentation of such carbon sources in favour of PAO.

Besides the presence and availability of VFA and other fermentable compounds, amino acids are also present in raw wastewaters. Their fermentation and consumption has been associated with the growth and proliferation of *Tetrasphera* (Nguyen *et al.,* 2011; Marques *et al.,* 2017; Liu *et al.,* 2019), which seems to be able to contribute to the P-removal process, but performing a different metabolism than *Accumulibacter* (Liu *et al.,* 2019). While their actual direct contribution to the EBPR process (as a fermentative organism and potential PAO) is still under study (Liu *et al.,* 2019; Rubio-Rincon *et al*., 2019), their presence has been associated with the stable operation of EBPR plants.

In full-scale WWTP, to ensure that PAO have enough carbon to perform the EBPR process, the addition of molasses to prefermenters has been evaluated (Zeng *et al.,* 2006), resulting in a higher availability of propionate and to an improved and stable EBPR process. Also, as presented in Section 6.6, certain process configurations have been developed to favour the self-production of VFA by fermenting (a fraction of) the RAS (Vollertsen *et al.,* 2006; Barnard *et al.,* 2017). This practice has led to a higher availability of different VFA species (Vollertsen *et al.,* 2006) and also to an increased EBPR performance. In another full-scale WWTP operated at high wastewater temperature (Ong *et al.,* 2014; Qiu *et al.,* 2019), the broad availability of different carbon sources and amino acids led to a stable EBPR operation, even though the temperature tended to be unfavourable for the EBPR process (Lopez-Vazquez *et al.,* 2009) (as discussed in Section 6.5.11). Overall, the availability of different VFA species and carbon sources contributes to an improved EBPR process stability, in contrast to the use of a single carbon source.

6.5.10 Influent COD/P ratio

Another factor affecting the metabolism of PAO is the influent P/COD ratio. Several studies have been conducted indicating that high P/COD ratios favour the metabolism of PAO while low P/COD ratios favour the metabolism of GAO. Schuler and Jenkins (2003a, 2003b) cultivated a PAO culture at different P/COD ratios. They observed a change in the metabolism of the enriched EBPR culture from a Phosphorus-Accumulating Metabolism (PAM) to a Glycogen-Accumulating Metabolism (GAM) as the P/COD ratio decreased. This implied a shift in the metabolism from the utilization of the TCA cycle at high P/COD ratios to a higher involvement of the glycolytic pathway as the P/COD ratio decreased. It is important to notice that in spite of the higher involvement of the TCA cycle at high P/COD ratios, the enriched EBPR culture continued to make use of the glycolytic pathway. Such observations were corroborated by similar research studies (Barat *et al.,* 2006; Zhou *et al.,* 2008; Acevedo *et al.,* 2012; Welles *et al.,* 2015, 2016). In studies performed in full-scale EBPR plants, Pijuan *et al.* (2008) and Lanham *et al.* (2014) also observed similar behaviour in particular at high P/COD ratios. Moreover, Welles *et al.* (2015, 2016) were able to cultivate an enriched PAO culture at a low influent P/COD ratio. They noticed that the PAO culture, due to the low intracellular polyphosphate content, made use of glycogen as the main intracellular compound for the anaerobic uptake of carbon, whereas after being exposed to a higher influent P/COD ratio, the PAO culture could shift their metabolism and take up orthophosphate aerobically.

The influence of the influent P/COD ratio is directly reflected in the anaerobic P-release-to-COD ratio. At higher P/COD ratios, the intracellular polyphosphate contents are higher which results in a higher anaerobic P-release-to-COD-consumption ratio. This seems to be caused by a higher involvement of the TCA cycle (Schuler and Jenkins, 2003a, 2003b; Pijuan *et al.,* 2008; Welles *et al.,* 2015, 2016). Meanwhile, at lower P/COD ratios, the intracellular polyphosphate contents decrease which increases the role of the glycolytic pathway and the anaerobic P-release-to-COD-consumption ratio decreases. In addition, Welles *et al.* (2015) observed that together with the phosphate release rates, acetate uptake rates also increased up to an optimal poly-phosphate/glycogen ratio of 0.3 P-mol/C-mol. At higher poly-phosphate/glycogen ratios (obtained at

influent P/C ratios above 0.051 P-mol/C-mol), the acetate uptake rates started to decrease. However, Welles *et al.* (2015) noticed that such metabolism was dependent on the specific type of microorganism present, with *Accumulibacter Type II* being more flexible than *Accumulibacter Type I*, and therefore more flexible and able to perform a GAM metabolism at low P/COD ratios with higher acetate uptake rates.

Overall, the influent P/COD ratio plays a major role in the EBPR process, affecting both the stoichiometry and the kinetics of known EBPR cultures. As such, these effects need to be considered when assessing or evaluating the EBPR process. For practical purposes and to comply with the required P-effluent discharge limits, in principle, EBPR systems tend to be designed considering a low influent P/COD ratio (a rather common practice since most of the EBPR designs and configurations aim at maximizing the influent COD provided to PAO).

6.5.11 pH effects

Smolders *et al.* (1994a) assessed the effects of pH finding a direct correlation between the anaerobic P-release/HAc-uptake ratio and the pH measured in the bulk liquid ($f_{PO4,rel}$ = 0.18 pH – 0.81, in gP/gCOD units). Such a relationship is a reflection of the increased energy required at higher pH levels for HAc uptake. Filipe et al. (2001a) amended such an equation ($f_{PO4,rel}$ = 0.15 pH – 0.53, in gP/gCOD units) suggesting that their research probably had a higher PAO abundance.

6.5.12 Temperature effects

With the application of mathematical modelling on biological processes, the role of temperature coefficients becomes more important. Mathematical models that include the presence of EBPR in activated sludge systems, such as Activated Sludge Model No. 2 (ASM2) (Henze *et al.,* 1994), University of Cape Town Activated Sludge Model (UCTPHO; Wentzel *et al.,* 1992) or the metabolic model of EBPR (TUDP model; Smolders *et al.,* 1994c, 1995) rely on stoichiometric and kinetic coefficients valid in a narrow temperature range or a single temperature value only. In ASM2, process coefficients are defined for two different temperatures (10 and 20 ºC). In this model the stoichiometric coefficients are temperature-independent, while the kinetic coefficients are affected by temperature changes. The processes in ASM2 are classified into four groups based on their temperature dependency (zero, low, medium and high dependency). Identical values at 10 and 20 °C were assigned to many of the coefficients. This was justified by the scarcity of data available or by the low sensitivity of the particular parameters to variations in temperature. In this classification, the EBPR processes are considered to have a low degree of temperature dependency in comparison with other processes incorporated in ASM2. ASM2 is in general recommended for application on wastewater treatment by activated sludge at temperatures between 10 and 25 °C, and the authors of ASM2 are cautious about its applicability outside this range. Similarly, the UCTPHO model has process parameters based on 20 °C. For other operational temperatures the adjustment of the values is computed from the input temperature and Arrhenius temperature constants. In the metabolic model all parameters are determined at 20 °C, but no information is available on their temperature dependency. It has been suggested that the temperature impact on the PAOs can be modelled with the same coefficients as for heterotrophic organisms. However, due to large differences in metabolism and the involvement of storage products, this can be erroneous. As municipal wastewater treatment plants, including those operating with EBPR, may experience mixed-liquor temperatures as low as 5 °C or as high as 35 °C, there was a significant need for a systematic study of the impact of temperature on EBPR systems which should also take into account the specific requirements of mathematical models and their application in different climates.

It was thought some years ago that *Acinetobacter* sp. are the microorganisms most responsible for

EBPR, and consequently, most of the scientific research concerning the impact of temperature was related to these particular bacteria. However, it was then shown that *Acinetobacter* plays a limited role in EBPR (Wagner *et al.,* 1994) and, therefore, information on the P metabolism of *Acinetobacter* alone is to be considered less relevant.

There are several publications reporting the effect of temperature on the efficiency (the difference in the influent and the effluent quality) of EBPR using activated sludge but with inconsistent results. Improved EBPR efficiency at higher temperatures (in the range 20-37 °C) was observed by Jones *et al.* (1987), Yeoman *et al.* (1988), McClintock *et al.* (1993) and Converti *et al.* (1995). In contrast, high or even only comparatively higher levels of P-removal efficiency at lower temperatures (in the range 5-15 °C) was reported by Sell *et al.* (1981), Kang *et al.* (1985b), Krichten *et al.* (1985), Barnard *et al.* (1985), Viconneau *et al.* (1985) and Florentz *et al.* (1987). However, when the kinetics of EBPR processes were studied, such inconsistencies did not exist. Increased P-release and/or P-uptake rates with increased temperature was reported by Shapiro and Levin (1967), Boughton *et al.* (1971), Spatzierer *et al.* (1985), and Mamais and Jenkins (1992). In addition to P-release and P-uptake rates, Mamais and Jenkins (1992) also reported increased growth and substrate consumption rates with an increase in temperature (10-33 ºC). The different results on the temperature effect on EBPR with activated sludge can be explained by the use of different substrates, activated sludge and/or measurement methods.

Temperature influences a variety of processes in activated sludge systems (lysis, fermentation, nitrification, etc.) which may influence EBPR processes. These effects complicate the determination of the effect of temperature on EBPR. In addition, most of the findings presented in the paragraph above are based on a black-box approach, comparing the influent and effluent phosphorus concentrations of wastewater treatment plants at different wastewater temperatures. At that time no structured study of the effect of temperature on stoichiometry and kinetics of the EBPR processes under defined laboratory conditions was available. All these factors explain conflicting results in the past, which were difficult to interpret correctly.

The effect of temperature on the stoichiometry of EBPR processes had not been studied in great detail until Brdjanovic *et al.* (1997, 1998c) carried out a systematic study on the effects of temperature changes on both the anaerobic and the aerobic stoichiometry and kinetics. This study comprised experiments to investigate the effects of short-term (hours) temperature changes on the physiology of the EBPR system, and long-term (weeks) temperature changes on the ecology of the EBPR system. Enriched PAO cultures fed with synthetic wastewater were used in an anaerobic-aerobic-settling sequencing batch reactor (SBR) under controlled (laboratory) conditions. The main results from this work are highlighted below.

6.5.12.1 Short-term temperature effects on the physiology of EBPR

P-removing sludge was enriched in an anaerobic-aerobic, acetate-fed, sequencing batch reactor (SBR) at 20 °C. Conversion of relevant compounds for biological phosphorus removal was studied at 5, 10, 20 and 30 °C in separate batch tests during the period of a few hours. The stoichiometry of the anaerobic processes was found to be insensitive to temperature changes while some effects on aerobic stoichiometry were observed. In contrast, temperature had a major influence on the kinetics of the processes under anaerobic as well as aerobic conditions. The anaerobic P release (or acetate uptake) rate showed a maximum at 20 °C. However, a continuous increase was observed in the interval 5-30 °C for the conversion rates under aerobic conditions. Based on these experiments, temperature coefficients for the different reactions were calculated. An overall anaerobic and aerobic temperature coefficient θ was found to be 1.078 and 1.057 (valid in the ranges 5 °C≤T≤20 °C and 5 °C≤T≤30 °C), respectively.

6.5.12.2 Long-term temperature effects on the EBPR process

Steady-state conversion of relevant compounds for EBPR was studied in one reactor subsequently at 20, 30, 20, 10 and 5 °C. The temperature coefficient for metabolic conversions obtained from long-term temperature tests was similar to the temperature coefficient observed in short-term (hours) tests (θ = 1.085 and θ = 1.078, respectively). Temperature had a moderate impact on the aerobic P-uptake process rate (θ = 1.031) during the long-term tests. However, a major temperature effect on other metabolic processes of the aerobic phase, such as PHA consumption (θ = 1.163), oxygen uptake (θ = 1.090) and growth (θ > 1.110), was observed. Different temperature coefficients were obtained for the aerobic phase from long-term and short-term tests, probably due to a change in population structure. This change was also visible from molecular ecological studies. The different temperature coefficient found for P uptake compared to the other metabolic processes of the aerobic phase underlines that in complex processes such as EBPR, it is dangerous to draw conclusions only from easily observable parameters (such as phosphate). Such consideration can easily lead to underestimation of the temperature dependency of other metabolic processes of the aerobic phase of EBPR. Meijer (2004) incorporated temperature coefficients obtained from studies of Brdjanovic *et al.* (1997, 1998c) into the TUDP model. Lopez-Vazquez *et al.* (2008b, c) repeated the original experiments of Brdjanovic *et al.* (1997, 1998c) and extended the TUDP model with coefficients for two additional temperatures, namely 15 ºC and 35 °C. In general, this study confirmed the results of Brdjanovic *et al.* (1997, 1998c) for the temperature range 5-30 ºC. In addition, Lopez-Vazquez *et al.* (2008c) also determined the temperature dependencies in the short- and long-term of a GAO culture enriched with *Competibacter* and then introduced them into the TUDelft model to assess the PAO-GAO competition at different temperatures (Lopez-Vazquez *et al.,* 2009).

6.5.13 Dissolved oxygen and aeration

The DO concentration plays an important role in the EBPR process. Carvalheira *et al.* (2014) defined that DO lower than 1.5 mg/l are preferable to favour the growth of PAO over GAO due to their higher DO affinity, whereas higher DO concentrations tend to favour GAO which can compete for C sources anaerobically with PAO. With more interest in reducing the energy costs of WWTP through the optimization of the aeration process and thus, operating the WWTP at low DO, such a practice is favourable for PAO.

On the other hand, while the system needs to comply with a minimum aerobic SRT to ensure the growth of PAO (Brdjanovic *et al.,* 1998b), excessive aeration can have a detrimental impact on the EBPR process due to the depletion of the intracellular PHA compounds as an energy source resulting in the aerobic hydrolysis of polyphosphate, and consequently on the aerobic release of P (Lopez *et al.,* 2006). This affects the effluent quality as the P effluent concentrations will rise. Extreme aeration events can take place after heavy rainfalls or during weekends (where the influent wastewater dilutes and the carbon source concentrations decrease leading to less PHA accumulation). To avoid such deleterious effects, the aeration needs to be adjusted and decreased during such conditions.

6.5.14 Inhibitory compounds

The absence or presence of certain compounds has proven to be deleterious for the EBPR process. Saito *et al.* (2004) observed that nitrite was inhibitory for the anoxic and aerobic P-uptake process. Rather than nitrite, Zhou *et al.* (2007) observed that the anoxic phosphorus uptake rates decreased when the free nitrous acid (FNA) concentrations increased from 0.002 to 0.02 mg HNO_2-N/l, being completely inhibited at 0.02 mg HNO_2-N/l. Concerning the aerobic P uptake, Pijuan *et al.* (2010) observed a 50 % inhibition on all anabolic processes at FNA concentrations of approximately $0.5 \cdot 10^{-3}$ mg HNO_2-N/l (equivalent to 2.0 mg NO_2^--N/l at pH 7.0),

while full inhibition occurred at FNA concentrations of approximately $6.0 \cdot 10^{-3}$ mg HNO_2-N/l.

Potassium limitation has also been shown to have a deleterious effect on the EBPR process (Brdjanovic *et al.,* 1996) while the excess of calcium (and other salts) for chemical P removal also leads to deterioration of the EBPR process (Barat *et al.,* 2006; Jobagy *et al.*, 2006) since P precipitates chemically and is no longer available to form the intracellular polyphosphate pools required by PAO. Thus, the wastewater composition has a direct impact on the efficacy of the EBPR process.

6.6 EBPR PROCESS CONFIGURATIONS

In this section, the EBPR optimisation concepts are first discussed and the development of the main EBPR activated sludge systems is then reviewed in a historical context.

6.6.1 Phosphorus removal optimization principles

An overview of EBPR and chemical phosphorus removal optimisation principles are presented in Figure 6.19. The principles of EBPR and P-removal process optimisation can be grouped into six categories. A number of configurations or processes that are based on these principles are identified by their specific names.

(i) Oxygen entrainment in the anaerobic reactor should be minimised. For this purpose, mixing vortexes, upstream cascades and screw pumps or air lift pumps should be avoided.

(ii) Nitrate (and nitrite) entrainment in the anaerobic reactor should be minimised. As explained in the next section, a number of named configurations were developed precisely for this purpose. To this end, an anaerobic–aerobic configuration (*e.g.* A/O configuration) can be improved by inserting an anoxic reactor in which aerobic sludge is recirculated for denitrification (*e.g.* A^2/O, modified Phoredox configurations). Also, the return activated sludge from the secondary settling tank can be denitrified either via an anoxic reactor on the sludge recycle line (*e.g.* JHB configuration) or via an anoxic reactor located downstream of the anaerobic zone from where another internal recirculation to the anaerobic reactor is located (*e.g.* UCT configurations). This anoxic reactor can be divided into two zones to provide return sludge denitrification in the first zone and aerobic sludge denitrification in the downstream anoxic reactor (*e.g.* MUCT configuration). Adding a second anoxic zone, downstream of the aerobic zone, is another way of reducing the nitrate concentration in both the effluent and return sludge (*e.g.* Modified Bardenpho configuration).

(iii) VFA uptake by PAOs in the anaerobic reactor should be maximized. Primary sludge fermentation is an efficient way to increase the VFA content of the influent even though it also contributes to an increased loading of ammonia in the activated sludge system. Sodium acetate or fermentable industrial wastes can be added directly to the anaerobic reactor or prefermentation tank. The hydraulic retention time of the anaerobic reactor or prefermenter can be increased to favour *in-situ* fermentation of the influent or added fermentable organic matter. RAS fermentation can also be employed to augment the organic matter to be consumed by PAOs (see Section 6.6.11).

(iv) Effluent particulate phosphorus should be minimized by removing total suspended solids efficiently. The particulate phosphorus content can reach as high as 18% gP/gTSS for enriched cultures. With a more typical 5% content, every 10 mgTSS/l in the effluent will contribute 0.5 mgP/l. Thus, efficient secondary clarification, avoiding floating sludge from denitrification in the settling tank, sand filtration, and even ultrafiltration (in a membrane bioreactor) are all means of reducing the effluent TSS concentration.

(v) Effluent soluble phosphorus should be minimized. Besides optimising the EBPR process, chemical coagulants such as iron (*e.g.*

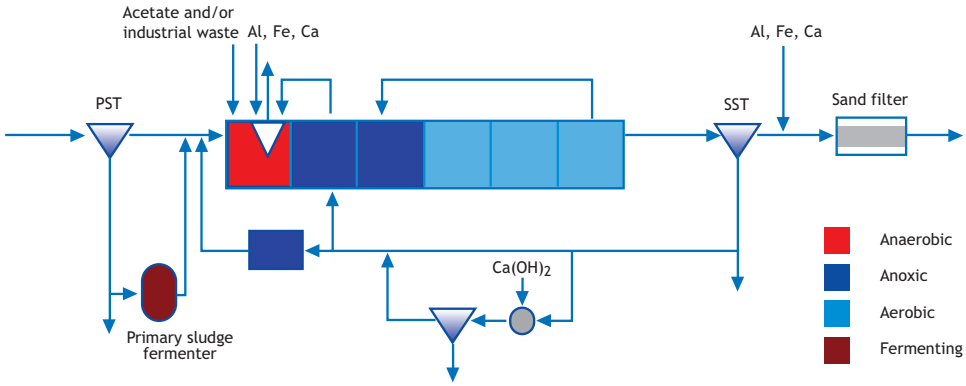


Figure 6.19 Overview of EBPR and P-removal process optimisation. Note: PST: primary settling tank; SST: secondary settling tank.

$FeCl_3$), aluminium (*e.g.* alum) or calcium (*e.g.* lime) salts can be added either in the mainstream for pre-, co- or post-precipitation (in the primary settling tank, in the activated sludge process, downstream of the secondary settling tank, respectively). Extracting the supernatant from the anaerobic tank or taking some sludge from the return activated sludge and coagulating them can also lead to lower effluent soluble phosphorus (*e.g.* the BCFS® process; Van Loosdrecht *et al.,* 1998). Sidestream lime precipitation of phosphate released anaerobically from the return sludge can also be carried out. More efficient phosphate release can be achieved in this sidestream tank by diverting some influent containing readily biodegradable COD (*e.g.* the PhoStrip® process). Should anaerobic or aerobic digestion be performed with the wasted secondary sludge, essentially all of the polyphosphates will be degraded and the phosphate released in solution. Phosphorus recovery in the form of struvite ($MgNH_4PO_4$) or hydroxyapatite [$Ca_{10}(PO_4)_6OH_2$], which can be used as fertilizers, are also means of reducing the loading of soluble phosphate back to the activated sludge process and, eventually, to the effluent.

(*vi*) Phosphorus uptake for cell synthesis can be maximized. Although more limited than the other optimisation concepts in its potential efficiency, maintaining the sludge retention time (SRT) as short as possible will result in an increase in phosphorus removal by cell synthesis. Another benefit of reducing the SRT will be that PAOs will degrade less polyphosphate for cell maintenance. The constraint on manipulating the SRT is maintaining a minimum sludge age to maintain nitrification, while stimulating PAOs.

6.6.2 EBPR process discovery

The removal of P by activated sludge systems in excess of that required for normal organism metabolism was first noted, independently, by two research groups, Srinath *et al.* (1959) in India and Alarcon (1961) in America. Although both groups demonstrated P uptake in aerobic batch tests, they proposed no explanation as to why sludges from certain plants exhibited enhanced P-uptake behaviour and others not, or whether the removal was a biological or physical/chemical phenomenon. Srinath *et al.* (1959) noted that an oxygen deficiency in the upstream zone of plug flow aeration tanks was associated with the occurrence of high phosphate concentration and that this 'problem' could be solved

by increasing aeration. These observations initiated research into EBPR which ultimately led to the full-scale implementation of EBPR technology.

Since then, a number of processes and configurations have been developed and different modifications and engineering plant-layouts have been proposed for their implementation all around the world. Here, the most fundamental EBPR plant configurations and their principles are presented in a chronological order (Figures 6.20A to 6.20I).

6.6.3 PhoStrip® system

The first structured investigation into the P uptake phenomenon was by Levin and Shapiro (1965). In extensive batch studies on the effect of oxygen tension, pH and inhibitors, they demonstrated the biological nature of EBPR removal. Further, in batch tests on two underflow recycle mixed-liquor samples, in which one was aerated and the other not, the aerated sample took up P while the unaerated sample released P. Shapiro and Levin (1965) focussed attention on the P release in anaerobic batch tests and found that the process of P release under anaerobic conditions could be reversed to a process of P uptake if the batch was subsequently aerated. Levin and Shapiro (1965) utilized the phenomena of P release under anaerobic conditions and P uptake under aerobic conditions to patent the first commercial system for P removal, the PhoStrip process (Figure 6.20G, marketed by Biospherics, USA).

Levin *et al.* (1972) report details of this system: 'The process is based on findings that the aeration of mixed liquor can induce activated sludge microorganisms to take up dissolved phosphorus in excess of the amount required for growth. If the air supply is turned off and the sludge organisms are permitted to consume all of the dissolved oxygen the phosphorus previously taken up is released to the liquid phase. However, when aeration is resumed, the microorganisms again take up the dissolved phosphorus'. The PhoStrip process consists of a single aeration tank with clarifier; a sidestream (typically 10-30 per cent of the influent flow rate) from the underflow of the clarifier passes to an anaerobic 'stripping tank' where the sludge settles and P is released. The 'stripped' sludge is returned to the activated sludge system, while the supernatant is dosed chemically (usually with lime) in a precipitator tank, to precipitate released P which is settled and wasted. The supernatant from the precipitator tank is returned to either the influent or the effluent flow.

The PhoStrip combines chemical and biological P removal, applies to non-nitrifying systems, and is a sidestream process. Later modifications proposed to the PhoStrip include the addition of a part of the influent flow to the stripper tank to promote P release (PhoStrip II; Levin and Della Sala, 1987), elutriation of the released P from the 'stripped' sludge by recycling around the stripper tank (Levin and Elster, 1985) and inclusion of an anoxic tank upstream of the aeration tank with recycle of mixed liquor from the aerobic to the anoxic tanks, to apply the PhoStrip principle to nitrifying activated sludge plants. Since the PhoStrip systems include chemical P removal, design procedures for this system will not be considered in this chapter. In the BCFS process the stripper function has been integrated in the anaerobic tank of activated sludge tanks (Van Loosdrecht *et al.,* 1998).

6.6.4 Modified Bardenpho

Although by the early 1970s the phenomenon of EBPR had been observed at a number of full-scale works (*e.g.* Vacker *et al.,* 1967; Scalf *et al.,* 1969; Witherow, 1970; Milbury *et al.,* 1971) and the first commercial EBPR system (the PhoStrip system) had been developed, there was little confidence in EBPR as a potential practical technology. Mulbarger (1970) went so far as to state 'specialized activated sludge plant design for high level P removal should be avoided and treated as a bonus when and if it occurs'. However, from research in the mid-1970s (Fuhs and Chen, 1975; Barnard, 1974a, 1974b, 1975a, 1975b, 1976a, 1976b) one conclusion emerged that made widespread exploitation of the EBPR phenomenon possible: biological P removal is stimulated by subjecting the activated sludge organisms to a

sequence of anaerobic and aerobic conditions. Quantification of the anaerobic state for design and operation, however, presented major problems.

Fuhs and Chen (1975), in a microbiological investigation of EBPR, concluded that the phenomenon is mediated by either a single organism group or several closely related groups. They implicated *Acinetobacter* as the principal organism genus. They concluded that 'anaerobic conditions preceding aerobiosis in sewage treatment could well be related to the appearance of *Acinetobacter* spp. During anaerobiosis, this flora would tend to produce compounds such as ethanol, acetate and succinate, which serve as a carbon source for *Acinetobacter* spp.'. However, Fuhs and Chen did not quantify the 'anaerobic conditions' and developed no practical method for implementation of EBPR.

The first practical mainstream system for EBPR was developed from the work of Barnard (1974a, b, 1975a, b, 1976a, 1976b) and Nicholls (1975). Barnard (1975a, 1975b) while investigating the nitrification/denitrification response of a system he developed for this purpose, the 4-stage Bardenpho system, noted that the system removed more P then expected. Barnard (1974a, 1974b) postulated that 'the essential requirement for phosphorus removal in biological systems is that during some stage before the final stage of the process, the sludge or mixed liquor must pass through an anaerobic stage, during which phosphates may or may not be released, followed by a well aerated aerobic stage, during which the phosphates will either be taken up by the organisms or be precipitated as a result of the change in redox potential'.

Noting the observations of Barnard (1974a), Nicholls (1975) experimented at full-scale with the Alexandra and Olifantsvlei activated sludge systems (Johannesburg, South Africa). He created anaerobic zones in different parts of the two activated sludge systems and concluded that 'good phosphate removal could be expected in the modified Bardenpho system (actually a 5-stage Bardenpho) when an anaerobic basin is placed prior to the activated sludge system'.

Barnard (1976a), in enlarging on the postulations he developed in 1974, concluded that the organism mass 'must pass through an anaerobic phase where the oxygen demand exceeds the supply of both oxygen or nitrates ...' He proposed to produce the anaerobic phase by including an anaerobic reactor prior to the inlet to the plant to 'allow the mixed liquor to become anaerobic through the action of the incoming sewage'. Barnard termed this principle the 'Phoredox' method, and applied it (amongst others) to the 4-stage Bardenpho system; he included an anaerobic reactor before the primary anoxic reactor in the 4-stage Bardenpho, the anaerobic reactor receiving the influent flow and underflow recycle from the secondary settling tanks; this configuration has become known as the 5-stage Modified Bardenpho (Figure 6.20C). Barnard also proposed that when less nitrogen removal is required, the second anoxic and reaeration reactors can be excluded, to give the 3-stage Modified Bardenpho (Figure 6.20A); this configuration has also been called the anaerobic/anoxic/aerobic (A^2O) [1] configuration. To explain the enhanced P removal phenomenon, Barnard (1976a) hypothesized that it is not the P release *per se* that stimulates the P removal, but that the release indicates that a certain low redox potential has been established in the anaerobic zone, *i.e.* that the low redox potential stimulates the enhanced P removal. Barnard (1976a) recognized the difficulties associated with redox potential measurement, and proposed that measurement of P release in the anaerobic zone could serve as a substitute to indicate that conditions necessary for enhanced P removal prevailed.

[1] The original nomenclature of Barnard for anaerobic and anoxic is adopted for use in this chapter; *i.e.* anoxic: a state in which nitrate is present but no oxygen; anaerobic: a state in which neither nitrate nor oxygen is present. The inadequacies of these definitions are apparent when attempting to compare the state of two reactors of the same size, one completely mixed and the other plug flow. A completely mixed anaerobic reactor, for example, will have no nitrate in the reactor; the equivalent plug flow reactor however may contain nitrate for a considerable portion of the reactor length, *i.e.* be partly 'anoxic', partly 'anaerobic' - the inadequacy arises in that no indication is given as to the intensity of the state.

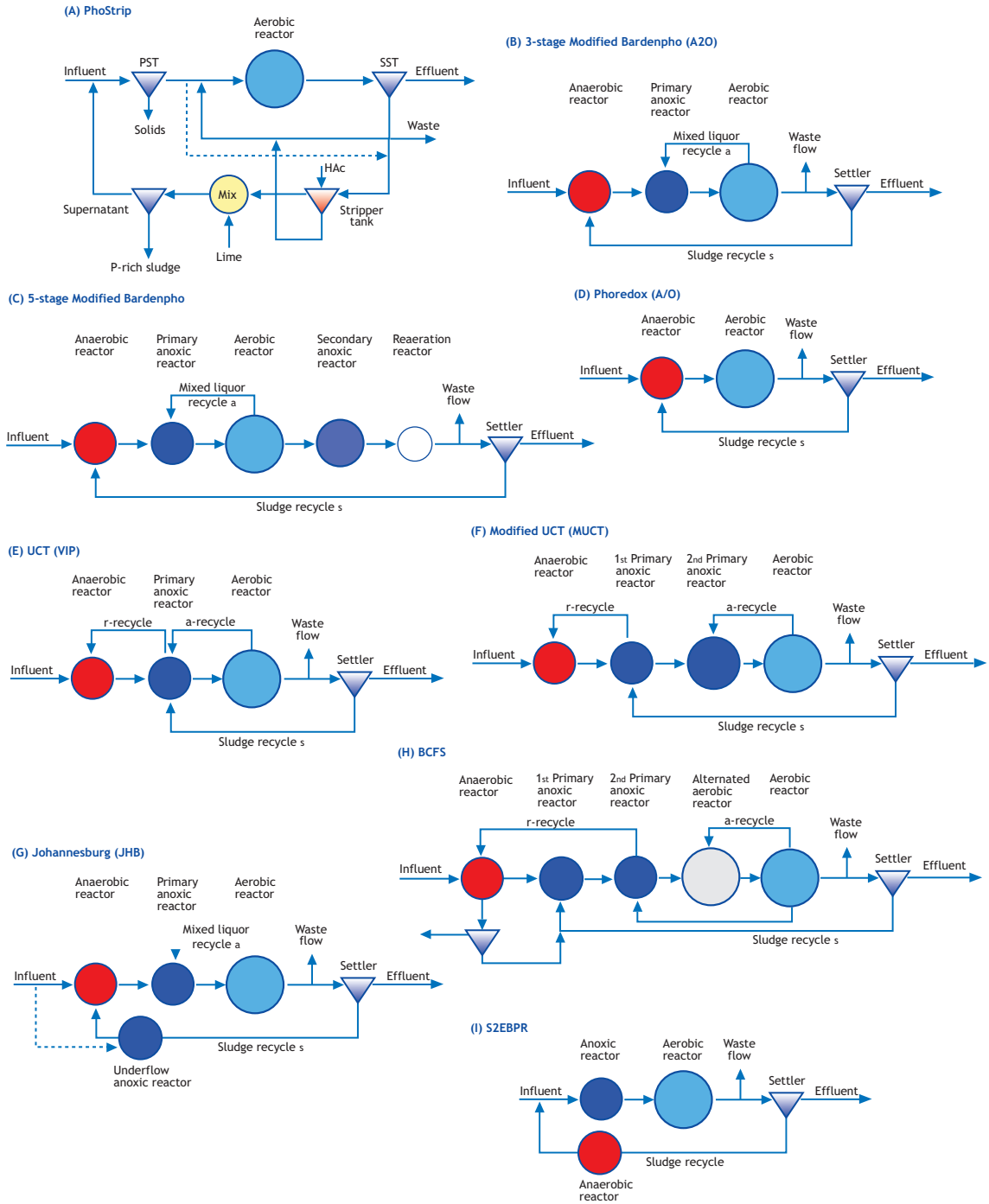


Figure 6.20 System configurations for EBPR: (A) PhoStrip, (B) 3-stage Modified Bardenpho (A2O), (C) 5-stage Modified Bardenpho, (D) Phoredox (A/O), (E) UCT (VIP), (F) MUCT (Modified UCT), (G), Johannesburg (JHB), (H) BCFS, and (I) S2EBPR.

In terms of Barnard's hypothesis, nitrate recycled via the underflow to the anaerobic reactor in the 5-stage Modified Bardenpho will restrain, in some degree, the level to which the redox potential can be lowered and, consequently, nitrate recycled can be expected to influence enhanced P removal adversely, as noted earlier by Barnard (1975b).

Barnard (1976a) apparently accepted that the Modified Bardenpho plant should reduce the nitrate sufficiently that any nitrate in the underflow would not prevent the attainment of the low redox potential necessary for P release in the anaerobic reactor. In any event he considered that nitrate entering the anaerobic reactor could be countered by increasing the retention time of this reactor. For design of the anaerobic reactor, Barnard (1976a) suggested a nominal retention time of one hour. At this stage no rational method for predicting nitrogen and phosphorus removal was available; for design, removals were estimated largely from experience gained in operating experimental systems.

The legal requirement for nitrification in South Africa focussed attention on nutrient removal systems (*i.e.* nitrogen and phosphorus) rather than phosphorus removal only. Consequently, considerable research effort was directed at investigating the Modified Bardenpho system. Early investigations into the 3- and 5-stage Modified Bardenpho systems (McLaren and Wood, 1976; Nicholls, 1978; Simpkins and McLaren, 1978; Davelaar *et al.,* 1978; Osborn and Nicholls, 1978) concurred that nitrate recycled to the anaerobic reactor had a deleterious effect on EBPR and identified that evaluation of the nitrate in the recycle to the anaerobic reactor could be crucial in assessing the success of a nitrifying system in stimulating P release and in determining the magnitude of the P removal. However, none of these investigations provided a reliable model to predict the magnitude of denitrification in order to quantify the nitrate recycled.

Marais and his group (Stern and Marais, 1974; Martin and Marais, 1975; Wilson and Marais, 1976; Marsden and Marais, 1977) recognized the importance of quantifying the nitrate removal. To obtain information on the magnitude and kinetics of denitrification, they replaced the completely mixed reactors in the 5-stage Bardenpho system by plug flow reactors and measured the nitrate along the reactor axes under constant flow and load conditions. Their findings on denitrification kinetics are reviewed in Chapter 5. With regard to relevance to P removal, they found that it was not possible to increase the anoxic zones in this system in order to ensure low nitrate in the effluent and underflow recycles; if, for a selected sludge age and temperature the unaerated mass fraction of the sludge was increased beyond a certain magnitude, the system stopped nitrifying. They showed that the maximum anoxic mass fraction allowable was determined by the maximum specific growth rate of the nitrifiers at the lowest temperature the system would be required to operate, and the sludge age. Limiting the anoxic mass fraction (to ensure nitrification) necessarily limits the magnitude of denitrification achievable. With the systems of the Marais group, when operated at unaerated mass fractions that allowed nitrification, the denitrification was incomplete and the effluent nitrate was high.

Taking note of the findings on the denitrification kinetics, Rabinowitz and Marais (1980) commenced a study on P removal using the Modified Bardenpho system treating unsettled municipal wastewater from the city of Cape Town. They selected the 3-stage Modified Bardenpho (Figure 6.20A) as the basic configuration in preference to the 5-stage (Figure 6.20C) because first, the wastewater source did not allow an unaerated mass fraction of greater than 40 per cent at 14 °C for a sludge age of 20 days if efficient nitrification was to be maintained, and second, taking account that the anaerobic reactor cannot contribute to the system denitrification potential, the 5-stage system could not reduce the nitrate to zero for the measured TKN/COD ratio of the wastewater. Consequently (as discussed earlier in

Chapter 5) the secondary anoxic reactor volume was added to the primary anoxic to obtain the maximum nitrate removal and hence the minimum nitrate concentration in the underflow recycle. The findings of this investigation (Rabinowitz and Marais, 1980) can be summarized as follows:

(i) When the nitrate concentration in the effluent (and underflow recycle) was low, usually P release and enhanced removal were observed. The enhanced P removal decreased quite disproportionately as the nitrate in the underflow recycle increased, a behaviour noted by previous workers (*e.g.* Barnard, 1975b; Simpkins and McLaren, 1978).

(ii) With different batches of wastewater having the same influent COD, with the same concentration of nitrate in the underflow recycle, one wastewater batch gave relatively high rates of P release and enhanced P removal whereas the next gave no (or little) P release and no (or little) enhanced P removal. The reason for this behaviour was not apparent.

In the 3-stage Modified Bardenpho configuration, P removal was disappointing; the system did not give enhanced P removal over lengthy periods of time, and when enhanced P removal was obtained, generally it tended to be low and erratic due to the effects of (*i*) and/or (*ii*) above. Increasing the anaerobic mass fraction during periods of low P removal was found to be counterproductive as this could only be done at the expense of anoxic mass fraction which in turn gave rise to increased nitrate in the recycle. It was finally concluded that for the wastewaters used in the experimental investigation, the Modified Bardenpho type system did not seem suitable for EBPR. This did not imply that the system might not be suitable for other wastewaters, but the investigation did bring to light that there were constraints, not adequately recognized before, that may prevent high P removal. For example, for any selected sludge age and minimum temperature, the requirement for complete nitrification imposes an upper limit on the unaerated mass fraction. This limitation on the unaerated mass fraction correspondingly limits the concentration of nitrate that can be removed. For the 5-stage Modified Bardenpho system, if the nitrate generated is higher than the denitrification achievable, nitrate will appear in the effluent and will be recycled to the anaerobic reactor. For the 3-stage Modified Bardenpho system, complete denitrification is not possible, and nitrate will always be present in the underflow recycle to the anaerobic tank. Recycling of nitrate for both systems will adversely influence the P removal.

Overall, the 3-stage and 5-stage Modified Bardenpho systems showed limitations to achieve stable and reliable P-removal efficiencies. Nevertheless, the work by Barnard led to the development of systems that appeared to incorporate the essential requirements for EBPR even though these requirements were not explicitly understood. This stimulated extensive research into this phenomenon, to gain experience on its behaviour, to delineate more precisely the factors influencing EBPR, and to develop design criteria.

6.6.5 Phoredox or anaerobic/oxic (A/O) system

In the Modified Bardenpho system, the configuration developed by Barnard for EBPR was heavily influenced by the legal requirement for nitrification in South Africa. Should nitrification not be required, the need for anoxic zones (to denitrify) and for long sludge ages (to ensure nitrification) falls away. These aspects were recognized by Barnard (1976a) who also applied the Phoredox method to a non-nitrifying activated sludge system. The configuration for this system was simplified to an anaerobic reactor, receiving the influent and underflow recycle, followed by an aerobic reactor (Figure 6.20B). The sludge age and aerobic tank are designed and controlled to prevent nitrification, *i.e.* short sludge age, high rate plant. This system has become known in South Africa as the Phoredox.

Timmerman (1979) proposed a system, essentially the same as the Phoredox system, which was designated the anaerobic/oxic (A/O) system. The

basic A/O configuration is identical to that of the Phoredox, but with the A/O it is specifically proposed that the anaerobic and aerobic zones are partitioned to give a configuration of a series of reactors that approaches plug flow conditions.

Although proposed conceptually in 1976, the requirement for nitrification in South Africa has prevented implementation of the Phoredox or A/O system. The performance of the system under South African conditions was investigated at laboratory-scale by Burke *et al.* (1986), who found difficulty in preventing nitrification at a temperature of 20 °C even at sludge ages as low as 3 days with an unaerated mass fraction of 50 %.

The A/O system has found wider application in the USA and has been investigated by several researchers (*e.g.* Hong *et al.,* 1983; Kang *et al.,* 1985a, etc.) with mixed results.

6.6.6 University of Cape Town (UCT; VIP) system

Rabinowitz and Marais (1980), in reviewing their unsuccessful endeavours to obtain EBPR consistently in the Modified Bardenpho system, came to the conclusion that, irrespective of other factors that may affect the P removal, the recycling of nitrate to the anaerobic reactor via the underflow recycle appeared to be of great significance (the deleterious effect of the presence of nitrate in the anaerobic reactor subsequently was demonstrated directly by Hascoet and Florentz, 1985, among others). If the nitrate in the underflow to the anaerobic reactor could be kept at a low concentration, then there was a high expectation that consistent EBPR could be obtained. The principal obstacle to attaining this desirable end in the Modified Bardenpho system appeared to be that the nitrate discharged to the anaerobic reactor is linked directly to the concentration of nitrate in the effluent. If, for any reason, the effluent nitrate concentration increased while the COD remained constant, *i.e.* if the influent TKN/COD ratio increased, the system appeared to offer little option to reduce this by operational means. The only operational means available was to reduce the magnitude of the underflow recycle, but this was a risky option as the settleability of the mixed liquor in the plants tended to be poorer than in purely aerobic systems. Accordingly, Rabinowitz and Marais (1980) investigated different system configurations that would shield the anaerobic reactor of any input of nitrate, that is, to make the anaerobic reactor independent of the effluent nitrate concentration. This led to the development of the University of Cape Town (UCT) system (Figure 6.20E).

In the UCT system, the underflow sludge recycle (s) is discharged into the primary anoxic reactor. A further recycle (the r-recycle) draws mixed liquor from the primary anoxic reactor and discharges it into the anaerobic reactor. Mixed liquor is also recycled from the aerobic to the primary anoxic reactor (the a-recycle). By manipulation of the a-recycle ratio, the nitrate in the anoxic reactor can be maintained at zero, and thus no nitrate will be recycled to the anaerobic reactor. Consequently, the anaerobic state in the reactor can be maintained irrespective of the effluent nitrate concentration, even if the influent TKN/COD ratio to the plant varies. This desirable condition is achieved in the UCT system at the expense of (*i*) the anaerobic reactor volume; in the UCT system to maintain the same fraction of sludge in the anaerobic reactor as in the Modified Bardenpho system, the volume of the anaerobic reactor in the UCT system would have to be increased by the proportion (1+r)/r, and (*ii*) the inability to achieve complete denitrification.

Laboratory-scale tests on the UCT system using waste flow from Cape Town showed improved EBPR in both magnitude and consistency over that obtained in the Modified Bardenpho systems. However perhaps the most important achievement from a research point of view was that with the UCT system it was possible to eliminate the confounding effect on P removal of nitrate in the recycle to the anaerobic reactor, so that other factors influencing EBPR could be studied with greater ease (Siebritz *et al.,* 1982). From experimental response data the effects of these other factors became clearly evident:

(*i*) for the same influent COD, one batch of sewage gave high levels of P removal, another gave low, an observation previously presumed to be due to the nitrate effect but not explicitly proven as such and (*ii*) the magnitude of the EBPR appeared to be linked to some characteristic of the wastewater, as yet not identified.

6.6.7 Modified UCT system

Experience with the UCT system (Siebritz *et al.,* 1980, 1982) indicated some problems in system control.

The mixed liquor a-recycle ratio needs to be carefully controlled so that the primary anoxic reactor is slightly underloaded with nitrate to avoid a nitrate discharge to the anaerobic reactor. However, under full-scale operation such careful control of the a-recycle ratio is not possible due to uncertainty in the TKN/COD ratio, particularly under cyclic flow and load conditions.

To simplify the operation of the UCT system, a modification was sought whereby careful control of the a-recycle would not be necessary. This led to a modification of the UCT system called the Modified UCT system (Figure 6.20F). In the Modified UCT system, the anoxic reactor is subdivided into two reactors, the first having a sludge mass fraction of approximately 0.10 and the second having the balance of anoxic mass fraction available. The first anoxic reactor receives the underflow s-recycle and the r-recycle to the anaerobic reactor is taken from it. The second anoxic reactor receives the a-recycle. The minimum a-recycle is that which introduces the minimum nitrate to the second anoxic reactor that is sufficient to load it to its denitrification potential. Any recycle higher than the minimum will not remove additional nitrate so that at higher recycles more nitrate is introduced than removed in the second anoxic reactor and nitrate will appear in the effluent from this reactor. This, however, is immaterial insofar as it affects the nitrate in the aerobic reactor which remains constant once $a > a_{min}$. Consequently, one can raise the a-recycle to any value greater than a_{min}, to give the required actual retention time, without affecting the nitrate recycled to the first anoxic reactor; careful control of the a-recycle is no longer necessary[2]. This improvement however is obtained at a cost (WRC, 1984): the maximum TKN/COD ratio to give zero nitrate to the anaerobic reactor is reduced from ± 0.14 in the UCT system to ± 0.11 in the Modified UCT system. However, a TKN/COD of 0.11 mgN/mgCOD includes most raw and settled municipal wastewaters. Furthermore, by making provision that the r-recycle can be taken from either the first or second anoxic reactor, the system can be operated either in a Modified UCT or a UCT configuration, as may be required.

Another variation of the UCT system has been proposed, namely the Virginia Initiative Plant (VIP; Daigger *et al.,* 1987). The basic configuration for this system is identical to that of the UCT, but two specific proposals are made, (*i*) multiple series of mixed reactors are used, and (*ii*) the system is operated at short sludge ages of 5 to 10 days.

6.6.8 Johannesburg (JHB) system

Taking note of the adverse influence of nitrate recycled to the anaerobic reactor in the 5-stage Modified Bardenpho system as reported by Barnard (1976a), Osborn and Nicholls (1978) in a pilot-scale study at Johannesburg Northern Works proposed to alter the configuration of the 5-stage Modified Bardenpho by moving the secondary anoxic zone from the mainstream flow and repositioning it in the underflow recycle stream. The resulting 4-stage system (anoxic, anaerobic, anoxic, and aerobic) has become known as the Johannesburg (JHB) system

[2] Although from a N and phosphate removal point of view, careful control of the a-recycle is not necessary, the appearance of nitrate and/or nitrite in the effluent from the second anoxic reactor has been linked to the problem of low F/M bulking in nutrient removal systems. Thus, to control low F/M bulking careful control of the a-recycle would be necessary which effectively eliminates the advantage of the MUCT over the UCT system. In BCFS systems the SVI is 100-120 *i.e.* this problem does not exist or the redox control is indeed effective.

(Burke *et al.,* 1986; Nicholls, 1987). In the JHB system (Figure 6.20D), by repositioning the secondary anoxic reactor in the underflow stream, the mass of nitrate that needs to be removed in the secondary anoxic zone to give zero nitrate discharge to the anaerobic reactor is reduced to s/(1+s) times that which needs to be removed in the secondary anoxic zone of the 5-stage Modified Bardenpho system (where s is the sludge recycle ratio with regard to the influent flow rate). That is, to protect the anaerobic reactor from recycling of nitrate, in the JHB system only the nitrate in the s-recycle (underflow) stream has to be removed whereas in the 5-stage Modified Bardenpho system the nitrate in the s-recycle plus effluent streams has to be removed. Also, by positioning the anoxic reactor in the underflow s-recycle the sludge concentration in the secondary anoxic reactor of the JHB system is increased by a factor (1+s)/s compared to the secondary anoxic of the 5-stage Modified Bardenpho system, enabling reduction in reactor size to achieve the same anoxic mass fraction. However, unlike the 5-stage Modified Bardenpho, the JHB system (as for the UCT) cannot achieve complete denitrification. Although the JHB system does overcome the problem in the UCT system of increased anaerobic volume for the same mass fraction, the denitrification is at a lower rate than the UCT primary anoxic reactor. Therefore protection of the anaerobic reactor from nitrate can only be achieved at lower influent TKN/COD ratios than the UCT system, although most wastewaters will fall into the range of operation of the JHB system. Extensive full-scale investigation on the performance of the JHB system has been reported (*e.g.* Nicholls, 1987; Pitman *et al.,* 1988; Pitman, 1991).

6.6.9 Biological-chemical phosphorus removal (BCFS® system)

A further adaptation of the MUCT system was developed in the late 20th century in the Netherlands. This system, named BCFS® (Figure 6.20H), was developed to support the biological process by phosphate stripping and potential recovery in the main line, stabilising the sludge-settling properties and optimising the control of nitrogen removal. In this system a third recycle is added from the aerated reactor to the first anoxic reactor in order to maximise denitrification and to be able to aerate the second anoxic reactor during peak flows. In this way both ammonium and nitrate can be better maintained at low effluent values (ammonium typically below 0.5 gN/l and nitrate around 5-8 mgN/l). The recycle flows are controlled by a simple redox electrode-based controller (Van Loosdrecht *et al.,* 1998). The compartmentation contributes to a stable low SVI (approximately 120 ml/g) (Kruit *et al.,* 2002).

Biological phosphorus removal can be supplemented by addition of precipitants to the anaerobic tank. Since phosphate concentrations are high in this tank the precipitants are used effectively. Dosing chemicals, however, should be done carefully. Too much precipitation will make the phosphate unavailable for PAOs and deteriorate the EBPR efficiency. A complicating factor is that the wastewater treatment plant will respond rapidly to changes in chemical addition whereas the biological phosphorus removal process might have a response time of several days if not weeks. In the BCFS process a small baffle is placed at the end of the plug flow anaerobic tank. The sludge will locally settle back into the anaerobic tank and a clear supernatant can be withdrawn for phosphate precipitation. The phosphorus can then be recovered (Barat and Van Loosdrecht, 2006) or the chemical sludge produced can be prevented from accumulating in the activated sludge which would limit the overall capacity of the plant by reducing the sludge age.

In order to efficiently construct all the tanks in these complex biological nutrient-removal systems, it is possible to change the design from rectangular tanks to one round tank divided into rings for the different aerobic/anoxic/anaerobic zones. In this way the amount of concrete needed is minimised since the inner walls require much less strength then the outer walls of the construction (see Figure 10.1).

6.6.10 Sidestream EBPR (S2EBPR) systems

Due to the relatively low concentrations of RBCOD and VFA present in raw wastewater to perform EBPR and N removal, the fermentation of the primary settled sludge (PST) and/or (a fraction) of the RAS has showed an increase in the EBPR efficiency in certain plants in North America since the 1970s. This has resulted in a robust operation of the EBPR plants leading to effluent P concentrations lower than 1 mg/l, and even below the detection limit (Barnard *et al.,* 2017). The increased and robust EBPR performance is associated with the hydrolysis and fermentation of the PST and/or RAS streams. Moreover, in Denmark in the 1990s, several sidestream EBPR (S2EBPR) configurations were implemented (Figure 6.20I) (Vollertsen *et al.,* 2006) as a means to increase the low availability of RBCOD and VFA in raw wastewater (accounting for only around 150 mg RBCOD/l and 1 mgVFA/l, respectively) (Vollertsen, 2002) to support the EBPR process. In the Danish S2EBPR configurations, a fraction of the RAS is directed (typically 4 to 7 %) to an anaerobic sidestream tank with a hydraulic retention time of 30 to 40 h. The anaerobic conditions and relatively long HRT and high solids concentration (of around 10,000 to 11,000 mgTSS/l) can lead to considerably high hydrolysis and fermentation activity. This can result in the production of sufficient VFA (*e.g.* 136-149 mgRBCOD/l) to promote the EBPR and even support the N-removal process (Vollertsen *et al.,* 2006). Furthermore, if needed, the RBCOD production can be increased through the addition of primary sludge, molasses or other carbon sources. Smolders *et al.* (1996) carried out a steady-state model analysis. They found out that the acetate requirements for a S2EBPR process (in a P- stripping tank) are lower than those requirements needed in a mainstream process and that the active PAO biomass required to support the EBPR process can be up to 10 times lower.

The increasingly stricter N and P discharge limits and the limited availability of RBCOD in raw wastewater have promoted the implementation of S2EBPR worldwide since 2000, contributing to a broad development and assessment of different configurations, and to deeper and more thorough physiological and molecular analysis. Whereas the increased EBPR performance appears to be linked to a higher abundance of *Tetrasphera* in several S2EBPR plants (due to its fermentative capabilities) (Stokholm-Bjerregaard *et al.,* 2017), *Accumulibacter* has still also been seen in relatively abundant quantities (Onnis-Hayden *et al.,* 2019; Wang *et al.,* 2019), although the role, interaction and contribution of these microorganisms is still not fully elucidated. Nevertheless, less GAO have been observed in S2EBPR systems, which could be reflected in a more stable EBPR performance (Stokholm-Bjerregaard *et al.,* 2017; Onnis-Hayden *et al.,* 2019; Wang *et al.,* 2019).

However, although the actual mechanisms that favour the EBPR process in S2EBPR processes are still unknown, overall the higher RBCOD availability, combined with the lower VFA and PAO biomass requirements to achieve EBPR, and the lower occurrence of GAO seem to contribute to the increased robustness of the S2EBPR systems.

6.7 MODEL DEVELOPMENT FOR EBPR

6.7.1 Early developments

When the first mainstream nitrification-denitrification EBPR (NDEBPR) system was proposed, the 5-stage Modified Bardenpho system (Barnard, 1976b), initial conceptualization extended little beyond recognition of (*i*) the necessity of an anaerobic/aerobic sequence of reactors, and (*ii*) the adverse influence of nitrate recycled to the anaerobic zone. Design procedures were based on empirically-based estimates for sizing denitrification and anaerobic reactors in terms of nominal hydraulic retention time and sizing of the anaerobic reactor appeared to be linked to reduction of the redox potential below some critical value. No rational method for predicting N and P removal was available and for design, removals were estimated largely from

experience gained in operating experimental systems similar to the proposed systems.

6.7.2 Readily biodegradable COD

In seeking an explanation for the different P release and enhanced P removal behavioural patterns in lab-scale Modified UCT and MLE systems, Siebritz *et al.* (1980, 1982) applied the concept of readily biodegradable COD (RBCOD) (see Section 6.4.5) developed in denitrification and aerobic studies (Dold *et al.,* 1980) to EBPR systems. They noted that the only evident difference between the Modified UCT and MLE systems lay in the concentration of RBCOD surrounding the organisms in the anaerobic reactor (S_{VFA}). In the Modified UCT system the RBCOD concentration in the anaerobic reactor (S_{VFA}) is the maximum possible as no nitrate is recycled to the anaerobic reactor. In contrast, in the MLE system sufficient nitrate is recycled to the anoxic reactor to utilize all the RBCOD, *i.e.* S_{VFA} = 0. Therefore, the different behavioural patterns of the processes would be consistently described if it is assumed that the concentration of RBCOD in the anaerobic reactor (S_S) surrounding the organisms is the key parameter determining whether or not P release and enhanced P removal takes place. In terms of our present understanding of EBPR, the parameter S_{VFA} is theoretical and cannot be measured; from the mechanisms of EBPR, the concentration of RBCOD surrounding the organisms in the anaerobic reactor does not equal S_{VFA} due to the conversion of the fermentable COD to VFAs by OHOs and the storage of VFAs by PAOs in the anaerobic reactor (see Section 6.4.5).

6.7.3 Parametric model

Extensive research over a year into the validity of the readily biodegradable COD (RBCOD) hypothesis by Siebritz *et al.* (1983) established that P release appears to be induced if the RBCOD in the anaerobic reactor, S_S, exceeds approximately 25 mg/l, and the P release and enhanced removal increases as (S_{VFA} - 25) increases. That is, the P removal is linearly related to the RBCOD concentration in the anaerobic reactor. This opened the way for enquiry into other factors affecting the P release and enhanced removal, and quantification of the enhanced P removal. They came to the conclusion that enhanced P removal depends on three factors (*i*) (S_{VFA} - 25), (*ii*) the fractional mass of sludge in the system passing through the anaerobic reactor, and (*iii*) the actual time a unit of sludge is retained in the anaerobic reactor.

They hypothesized that if any one of these is zero, no EBPR is obtained. Empirically these three factors are combined in a phosphorus-removal propensity factor. It was found that the mass of phosphorus in the sludge relative to the active mass could be functionally related to the P-removal propensity factor. Further investigation showed that in the modified Bardenpho and UCT (and although not considered, JHB) systems, with their respective recycles and their interactive effects on the anaerobic retention time, the factors (*ii*) and (*iii*) above could be combined by a single parameter, that is, the three parameters could be reduced to two key parameters: (*i*) (S_{VFA} - 25) and (*ii*) the anaerobic mass fraction, defined by (mass of sludge in the anaerobic reactor)/(total mass of sludge in the system).

Based on this observation, EBPR was formulated empirically in terms of the two key parameters and the mass of sludge (active, endogenous and inert) wasted per day, to give the parametric model.

Extensive testing of the concepts embodied in the parametric model did, in general, verify the utility of the model. At laboratory-scale, employing the UCT system, the concepts were tested at different sludge ages, temperatures, anaerobic mass fractions and influent COD concentrations in which the RBCOD fraction of the influent (unsettled municipal sewage) was augmented by addition of glucose or acetate. All these tests gave results consistent with the predictions based on the RBCOD concept embodied in the parametric model.

At full-scale, in a joint research project with Johannesburg City Council on the Goudkoppies and

Northern Works, analysis of the systems in terms of these concepts provided a consistent explanation when efficient or inefficient P removal was obtained (Nicholls, 1982). Thus, for the first time the parametric model allowed a quantitative approach to the design of N and P removal plants, and a basis for evaluating the performance of existing plants (Ekama *et al.,* 1983). For a detailed treatise on the parametric model the reader is referred to WRC (1984).

6.7.4 Comments on the parametric model

The parametric model described above was developed from observed data on experimental systems operated under a range of conditions, as follows:

- Influent COD concentrations: 250-800 mgCOD/l
- Readily biodegradable COD: 70-220 mgCOD/l, *i.e.* representing a f_{SS} fraction of between 0.12-0.27 mgRBCOD/mgCOD_{total}
- TKN/COD ratio: 0.09-0.14 mgN/mgCOD
- Sludge age: 13 and 25 days
- Temperature: 14 °C and 20 °C
- Anaerobic mass fraction: 0.10-0.20 gVSS_{AN}/gVSS_{sys}

Observations under these conditions formed the basis for structuring the formulations for estimating the enhanced P removal, and the equations thus derived were calibrated against the observed data. A comparison of the theoretically predicted and experimentally measured P removal data for the conditions set out above show a good correlation. However, despite the evident utility of the parametric model, it is still an empirical one; it links observable parameters but does not provide any explanation as to why these parameters are important to the phenomenon and it is independent of any formal hypothesis on the biological mechanisms driving the process. Accordingly, application of the parametric model had to be limited strictly to within the ranges of system parameters and wastewater characteristics listed above. What was required was a model with a more fundamental basis.

Essentially, up to this time the description of nitrification denitrification biological P removal (NDEBPR) system behaviour did not recognize the presence of any specific organism implicated in EBPR. The parametric model in fact considered the active sludge as a whole, to constitute a surrogate sludge with a propensity for P removal; variation in EBPR between different systems was hypothesized to be due to changes in the propensity for P removal of this surrogate sludge, caused by changes in influent RBCOD concentration, anaerobic mass fraction and/or nitrate discharge to the anaerobic reactor. However, parallel research in the natural sciences had identified specific organism groups that have the propensity to store large quantities of P in the form of polyphosphates. This led to a shift in the approach to modelling EBPR in NDEBPR systems, from a surrogate sludge to specific organism groups that are responsible for EBPR process, generically termed polyphosphate organisms (Wentzel *et al.,* 1986), bio-P organisms (Comeau *et al.,* 1986) and phosphorus-accumulating organisms (PAOs; Henze *et al.*, 1999).

6.7.5 NDEBPR system kinetics

Wentzel *et al.* (1988) set out to develop a general model that describes NDEBPR system behaviour. They assumed that in an NDEBPR system treating municipal wastewater, a mixed culture would develop which could be categorized into three groups of organisms: (*i*) heterotrophic organisms able to accumulate polyphosphate, termed phosphorus-accumulating organisms (PAOs), (*ii*) heterotrophic organisms unable to accumulate polyphosphate, termed ordinary heterotrophic organisms (OHOs), and (*iii*) autotrophic organisms mediating nitrification, termed nitrifying organisms (NIT). Wentzel *et al.* (1985, 1988) recognized that development of an activated sludge model to describe the behaviour of NDEBPR systems would require inclusion of all three organism groups, and their interactions. With regard to OHOs and NIT, they accepted the nitrification denitrification (ND) steady-state model described earlier and the general ND kinetic model (Dold *et al.,* 1991) (chapters 4 and

5). These models now needed to be extended to incorporate PAO behaviour in order to develop models that would include all three organism groups: general NDEBPR, steady state, and kinetic models. To achieve this objective, the kinetic and stoichiometric characteristics of the PAOs in the activated sludge environment needed to be established. From attempts to obtain information on the characteristics of the PAOs using mixed liquor from NDEBPR systems treating municipal wastewaters, Wentzel *et al.* (1988) concluded that, in these mixed culture systems, the OHO behaviour tends to dominate and mask the PAO behaviour. Accordingly, they endeavoured to isolate the PAO characteristics by enhancing the growth of the PAOs in the mixed-culture activated sludge systems. By enhanced culture is meant a culture in which (*i*) the growth of PAOs is favoured to the extent that they become the dominant primary organism and their behaviour dominates the system response, and (*ii*) the growth of competing organisms is curtailed but not positively excluded; neither are predation or other interaction effects. Wentzel *et al.* (1988) proposed to achieve a PAO-enhanced culture by taking mixed liquor from a mixed-culture NDEBPR system and selecting a substrate and a set of environmental conditions in the activated sludge system that would greatly favour PAO growth and enrichment.

6.7.6 Enhanced PAO cultures

6.7.6.1 Enhanced culture development

From the biochemical models, Wentzel *et al.* (1988) were able to identify conditions to be imposed in an NDEBPR activated sludge system to produce an enhanced PAO culture: an anaerobic/aerobic sequence with an adequate anaerobic mass fraction; influent fed to the anaerobic reactor with acetate as substrate and with adequate macro- and micronutrients, in particular Mg^{2+}, K^{+} and to a lesser degree Ca^{2+}, and pH control in the aerobic reactor. Using the UCT and 3-stage Modified Bardenpho systems, with system sludge ages ranging from 7.5 to 20 days, they developed enhanced cultures of PAOs with greater than 90 % of the organisms cultured aerobically being identified as *Acinetobacter* spp. using the Analytical Profile Index (API) 20NE procedure[3]. The response of the enhanced culture systems indicated that significant concentrations of PAOs developed. For example, the UCT system (anaerobic mass fraction 15%, sludge age 10 days and influent of acetate at 500 mgCOD/l) gave phosphate release of ≈ 253 mgP/l, phosphate uptake of ≈ 314 mgP/l and phosphate removal of ≈ 61 mg/l, all as mgP/l influent flow. This EBPR behaviour was much higher than observed in mixed culture NDEBPR systems with municipal wastewater influent of 500 mgCOD/l giving a phosphate release of ≈ 45 mg/l, phosphate uptake of ≈ 57 mg/l and phosphate removal of ≈ 12 mgP/l. The enhanced culture of mixed liquor in the aerobic zone contained ≈ 0.38 mgP/mgMLVSS and had a VSS/TSS ratio of ≈ 0.46 mgVSS/mgTSS, much higher than for mixed culture systems at a P/MLVSS ratio of ≈ 0.1 and a VSS/TSS fraction of ≈ 0.75. The low VSS/TSS ratio for the enhanced culture systems was due to the large amounts of polyphosphate with associated counter ions stored by the PAOs (Ekama and Wentzel, 2004).

6.7.6.2 Enhanced culture kinetic model

From experimental observations on the enhanced culture steady-state systems, and on batch tests in which mixed liquors drawn from the steady-state systems were subjected to a wide variety of conditions, Wentzel *et al.* (1989a) elucidated the characteristics and kinetic response of the active PAO biomass. Two characteristics of the PAOs in the enhanced cultures were of particular interest:

(i) Uncertain capacity to denitrify so that no provision for this process needed to be made in modelling PAO behaviour (the uncertain denitrification activity of PAOs has implications

[3]The API 20NE procedure has subsequently been shown to overestimate *Acinetobacter* spp. numbers due to the testing technique (Venter *et al.,* 1989) and selection in culturing (*e.g.* Wagner *et al.,* 1994). However, for the development of the design and simulation models, exact identification of the PAOs in the enhanced cultures has been of minor consequence as the models are based on quantitative experimental observations.

for modelling denitrification in mixed-culture NDEBPR systems, see Section 6.11).

(ii) An extremely low endogenous mass loss rate, 0.04 mgAVSS/mgAVSS.d which is much lower than that of OHOs in an aerobic activated sludge system at 0.24 mgAVSS/mgAVSS.d (McKinney and Ooten, 1969; Marais and Ekama, 1976). A similar observation had been made by Wentzel *et al.* (1985) in studies on mixed-culture NDEBPR systems treating municipal wastewaters; they noted from plots of phosphate uptake *versus* phosphate release for various sludge ages that, for a given phosphate release, the phosphate uptake was relatively insensitive to sludge age. To explain this observation, Wentzel *et al.* (1985) proposed that the PAOs 'suffer little or no endogenous mass loss'. The high specific endogenous mass loss rate with OHO systems had been attributed to a high rate of predation and regrowth, formulated as death regeneration in the ND kinetic model by Dold *et al.,* (1980). However, the low specific endogenous mass loss rate with PAOs in enhanced culture systems and the observations of Wentzel *et al.* (1985) led Wentzel *et al.* (1989a) to conclude that PAOs are not predated to the same degree as OHOs. Accordingly, in modelling PAO endogenous mass loss, Wentzel *et al.* (1989a) used the classical endogenous respiration approach, except that provision was made for situations where no external electron acceptor is available.

Taking note of the above, Wentzel *et al.* (1989a) developed a conceptual model for PAO behaviour in the enhanced cultures incorporating the characteristics, processes and compounds identified as important from the experimental investigation. Using the conceptual model as a basis, Wentzel *et al.* (1989a) formulated mathematically the process rates and their stoichiometric interactions with the compounds, to develop a kinetic model for the enhanced cultures of PAO. As recommended by the IAWPRC Task Group (Henze *et al.,* 1987), this model was presented in a matrix format with the kinetic and stoichiometric constants of the enhanced cultures being quantified by a variety of experimental procedures (Wentzel *et al.,* 1989b). Thus the PAO model, when integrated with the OHO and NIT simulation model, became known as UCTPHO (Wentzel *et al.,* 1992).

With these constants, application of the kinetic model to the various test responses observed with the enhanced cultures gave a good correlation between observations and simulations (figures 6.21 to 6.23). The model was then applied to simulate the steady-state behaviour of the enhanced culture UCT and 3-stage Modified Bardenpho systems, for which good correlation was obtained again (Wentzel *et al.,* 1989b).

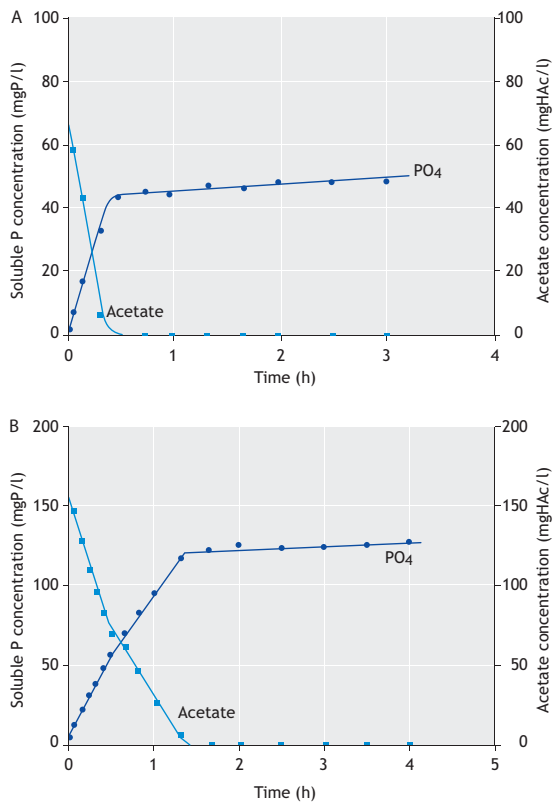


Figure 6.21 Experimentally observed and simulated total soluble phosphorus (PO_4) and acetate concentration-time profiles with the anaerobic addition of (A) 0.11 $mgCOD_{acetate}$/mgVSS and (B) 0.265 mgCOD/mgVSS to a mixed liquor drawn from an enhanced Bardenpho culture system (after Wentzel *et al.,* 1989b).

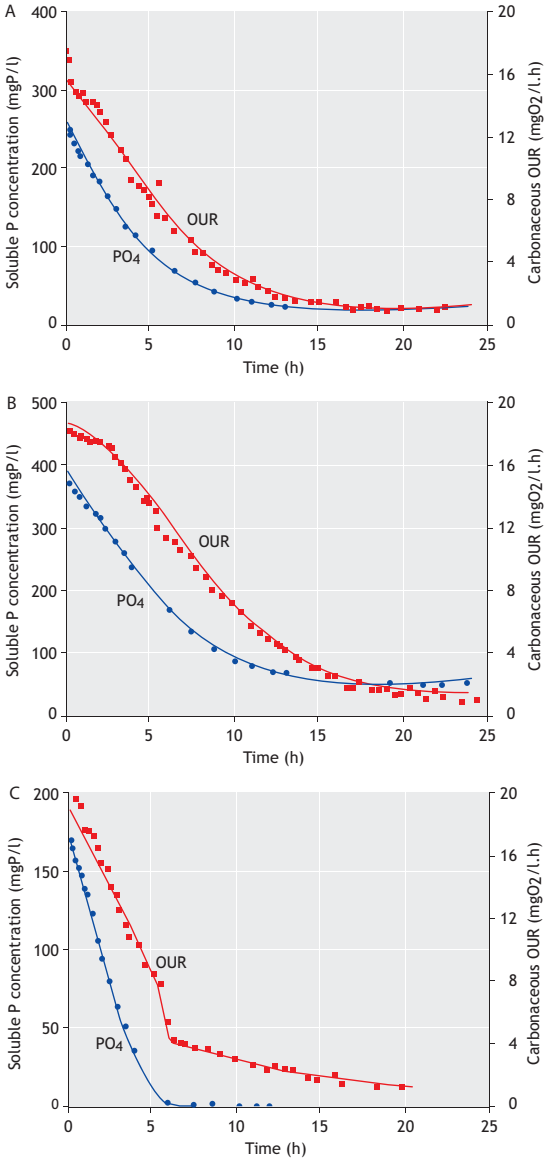


Figure 6.22 Experimentally observed and simulated total soluble phosphorus (PO_4) concentration and carbonaceous oxygen utilisation rate (OUR)-time profiles on aeration following the anaerobic acetate addition of (A) 0.207 mgCOD/mgVSS, (B) 0.363 mgCOD/mgVSS and (C) 0.22 mgCOD/mgVSS to mixed liquor drawn from an enhanced Bardenpho culture system. The PO4 concentration fell to zero during the course of the (C) test (after Wentzel *et al.*, 1989b).

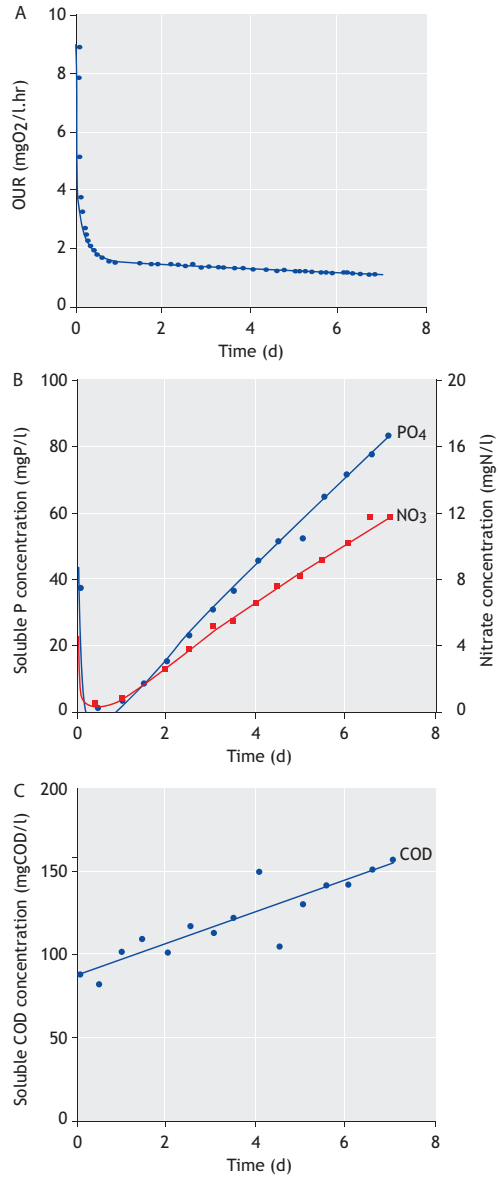


Figure 6.23 Experimentally observed and simulated (A) oxygen utilization rate (OUR), (B) total soluble phosphorus (PO_4) and nitrate (NO_3) concentrations and, (C) filtered COD concentrations with time in a batch digestion of mixed liquor from an enhanced culture system (after Wentzel *et al.*, 1989a).

6.7.6.3 Simplified enhanced culture steady state model

Wentzel *et al.* (1990) simplified the enhanced culture kinetic model to develop a steady-state model for the enhanced culture systems under constant flow and load conditions. From an examination of the kinetics of the processes under steady-state conditions, they found many of the processes to be virtually complete; these kinetic relationships no longer served any function and could be replaced by stoichiometric relationships. For example:

- The anaerobic mass fractions provided in the enhanced culture systems were sufficient to ensure that all the acetate substrate was sequestered in the anaerobic zone, *i.e.* the kinetics of acetate storage need not be incorporated.
- Virtually all the substrate sequestered in the anaerobic zone was utilized in the subsequent aerobic zone, *i.e.* the kinetics of PHA substrate utilization (and polyphosphate storage) did not need to be incorporated.

They noted that these simplifications implied that for a given sludge age, a constant relationship exists between the mass of acetate fed to the system and the mass of PAOs formed with stored polyphosphate. Further, they made an assumption which simplified the development of the steady state model:

- P release for anaerobic maintenance energy requirements is always small compared to phosphate release for VFA storage energy requirements, *i.e.* the kinetics of phosphate release for anaerobic maintenance energy did not need to be incorporated.

They further rationalized that the simplifications and assumptions imply that under steady state the polyphosphate content of the PAOs in the activated sludge is constant at 0.38 $gP/gVSS_{PAO}$, and independent of sludge age. What does vary is the relative proportion of PAOs (with stored polyphosphate) in the activated sludge. Taking due account of the simplifications and assumptions, Wentzel *et al.* (1990) developed a number of steady-state equations for the enhanced cultures, for PAO active and endogenous masses and for phosphate release, uptake and removal due to these masses. These equations provided the means for quantifying the PAO population in mixed-culture NDEBPR systems receiving municipal wastewaters as influent.

6.7.7 Steady-state mixed-culture NDEBPR systems

6.7.7.1 Mixed-culture steady-state model

Having developed the steady-state model for enhanced culture systems, Wentzel *et al.* (1990) extended this model to incorporate mixed cultures of PAOs and OHOs present in NDEBPR systems receiving domestic wastewater as influent, to give a steady-state mixed-culture model. This extension proved possible because (*i*) enhanced cultures rather than pure cultures were used to establish the kinetic and stoichiometric characteristics of the PAOs. In the enhanced cultures, PAOs present in mixed-culture activated sludge were enriched and no single species were artificially selected (as in pure cultures), (*ii*) competing organisms and predators were not artificially excluded (as in pure cultures) so that the PAOs were subjected to the same selective pressures in enhanced as in mixed cultures, (*iii*) the PAOs were also subjected to the same conditions present in mixed-culture activated sludge systems (*e.g.* anaerobic/aerobic sequencing, long SRT > 5 days, etc.), and (*iv*) the PAOs exhibited the same behavioural patterns in the enhanced cultures as they did in mixed-culture activated sludge systems (*i.e.* P release/uptake, PHA/polyphosphate accumulation, etc.). In fact, the similar, though 'magnified' behaviour of the enhanced culture compared to the mixed-culture systems was one criterion used to establish that the correct enhanced cultures had been established.

In extending the model one aspect that emerged was the difference in the endogenous mass loss rate between PAO-enhanced culture sludges and the 'normal' aerobic-OHO activated sludge. As noted earlier, the high rate of specific endogenous mass

loss with OHO systems had been attributed to a high rate of predation and regrowth, formulated as death regeneration in the ND kinetic model by Dold *et al.* (1980). However, the low rate of specific endogenous mass loss with PAOs in the enhanced-culture systems led Wentzel *et al.* (1989a) to conclude that the PAOs were not predated to the same degree as OHOs, and to adopt an endogenous respiration approach in modelling PAO endogenous mass loss[4]. The low predation rate on the PAOs, and the fact that the PAOs and OHOs essentially do not compete for the same substrate, implied that PAO and OHO populations act virtually independently of each other in 'normal' mixed-culture NDEBPR systems. In developing the steady-state model for mixed-culture NDEBPR systems, Wentzel *et al.* (1990) noted that this implied that analysis of the two population groups could be largely separated. However, two significant interactions were identified for inclusion in the mixed-culture NDEBPR steady-state model, both in the anaerobic reactor, as follows:

(i) In many 'normal' municipal wastewaters the acetate or other volatile fatty acid (VFA) content is small or not present (Wentzel et al., 1988). Wentzel et al. (1985) showed that in the anaerobic reactor the RBCOD component of the influent is converted into VFAs by acid fermentation by the OHOs, thereby making VFAs available to the PAO mass for storage. The rate of conversion is much slower than the rate of storage for PHA, so that the rate of conversion controls the rate of storage. Hence, the mass of VFA substrate that becomes available in the anaerobic reactor to the PAOs is governed by the kinetics of conversion mediated by the OHOs. The work of Meganck *et al.* (1985) and of Brodisch (1985) supported this conversion hypothesis as they showed that anaerobic/aerobic systems develop organisms which convert sugars and similar compounds into VFAs in the anaerobic reactor.

(ii) If nitrate (or oxygen) is recycled to the anaerobic reactor, RBCOD is utilized preferentially by the OHOs with nitrate (or oxygen) as the external electron acceptor, thereby reducing the mass of RBCOD converted to VFAs.

Wentzel *et al.* (1985) recognized the above points and formulated a kinetic model for conversion of RBCOD to VFAs, and hence for storage of these VFAs. Wentzel *et al.* (1990) accepted this model, but made provision to include situations where VFAs are present in the influent by noting that:

- The RBCOD needs to be subdivided into two fractions, VFAs/RBCOD (*e.g.* acetate) and fermentable S_F/RBCOD (*e.g.* glucose). Both these fractions will be measured as RBCOD in conventional bioassay (*e.g.* Ekama *et al.,* 1986; Wentzel *et al.,* 1995) and filtration (*e.g.* Dold *et al.,* 1986; Mamais *et al.,* 1993; Wentzel *et al.,* 1995) tests, *i.e.*:

  RBCOD = VFAs + fermentable COD (6.1a)

  or, in symbols

  $$S_S = S_{VFA} + S_F \quad (6.1b)$$

- The rate of VFA storage is so rapid that all influent VFAs will be sequestered by the PAOs in the anaerobic reactor for anaerobic mass fractions greater than 10 % and sludge ages greater than 10 days (this can be verified from the kinetics of storage).
- The fermentable COD is converted into VFAs by the OHOs in the anaerobic reactor, and the resultant VFAs are available for storage by the PAOs. The model for conversion is given by Wentzel *et al.* (1985).

This theory provided Wentzel *et al.* (1990) with the means of calculating the mass of VFA substrate (from the influent and from conversion of fermentable COD) sequestered by the PAOs in the anaerobic reactor. Knowing the mass of substrate

[4] From subsequent simulations with the steady-state mixed culture model, it was found that if the PAOs were subjected to a high predation rate, then significant EBPR in the mixed-culture NDEBPR system would not be possible; the rate of death of the PAOs would be so high that no significant mass of these organisms could accumulate in the system, and EBPR would be near zero.

sequestered by the PAOs, the mass of substrate remaining that is available to the OHOs could be calculated. In effect, Wentzel *et al.* (1990) split the biodegradable influent COD into two fractions, one eventually to be utilized by the PAOs and the other to be utilized by the OHOs. Because of the independent action of these two groups of organisms, they could use:

(i) The simplified PAO-enhanced culture steady-state model for calculating the PAO active and endogenous masses formed from the sequestered substrate, and the P release, uptake and removal performed by these masses.

(ii) The steady-state activated sludge model (Marais and Ekama, 1976; WRC, 1984, Chapter 4) to calculate the OHO active and endogenous masses formed from the remaining substrate, the rate of conversion of fermentable COD to VFAs in the anaerobic reactor, the inert VSS accumulated from the influent, and the P requirement of, and hence P removal associated with, the active, endogenous and inert masses. Note that in this steady-state activated sludge model, the endogenous mass loss is modelled using the classical endogenous respiration approach; this approach is simpler and under steady-state conditions gives results very close to the death regeneration approach.

The total P removal for the system was calculated by summation of the individual P removals.

Wentzel *et al.* (1990) evaluated the predictive power of the steady-state mixed-culture EBPR model against observations made of 30 laboratory scale NDEBPR systems over a six-year period. The system configurations were Phoredox, 3-stage Modified Bardenpho, UCT, MUCT and Johannesburg with system sludge ages ranging from 3 to 28 days. For the evaluation, the measured nitrate in the recycle to the anaerobic zone was used to estimate the fermentable COD removal in the anaerobic zone by the OHOs with nitrate as the external electron acceptor. The fermentable COD remaining was available for conversion in the anaerobic reactor to VFAs, for storage as PHA by the PAOs. Plots of the predicted and measured P releases, P removals and VSS concentrations (figures 6.24 to 6.26) show good correlation.

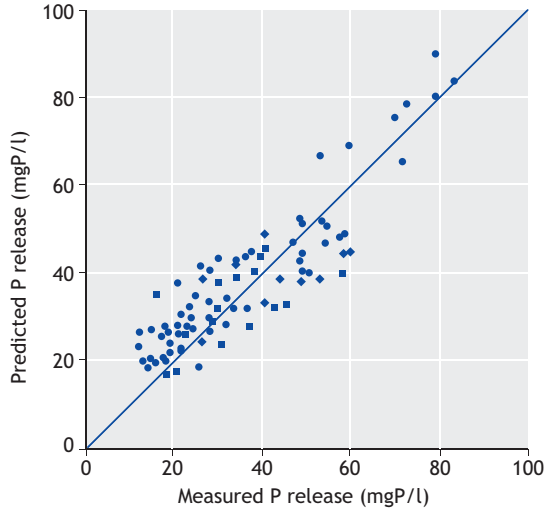


Figure 6.24 Predicted *versus* measured P release in a variety of EBPR systems with various configurations for SRTs from 3 to 28 d (after Wentzel *et al.*, 1990).

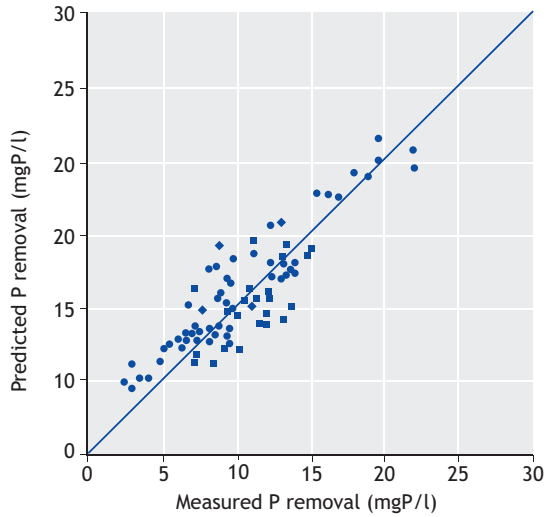


Figure 6.25 Predicted *versus* measured P removal in a variety of EBPR systems with various configurations for SRTs from 3 to 28 d (after Wentzel *et al.*, 1990).

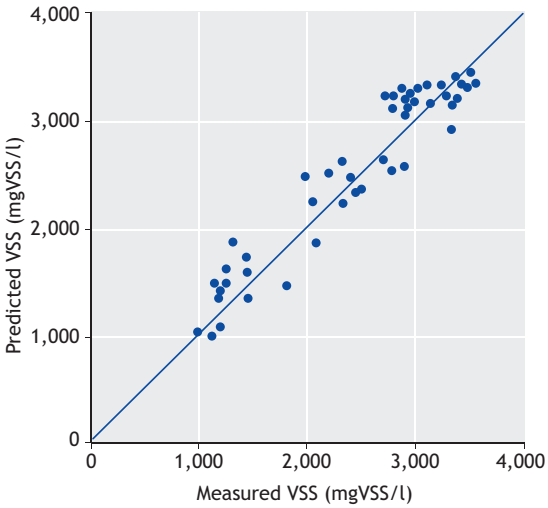


Figure 6.26 Predicted *versus* measured VSS concentration in a variety of biological enhanced P-removal systems with various configurations for SRTs from 3 to 28 d (after Wentzel *et al.*, 1990).

6.8 MIXED-CULTURE STEADY-STATE MODEL

6.8.1 Principles of the model

The fundamental principle underlying the mixed-culture steady-state model is to divide the activated sludge between three population groups:

1. NIT, the nitrifiers;
2. OHOs, the ordinary heterotrophic organisms; and
3. PAOs, the phosphorus-accumulating organisms.

Then, knowing the P content of the sludge fractions generated by each population group (active, endogenous and inert), the P removal for each sludge fraction can be calculated and the system P removal will be given by the summation of the individual P removals.

Procedures for quantification of the NIT are presented in Chapter 5; these procedures can be retained unmodified for nitrifying and denitrifying EBPR systems provided the unaerated mass fraction (f_{xa}) is extended to include both the anoxic and anaerobic reactors. The relatively small contribution that the NIT make to the sludge mass (< 3 per cent) means that the P removal due to this population group can be neglected.

With regard to the OHOs and the PAOs, the principle is to split the biodegradable COD between the two population groups and to calculate the masses that result from the two COD fractions (Figures 6.10 and 6.27); knowing the P content of each mass, then the P removal can be calculated. Procedures for quantification of the OHOs (including inert mass) are presented in Chapter 4; these can be applied to nitrifying and denitrifying EBPR systems, but need to be modified to take account of the biodegradable COD reduction due to COD storage by the PAOs, see below. In this section, procedures will be presented for quantification of the PAOs and OHOs and for dividing the biodegradable COD between the PAOs and OHOs.

The relationship between the flux of the influent biodegradable COD components, their fate in the treatment system and the active biomass produced is illustrated in Figure 6.28 and explained in the following sections.

The sludge biomass is composed of active and inactive particulate fractions. The active fractions include the biomass components of PAOs, OHOs and other biomasses such as nitrifiers that do not need to be calculated in this design example. Inactive components include particulate inert organics and particulate inorganics from the influent, and particulate endogenous residues generated by cell decay.

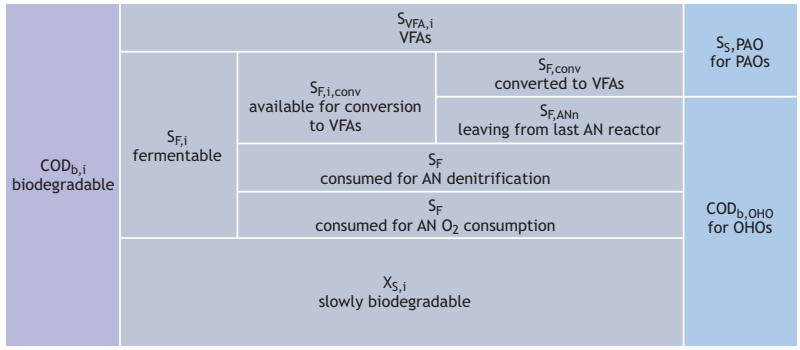
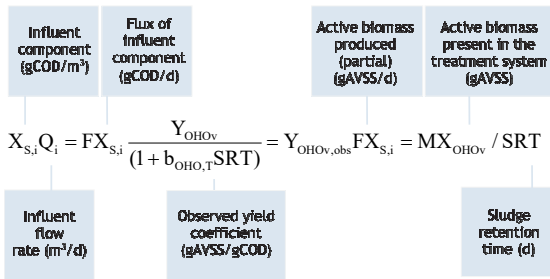


Figure 6.27 Division of influent biodegradable COD between PAOs and OHOs.



(6.1)

Figure 6.28 Relationships between the influent components, flux and biomass produced and present in the system.

6.8.2 MASS EQUATIONS

6.8.2.1 PAOs

Biological active mass:

$$MX_{PAOv} = \frac{Y_{PAOv}}{(1+b_{PAO,T}SRT)}FS_{S,PAO}SRT \qquad (6.2)$$

where:

MX_{PAOv}	biological active mass of PAO (gAVSS)
Y_{PAOv}	PAO biomass yield (gAVSS/gCOD)
$FS_{S,PAO}$	daily mass of substrate stored by PAOs in the anaerobic reactor (gCOD/d)
$b_{PAO,T}$	specific endogenous mass loss rate constant of PAO at temperature T (gEVSS/gVSS.d)
SRT	sludge age (d)

Endogenous mass:

$$MX_{E,PAOv} = f_{XE,PAO}b_{PAO,T}MX_{PAOv}SRT \qquad (6.3)$$

where:

$MX_{E,PAOv}$	PAO endogenous mass (gEVSS)
$f_{XE,PAO}$	fraction of endogenous particulate residue of PAOs (gEVSS/gAVSS)

6.6.2.2 OHOs

Biological active mass:

$$MX_{OHOv} = \frac{Y_{OHOv}}{(1+b_{OHO,T}SRT)}FCOD_{b,OHO}SRT \qquad (6.4)$$

where:

MX_{OHOv}	OHO active biomass (gAVSS)
$FCOD_{b,OHO}$	daily mass of biodegradable substrate available to OHOs (gCOD/d) $= FCOD_{b,i} - FS_{S,PAO}$
$FCOD_{b,i}$	daily mass of influent biodegradable COD (gCOD/d) $= FCOD_i (1 - f_{SU} - f_{XU})$
Y_{OHOv}	OHO yield (gAVSS/gCOD)
$b_{OHO,T}$	specific endogenous mass loss rate constant of OHO at temperature T (/d)

Endogenous mass:

$$MX_{E,OHOv} = f_{XE,OHO} b_{OHO,T} MX_{OHOv} SRT \quad (6.5)$$

where:

$MX_{E,OHOv}$ mass of endogenous residue in the system (gEVSS)

$f_{XE,OHO}$ fraction of endogenous particulate residue of OHOs (gEVSS/gAVSS)

6.8.2.3 Inert mass

Inert organic matter from the influent accumulates in the system:

$$MX_{Uv} = \frac{f_{XU,CODi} FCOD_i SRT}{f_{cv}} \quad (6.6)$$

where:

MX_{Uv} mass of inert organic matter in the system coming from the influent (gIVSS)

$f_{XU,COD,i}$ fraction of influent COD that is particulate and unbiodegradable

$FCOD_i$ daily mass of influent total COD

6.8.3 Division of biodegradable COD between PAOs and OHOs

- From the mechanisms for EBPR (Section 6.3 above), only VFA substrate can be stored by the PAOs in the anaerobic reactor. Accordingly, the influent RBCOD ($S_{S,i}$) needs to be subdivided into two fractions, namely VFAs ($S_{VFA,i}$) and fermentable COD ($S_{F,i}$). Thus, $S_{S,i} = S_{VFA,i} + S_{F,i}$.

The VFAs in the influent ($S_{VFA,i}$) are directly available to the PAOs for storage in the anaerobic reactor. Wentzel *et al.* (1985) have shown that the fermentable component ($S_{F,i}$) is converted to VFAs in the anaerobic reactor by the OHOs, thereby making additional VFAs available to the PAOs for storage. The rate of conversion is much slower than the rate of storage, so that the rate of conversion controls the rate of storage of generated VFAs. Hence, the mass of VFA substrate that becomes available in the anaerobic reactor is governed by the kinetics of conversion and by the mass of VFA substrate present in the influent. Should VFAs be present in the influent, it can be assumed that all these VFAs will be stored in the anaerobic reactor by the PAOs.

6.8.3.1 Kinetics of conversion of fermentable organics to VFAs

The conversion model proposed by Wentzel *et al.* (1985) is followed. It is hypothesized that:

- only fermentable COD (S_F) can be converted to a form suitable for storage by the PAOs (*i.e.* VFAs); within the timescale of residence of the mixed liquor in the anaerobic reactor the conversion of slowly biodegradable COD (X_S) to VFAs is assumed to be negligible (see Section 6.3.6.1).
- the conversion is performed by the OHO mass in the anaerobic reactor.
- all VFAs generated from conversion of fermentable COD are immediately stored by the PAOs.
- all fermentable COD not converted to VFAs in the anaerobic reactor is utilized subsequently for OHO metabolism.
- the rate of conversion of fermentable COD is given by:

$$\frac{dS_{F,AN}}{dt} = -k_{F,T} S_{F,AN} X_{OHOv,AN} \quad (6.7)$$

where:

$dS_{F,AN}/dt$ rate of conversion of fermentable organics (gCOD m^3/d)

$k_{F,T}$ first-order fermentation rate constant at temperature T (0.06 m^3/gVSS.d at 20 °C)

$S_{F,AN}$ fermentable COD concentration in the anaerobic reactor (gCOD/m^3)

$X_{OHOv,AN}$ concentration of OHOs in the anaerobic reactor (gAVSS/m^3)

- all VFAs present in the influent to the anaerobic reactor will be immediately stored by the PAOs.

6.8.3.2 Effect of recycling nitrate or oxygen

Should nitrate or oxygen enter the anaerobic reactor via recycle or with the influent, the conversion of fermentable COD to VFAs is further complicated. It is hypothesized that any oxygen or nitrate entering the anaerobic reactor is utilized as the electron acceptor by the OHOs with RBCOD (S_S) as the electron donor (substrate). It is not clear whether the fermentable COD or the influent VFAs will be used preferentially as the electron donor. For the purpose of the steady-state mixed-culture model it is assumed that the influent fermentable COD will serve as the electron donor. The implication is that the VFAs generated by conversion are no longer released, but are metabolized directly by the OHOs, until the oxygen or nitrate is depleted. In the conversion model this can be accommodated by reducing the amount of fermentable COD available for conversion as follows:

$$S_{F,i,conv} = S_{F,i} - 8.6\,(s\,S_{NO3,s} + S_{NO3,i}) - 3.0\,(s\,S_{O2,s} + S_{O2,i}) \quad (6.8)$$

where:

$S_{F,i,conv}$ fermentable COD available for conversion per volume of influent ($gCOD/m^3$)

$S_{F,i}$ fermentable COD influent concentration ($gCOD/m^3$)

s sludge recycle ratio to anaerobic reactor based on influent flow

$S_{NO3,s}$ nitrate concentration in the sludge recycle to the anaerobic reactor ($gNO_3\text{-}N/m^3$)

$S_{O2,s}$ oxygen concentration in the sludge recycle to the anaerobic reactor (gO_2/m^3)

$S_{NO3,i}$ nitrate concentration in the influent to anaerobic reactor ($gNO_3\text{-}N/m^3$)

$S_{O2,i}$ oxygen concentration in the influent to anaerobic reactor (O_2/m^3)

8.6 mass of COD removed per unit of nitrate denitrified ($gCOD/gNO_3\text{-}N$);
$2.86 / (1 - f_{cv} \cdot Y_{OHOv}) =$
$2.86/(1 - 1.48 \cdot 0.45) = 8.6$

3.0 mass of COD removed per unit of oxygen utilized ($gCOD/gO_2$);
$1 / (1 - f_{cv} \cdot Y_{OHOv}) =$
$1/ (1 - 1.48 \cdot 0.45) = 3.0$

6.8.3.3 Steady-state conversion equations

Steady-state equations for the conversion of fermentable COD to VFAs can be developed by applying eqs. 6.7 and 6.8 in mass balances for the n^{th} anaerobic reactor in a series of N anaerobic reactors of equal volume. This yields an equation to calculate the concentration of fermentable COD in the effluent from the n^{th} anaerobic reactor:

$$S_{F,ANn} = \frac{S_{F,i,conv} / (1+s)}{\left[1 + k_{F,T} \frac{f_{xa}}{N} \frac{MX_{OHOv}}{Q_i (1+s)}\right]^n} \quad (6.9)$$

where:

$S_{F,ANn}$ concentration of fermentable COD in the effluent of the n^{th} anaerobic reactor ($gCOD/m^3$)

f_{xa} anaerobic mass fraction (gVSS/gVSS)

N total number of anaerobic reactors of equal volume in the series n = 1,2.....N

MX_{OHOv} mass of OHOs in the whole NDEBPR system (gAVSS)

Q_i influent flow rate (m^3/d)

Eq. 6.9 provides the means to calculate the fermentable COD converted to VFAs in a series of N anaerobic reactors, *i.e.*:

$$FS_{F,CONV} = Q_i[S_{F,i,conv} - (1+s)S_{F,ANn}] \quad (6.10)$$

where:

$FS_{F,CONV}$ daily mass of fermentable COD converted to VFAs in the anaerobic reactors (gCOD/d)

However, to calculate $S_{F,ANn}$ the term MX_{OHOv}/Q_i needs to be determined.

Now, MX_{OHOv} is synthesized from the total mass of biodegradable influent COD less the mass of COD stored by the PAOs. From the mechanisms of EBPR and the hypothesis for conversion, all the VFAs generated by conversion and all the VFAs in the influent are stored by the PAOs., *i.e.* the mass of COD stored by the PAO, $FS_{S,PAO}$, is given by:

$$FS_{S,PAO} = FS_{F,CONV} + Q_i S_{VFA,i} \quad (6.11)$$

$$FS_{S,PAO} = Q_i[S_{F,i,conv} - (1+s)\,S_{F,ANn}] + Q_i\,S_{VFA,i} \quad (6.12)$$

where:

$FS_{S,PAO}$ daily mass of S_S stored by the PAOs (gCOD/d)

The COD available to the OHOs, is the biodegradable COD not stored by the PAOs:

$$FCOD_{b,OHO} = FCOD_{b,i} + FS_{S,PAO} \quad (6.13)$$

where:

$FCOD_{b,OHO}$ daily mass of biodegradable COD available to the OHOs (gCOD/d)

Accordingly, and as presented earlier, the equation to estimate the mass of ordinary heterotrophic organisms takes account of the reduced COD available:

$$MX_{OHOv} = \frac{Y_{OHOv}}{(1 + b_{OHO,T}\,SRT)}\,FCOD_{b,OHO}\,SRT \quad (6.14a)$$

The production of OHOs can also be expressed as mass synthesized per volume of influent by substituting eqs. 6.12 and 6.13 into eq. 6.14a and dividing by the influent flow rate:

$$\frac{MX_{OHO}}{Q_i} = \frac{Y_H}{(1 + b_{OHO,T}\,SRT)} \cdot (COD_{b,i} - (1+s)\,S_{F,ANn} + S_{VFA,i})\,SRT \quad (6.14b)$$

where:

MX_{OHOv}/Q_i equivalent concentration of OHOs produced per volume of influent ($gAVSS/m^3$)

Eqs. 6.9 and 6.14a need to be solved simultaneously to calculate the concentration of fermentable COD ($S_{F,ANn}$) leaving the last anaerobic reactor (ANn); the following iterative procedure can be used:

- Assume $S_{F,ANn} = 0$ mgCOD/l.
- Calculate MX_{OHOv} using Eq. 6.14a.
- Using the calculated value for MX_{OHOv}, calculate $S_{F,ANn}$ from Eq. 6.9.
- Recalculate MX_{OHOv} using the calculated value for $S_{F,ANn}$.
- Repeat the last two steps until $S_{F,ANn}$ and MX_{OHOv} are constant.

Similar equations could be derived for the behaviour of denitrifying PAOs (DPAOs) for anoxic conditions. However, the interaction with strictly aerobic PAOs and ordinary denitrifiers would require that the kinetics of substrate consumption and storage by each group of microorganisms be considered, a task that can best be managed through dynamic modelling.

6.8.3.4 Implications of conversion theory

The conversion theory set out above provides the means for calculating the mass of VFAs generated per day by the OHOs. Accepting that all the VFAs from conversion and from the influent are stored by the PAOs, the mass of substrate available to the OHOs is the remaining biodegradable COD. In effect the influent biodegradable COD is split into two fractions, one to be utilized by the PAOs and the other to be utilized by the OHOs. Because of the independent action of the two groups of organisms, the equations set out earlier (eq. 6.1 to 6.3) can be applied to calculate the active and endogenous PAO masses, and the equations in Chapter 4 can be used to calculate the OHO active, endogenous and inert masses appropriately modified as in eqs. 6.4 to 6.6. Then, knowing the P content of each of these mass

fractions, the P removal due to each can be calculated (see below).

6.8.4 P release

The phosphorus release by PAOs as a result of VFA storage does not need to be quantified for the steady-state design of EBPR systems but nevertheless can be useful information to obtain. From the mechanisms for P removal (Section 6.3), for every mole of VFAs stored by PAOs, it is considered that one mole of P is released (recognising that this ratio is pH-dependent, Smolders *et al.,* 1994a; Filipe *et al.,* 2001c) to provide energy to polymerise and store the VFAs as PHA. Accordingly, the P release will be given by:

$$FS_{PO4,rel} = f_{PO4,rel}\ FS_{S,PAO} \qquad (6.15a)$$

where:

$FS_{PO4,rel}$	daily mass of P release by PAOs (gP/d)
$f_{PO4,rel}$	ratio P release/VFA uptake = 1.0 molP/molCOD = 0.5 gP/gCOD at pH 7.0

or, in concentration units:

$$S_{PO4,rel} = f_{PO4,rel}\ \frac{FS_{S,PAO}}{Q_i} \qquad (6.15b)$$

where:

$S_{PO4,rel}$	P released (gP/m^3 of influent)
$S_{S,PAO}$	concentration of readily biodegradable COD stored by PAOs (gCOD/m^3)

$$S_{PO4,rel} = f_{PO4,rel}\ \frac{FS_{S,PAO}}{Q_i} \qquad (6.15c)$$

If the $f_{PO4,rel}$ coefficient needs to be estimated as a function of the pH in the bulk liquid, the following expression developed by Smolders *et al.* (1994a) could be applied:

$$f_{PO4,rel} = 0.18\ pH - 0.81 \qquad (6.15d)$$

where:

$f_{PO4,rel}$	ratio P release/VFA uptake in gP/gCOD
pH	pH measured in the bulk liquid of the anaerobic stage of an EBPR system.

6.8.5 P removal and effluent total phosphorus concentration

The P removal is calculated for the individual sludge fractions, with the total P removal being given by the summation of the individual P removal of each biomass. Overall, only the PAO biomass, MX_{PAOv}, is able to store higher P concentrations (of up to 0.38 g P/gVSS of active PAO biomass) than those needed for biomass synthesis. On the other hand, the rest of the biomasses (namely MX_{OHOv}, $MX_{E,OHOv}$, $MX_{E,XUv}$, and even the endogenous PAO biomass $MX_{E,PAOv}$) accumulate the typical P content observed in VSS, f_p, of 0.03 gP/gVSS. It is important to notice that the endogeneous PAO biomass, $MX_{E,PAOv}$, being an endogenous residue, contains only the P present in the residue (presumably used to make the cell tissues and other organic compounds) and therefore is far less than the $f_{P,PAO}$ fraction that the active PAO biomass can actually store.

Thus, the different biomasses contribute to the overall potential P removal as follows:

PAOs:

$$\Delta P_{PAO} = f_{P,PAO}\ \frac{MX_{PAOv}}{Q_i \cdot SRT} \qquad (6.16)$$

where:

ΔP_{PAO}	P removal due to PAOs (gP/m^3)
$f_{P,PAO}$	fraction of PAO active mass that is P = 0.38 gP/gVSS with regard to the active PAO biomass, MX_{PAOv}

OHOs:

$$\Delta P_{OHO} = f_p \frac{MX_{OHOv}}{Q_i \cdot SRT} \quad (6.17)$$

where:

ΔP_{OHO} P removal due to OHOs (gP/m^3)

f_p fraction of OHO active mass that is P = 0.03 gP/gVSS with regard to the active OHO biomass, MX_{OHOv}, which corresponds to the typical P content present in VSS

Endogenous residue mass (from any biomass, including PAOs and OHOs) is defined by:

$$\Delta P_{XE} = f_p \frac{MX_{E,OHOv} + MX_{E,PAOv}}{Q_i \cdot SRT} \quad (6.18)$$

where:

ΔP_{XE} P removal due to endogenous residue mass (gP/m^3)

f_p fraction of endogenous mass that is P (gP/gVSS) = 0.03 gP/gVSS of endogenous residues ($MX_{E,OHOv}$ and $MX_{E,PAOv}$), which is similar to the typical P content present in VSS

The influent inert mass is:

$$\Delta P_{XU} = f_p \frac{MX_U}{Q_i \cdot SRT} \quad (6.19)$$

where:

ΔP_{XU} P removal due to influent unbiodegradable particulate organics, $X_{U,i}$ (gP/m^3)

f_p fraction of influent unbiodegradable particulate organics that is P (gP/gVSS) = 0.03 gP/gVSS of $X_{U,i}$, corresponding to the typical P content present in VSS

The total P removal potential by the system, neglecting chemical phosphorus precipitation (typically due to aluminium, calcium or iron salts present in the influent or added to the system), is:

$$\Delta P_{SYS,pot} = \Delta P_{PAO} + \Delta P_{OHO} + \Delta P_{XE} + \Delta P_{XU} \quad (6.20)$$

where:

$\Delta P_{SYS,pot}$ potential total P removal by the system (gP/m^3)

The actual P removal by the system is the lowest of the total P removal potential and the influent total phosphorus:

$$\Delta P_{SYS,actual} = \min(\Delta P_{SYS,pot}; P_i) \quad (6.21)$$

where:

$\Delta P_{SYS,actual}$ actual total P removal for the system (gP/m^3)

Any suspended solids in the effluent contributes to increasing the particulate phosphorus concentration in the effluent:

$$X_{P,e} = f_{P,TSS}\, TSS_e \quad (6.22)$$

where:

$f_{P,TSS}$ average P content of the activated sludge (gP/m^3)

TSS_e total suspended solids concentration of the effluent ($gTSS/m^3$)

The effluent total P concentration is calculated by subtracting the actual total P removal for the system and adding any particulate P contributed by the suspended solids in the effluent:

$$P_e = P_i - \Delta P_{SYS,actual} + X_{P,e} \quad (6.23)$$

where:

P_i influent total P concentration (gP/m^3)

P_e effluent total P concentration (gP/m^3)

6.8.6 VSS and TSS sludge masses and P content of TSS

6.8.6.1 Actual P content in active PAO biomass

Although the active PAO biomass, MX_{PAOv}, can have the potential to remove a high P concentration, logically the P concentration that MX_{PAOv} can remove (store intracellularly) cannot be higher than the concentration present in the wastewater influent. This principle is similar to the one that defines the total P removal potential ($\Delta P_{SYS,pot}$) and the actual P removal ($\Delta P_{SYS,actual}$) of the system. As such, the actual P content of the active PAO biomass, $f_{P,PAO,act}$, can be estimated as the difference between the actual P mass removed by the system minus the P mass accumulated by the VSS biomass excluding the contribution of MX_{PAOv}, as follows:

$$f_{P,PAO,act} = \frac{\left[Q_i \cdot SRT \cdot \Delta P_{SYS,actual}\right] - \left[f_p (MX_{VSS} - MX_{PAOv}\right]}{MX_{PAOv}} \quad (6.24a)$$

where:

$f_{P,PAO,act}$ Actual P content stored by the active PAO biomass (gP/gVSS of active PAO biomass, MX_{PAOv})

For design purposes, it is important to estimate the actual P content stored by PAO $f_{P,PAO,act}$ since it can affect the estimation of different parameters of utmost importance: (*i*) the determination of MX_{FSS}; (*ii*) the total mass MX_{TSS} accumulated in the system; (*iii*) as a consequence of the influence on MX_{FSS} and MX_{TSS}, it can affect the calculation of the total volume of the plant V_R, and (*iv*) also the estimation of the $f_{P,TSS}$ content which can affect the correct estimation of the actual total P concentration present in the effluent.

6.8.6.2 VSS sludge mass

The VSS sludge mass in the system is calculated in the same fashion used for aerobic and anoxic/aerobic systems, by summing the contributions of the individual VSS fractions, *i.e.*:

$$MX_{VSS} = MX_{PAOv} + MX_{OHOv} + MX_{E,PAOv} + MX_{E,OHOv} + MX_{Uv} \quad (6.24b)$$

$$MX_{VSS} = V_R \; VSS \quad (6.24c)$$

where:

MX_{VSS}	VSS mass in system (gVSS)
VSS	VSS concentration in system ($gVSS/m^3$)
V_R	system process volume (m^3)

As for aerobic and anoxic/aerobic systems, the TSS sludge mass in the system is calculated from the VSS via the VSS/TSS ratio. However, for the PAO mixed-liquor fractions the VSS/TSS ratio will differ substantially from the value for the OHO fractions. This is due to the large amount of inorganic polyphosphate stored internally in the PAOs, with associated counterions. The counterions are required to neutralise the negative charges on the polyphosphate, thereby stabilising it. These counterions are principally Mg^{2+} and K^{+}, and to a lesser extent Ca^{2+} (Fukase *et al.,* 1982; Arvin *et al.,* 1985; Comeau *et al.,* 1986; Wentzel *et al.,* 1989a).

6.8.6.3 FSS sludge mass

The fixed (inorganic) suspended solids (FSS) sludge mass in the system comes from various sources (Ekama and Wentzel, 2004):

- Intracellular components of active biomass contain salts that are left as inorganic residue by combustion at 550 ºC. A fraction of 0.15 gFSS/gVSS is considered for OHOs. Nitrifiers have a similar FSS fraction but they can often be neglected as they normally compose less than 2 % of the biomass;
- PAOs contain both the standard 0.15 gFSS/gVSS fraction plus their polyphosphates and cationic counterions that contribute considerably to the FSS content of the PAOs. For aerobic PAOs containing 38% gP/gVSS, an FSS content of 1.30

gFSS/gVSS is reported by Ekama and Wentzel (2004). However, if the PAO cells have not reached their maximum intracellular P storage of 38% gP/gVSS, it can be assumed that the FSS content can be reduced proportionally. This aspect is taken into account by considering the actual P content stored by the active PAO biomass ($f_{P,PAO,act}$) with regard to their maximum P content ($f_{P,PAO}$) in the determination of MX_{FSS} (see Eq. 6.24d).

- Endogenous and inert organic residues are considered not to contain inorganics as the salt content of these components should have been dissolved upon cell lysis.
- Slowly biodegradable particulate organic matter is also considered not to contain inorganics.
- Influent FSS which is accumulated onto the activated sludge.
- Precipitation of minerals and dissolution of FSS are neglected. Should chemical precipitation take place, however, mineral accumulation into the sludge should be considered.

Thus, the FSS sludge mass in the system is given by:

$$MX_{FSS} = f_{FSS,OHO} MX_{OHOv} + f_{FSS,PAO} \frac{f_{P,PAO,act}}{f_{P,PAO}} MX_{PAOv} + Q_i \cdot SRT \cdot X_{FSS,i} \quad (6.24d)$$

where:

MX_{FSS} mass of fixed suspended solids in the system (gFSS)

$f_{FSS,OHO}$ fraction of FSS in the OHO active biomass = 0.15 gFSS/gVSS (giving a $f_{VT,OHO}$ of 0.87 gVSS/gTSS)

$f_{FSS,PAO}$ fraction of FSS in the PAO active biomass = 1.30 g FSS/gAVSS for aerobic PAOs (giving a $f_{VT,PAO}$ of 0.44 gVSS/gTSS)

$FX_{FSS,i}$ daily mass of influent FSS (gFSS/d)

6.8.6.2 TSS sludge mass and sludge VSS/TSS ratio

The TSS sludge mass in the system is given by the sum of VSS and FSS:

$$MX_{TSS} = MX_{VSS} + MX_{FSS} \quad (6.25a)$$

$$MX_{TSS} = V_R \; X_{TSS} \quad (6.25b)$$

where:

MX_{TSS} mass of total suspended solids in the system (gTSS)

and the sludge VSS to TSS ratio:

$$f_{VT} = \frac{MX_{VSS}}{MX_{TSS}} \quad (6.25c)$$

where:

f_{VT} VSS/TSS ratio for the sludge.

6.8.6.4 P content of TSS

The average phosphorus content of the biomass is calculated by considering each mass contributing to the TSS, and in particular, the actual P content stored by the active PAO biomass since it may contribute with the highest P content. The fraction of phosphorus in the fixed suspended solids can vary significantly depending on the presence of aluminium, iron and calcium salts either present in the influent or added to the system for phosphorus precipitation.

$$f_{P,TSS} = \frac{f_p \cdot (MX_{OHOv} + MX_{E,OHOv} + MX_{E,PAOv} + MX_{Uv})}{MX_{TSS}} + \frac{f_{P,PAO,act} \cdot MX_{PAOv}}{MX_{TSS}} + \frac{f_{P,FSS} \cdot MX_{FSS}}{MX_{TSS}} \quad (6.26)$$

where:

$f_{P,TSS}$ P fraction of total suspended solids mass (gP/gTSS)

$f_{P,FSS}$ P fraction of fixed (inorganic) suspended solids mass (gP/gFSS)

= 0.02 gP/gFSS (proposed value; it would need to be corrected should there be a significant presence of salts that coagulate P such as Al, Fe or Ca salts).

6.8.7 Process volume requirements

As set out in Chapter 4, process volume requirements are determined from the mass of sludge in the system and the selected required sludge concentration either as TSS or as VSS:

$$V_R = MX_{TSS} / X_{TSS,OX} \quad (6.27a)$$

where:

V_R process volume (m^3)

$X_{TSS,OX}$ selected desired TSS concentration in the aerobic reactor ($gTSS/m^3$)

or, alternatively:

$$V_R = MX_{VSS} / X_{VSS,OX} \quad (6.27b)$$

where:

$X_{VSS,OX}$ the selected desired VSS concentration in the aerobic reactor ($gVSS/m^3$)

The process volume requirements (V_R) is the effective volume, *i.e.* the volume that would be required if the sludge was at uniform concentration throughout the system. However, with some nitrifying and denitrifying EBPR system configurations, this is not true and the sludge concentrations differ between the different zones. For example, the sludge concentration in the anaerobic zone of the UCT/MUCT configuration is reduced by the factor s/(1 + s) compared to the other zones (anoxic and aerobic). In these cases the volume must be adjusted to take this into account.

6.8.8 Nitrogen requirements for sludge production

The form of the equation for calculating the nitrogen requirement for sludge production is:

$$FN_s = f_n \, MX_{VSS} / SRT \quad (6.28a)$$

where:

FN_s daily mass of nitrogen required for sludge production (gN/d)

f_n nitrogen content of the sludge = 0.10 gN/gVSS

However, for the EBPR system the term MX_{VSS} needs to take account of the changes in VSS constituents, that is, it must be calculated using Eq. 6.24a.

Expressed on the basis of influent concentration, the nitrogen requirement for sludge production is:

$$TKN_{i,s} = FN_s / Q_i \quad (6.28b)$$

6.8.9 Oxygen demand

6.8.9.1 Carbonaceous oxygen demand

The carbonaceous oxygen demand (FO_c) is given by the sum of oxygen demands due to the PAOs and OHOs. From a COD mass balance point of view, any removed COD not converted into biomass or endogenous residue is consumed for energy production. For example, 1 unit of biodegradable COD (COD_b; such as S_{VFA}) removed will produce ($f_{cv} \cdot Y_{PAOv}$) units of X_{PAO} with energy provided by respiring ($1 - f_{cv} \cdot Y_{PAOv}$) of COD_b. The factor f_{cv} (gCOD-active biomass/gVSS-active biomass) is used to convert the units of Y_{PAOv} from gVSS-active biomass/gCOD-substrate into gCOD-active biomass/gCOD-substrate. Thus, 1 unit of COD_b equals ($f_{cv} \cdot Y_{PAOv} + 1 - f_{cv} \cdot Y_{PAOv}$) and the COD mass balance is maintained.

Oxygen demand for PAOs

The oxygen demand for PAOs comes from respiration to provide energy for biomass synthesis and for endogenous respiration.

$$FO_{PAO} = FO_{PAO\,synthesis} + FO_{PAO\,endogenous\ respiration} \quad (6.29a)$$

$$FO_{PAO} = (1 - f_{cv}\, Y_{PAOv})\, FS_{S,PAO} + f_{cv}\,(1 - f_{E,PAO})\, b_{PAO,T}\, MX_{PAOv} \quad (6.29b)$$

or, more explicitly as a function of the daily mass of substrate stored by the PAOs:

$$FO_{PAO} = FS_{S,PAO}\left[(1 - f_{cv}\, Y_{PAOv})\right] + \left[f_{cv}(1 - f_{XE,PAO}) b_{PAO,T} \cdot \frac{Y_{PAOv}}{(1 + b_{PAO,T}\, SRT)}\, SRT \right] \quad (6.29c)$$

where:

FO_{PAO} daily mass of oxygen consumed by PAOs (gO_2/d)

f_{cv} COD/VSS ratio of the sludge (gCOD/gVSS)

Oxygen demand for OHOs

Similarly, for OHOs:

$$FO_{OHO} = FO_{OHO\,synthesis} + FO_{OHO\,endogenous\ respiration} \quad (6.30a)$$

$$FO_{OHO} = (1 - f_{cv}\, Y_{OHOv})\, FCOD_{b,OHO} + + f_{cv}\,(1 - f_{E,OHO})\, b_{OHO,T}\, MX_{OHOv} \quad (6.30b)$$

or, more explicitly as a function of the daily mass of substrate stored by the OHOs:

$$FO_{OHO} = FCOD_{b,OHO}\left[(1 - f_{cv}\, Y_{OHOv})\right] + \left[f_{cv}\,(1 - f_{XE,OHO}) \cdot b_{OHO,T} \frac{Y_{OHOv}}{(1 + b_{OHO,T}\, SRT)}\, SRT \right] \quad (6.30c)$$

where:

FO_{OHO} daily mass of oxygen consumed by OHOs(gO_2/d)

Total oxygen demand

The total carbonaceous oxygen demand (gO_2/d) is:

$$FO_c = FO_{PAO} + FO_{OHO} \quad (6.31a)$$

where:

FO_c daily mass of carbonaceous oxygen demand (gO_2/d)

Now, assuming that $Y_{PAOv} \approx Y_{OHOv}$, that ($FS_{F,PAO} + FCOD_{b,OHO}$) $\approx FCOD_{b,i}$ and that $f_{XE,PAO}$ (0.20) $\approx f_{XE,OHO}$ (0.25), Eq. 6.31b may be simplified (gO_2/d):

$$FO_c = (1 - f_{cv}\, Y_{OHOv})\, FCOD_{b,i} + f_{cv}\,(1 - f_{XE,OHO}) \cdot (b_{PAO,T}\, MX_{PAOv} + b_{OHO,T}\, MX_{OHOv}) \quad (6.31b)$$

6.8.9.2 Nitrification oxygen demand

Taking due account of the change in nitrogen requirements for sludge production (FN_S) and nitrification capacity (NIT_c), the nitrification oxygen demand FO_{NIT} is given in Chapter 5.

6.8.9.3 Total oxygen demand

For a non-nitrifying EBPR system the total oxygen demand FO_t is given by FO_c, while for a nitrifying EBPR system, FO_t is given by the sum of FO_c and FO_{NIT}. Including nitrification in the EBPR system necessarily means that denitrification must also be included; the effect of nitrification and denitrification on the total oxygen demand will be considered later.

$$FO_t = FO_c + FO_{NIT} \quad (6.31c)$$

where:

FO_t daily mass of total oxygen demand (gO_2/d).

6.9 DESIGN EXAMPLE

6.9.1 Steady-state design procedure

The procedure for the steady-state design of an EPBR process is shown in Figure 6.29. First, the wastewater needs to be characterized in terms of its flow rate and daily fluxes of COD, nitrogen, phosphorus, inorganic solids and oxygen concentration. A treatment configuration is selected which is operated at a given SRT, temperature and with appropriate kinetic and stoichiometric constants. Then, the influent RBCOD is divided between PAOs and OHOs which allows the calculation of their biomass (and endogenous residue) production as VSS and the phosphorus-removal capacity of the system. From total VSS and TSS estimation, the bioreactor process volume can be calculated as well as the nitrogen and oxygen requirements. Finally, a calculation check can be made with the COD mass balance.

6.9.2 Information provided

The raw wastewater (without primary settling) to be treated has a similar composition to that presented in chapters 4 and 5 on organic matter and nitrogen removal, respectively. The influent composition COD fractions are summarized in Tables 6.2 and 6.3. A flow rate of 15 MLD is selected for ease of transposition. The total influent COD is 750 g/m^3 and the influent total phosphorus is 17 g/m^3. The fractionation of the influent COD is illustrated in Figure 6.30. The kinetic and stoichiometric parameters are presented in Table 6.4.

The EBPR process selected (Table 6.5) is a Johannesburg configuration which is operated at 14 °C, with 2 anaerobic zones, an SRT of 20 days, an anaerobic mass fraction of 0.10, a sludge recycle ratio of 0.75 with respect to the influent flow, an aerobic to anoxic recycle ratio of 1.5, a sludge recycle entering the anaerobic zone containing no dissolved oxygen but 0.5 gNO_3-N/m^3, a total suspended solids in the effluent of 5 mg/l, and a design aerobic mixed-liquor solids concentration of 4,000 gTSS/m^3.

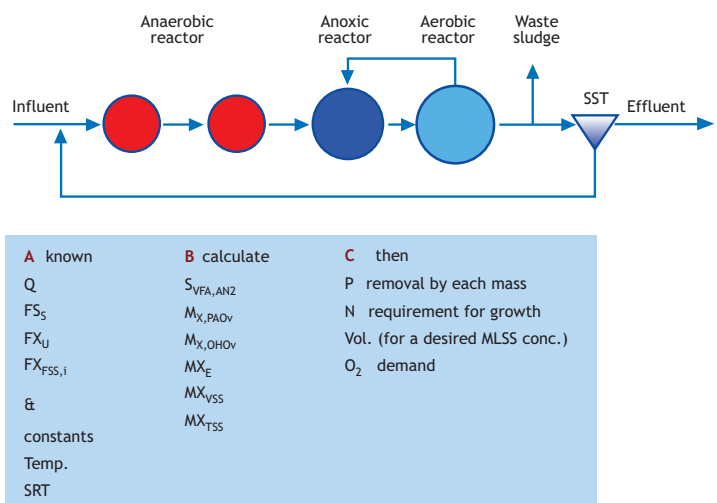


Figure 6.29 Design procedure overview for the EBPR system. A Johannesburg configuration is illustrated. The anaerobic reactor is divided into two cells (not illustrated).

Table 6.2 Influent characteristics for the EBPR design example (raw wastewater).

Description	Symbol	Value	Units	Calculations
Flow rate	Q_i	15	MLD	
Total COD	COD_i	750	$gCOD/m^3$	
COD concentrations				
- readily biodegradable COD	$S_{S,i}$	146	$gCOD/m^3$	$= 750 \cdot 0.195$
- volatile fatty acids	$S_{VFA,i}$	22	$gCOD/m^3$	$= 146 \cdot 0.15$
- fermentable COD	$S_{F,i}$	124	$gCOD/m^3$	$= 146 - 22$
- slowly biodegradable COD	$X_{S,i}$	439	$gCOD/m^3$	$= 750 \cdot (1 - 0.195 - 0.07 - 0.15)$
- inert soluble COD	$S_{U,i}$	53	$gCOD/m^3$	$= 750 \cdot 0.07$
- inert particulate COD	$X_{U,i}$	113	$gCOD/m^3$	$= 750 \cdot 0.15$
Nitrate	$S_{NO3,i}$	0	gN/m^3	
Dissolved O_2	$S_{O2,i}$	0	gO_2/m^3	
Total P	P_i	17.0	gP/m^3	
Fixed (inorganic) SS	$X_{FSS,i}$	49	$gFSS/m^3$	
P fraction of influent FSS	$f_{P,FSS,i}$	0.02	gP/gFSS	
Alkalinity	S_{Alk}	250	$gCaCO_3/m^3$	

Table 6.3 COD fractions of raw wastewater for the EBPR design example.

Description	Symbol	COD fractions	Units
Type of WW		Raw	
COD fractions			
Fraction of RBCOD	$f_{SS,CODi}$	0.195	g/gTCOD
S_{VFA} fraction of RBCOD	$f_{SVFA,SSi}$	0.15	$g/gCOD_{SS}$
Fraction of soluble unbiodegradable COD	$f_{SU,CODi}$	0.07	g/gTCOD
Fraction of particulate unbiodegradable COD	$f_{XU,CODi}$	0.15	g/gTCOD

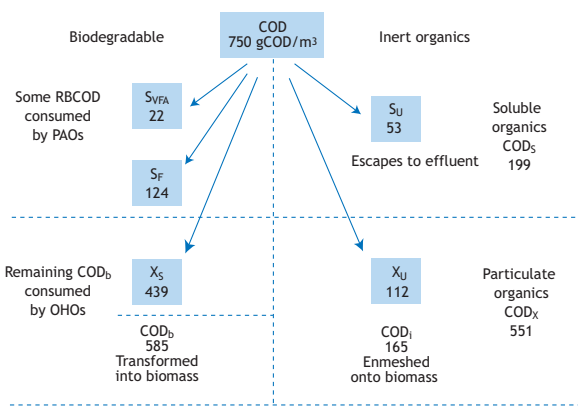


Figure 6.30 Influent COD fractionation for the EBPR design example.

Table 6.4 Kinetic and stoichiometric parameters for the EBPR design example.

	Parameter	Symbol	Value	Units
Kinetic	*OHO*			
	First-order fermentation rate constant at T = 20 ºC	$k_{F,20}$	0.06	m^3/gVSS.d
	Temperature coefficient for $k_{F,T}$	$\theta_{k,F}$	1.029	
	First-order fermentation rate constant at temperature T[(a)]	$k_{F,T}$	0.051	m^3/gVSS.d
	Specific endogenous mass loss rate of the OHOs at 20 ºC	$b_{OHO,20}$	0.24	gVSS/gVSS.d
	Temperature coefficient for $b_{OHO,T}$	$\theta_{b,OHO}$	1.029	
	Specific endogenous mass loss rate of the OHOs at temperature T	$b_{OHO,T}$	0.202	gVSS/gVSS.d
	PAO			
	PAO-specific endogenous mass loss rate constant at T = 20 ºC	$b_{PAO,20}$	0.04	gVSS/gVSS.d
	Temperature coefficient for $b_{PAO,T}$	$\theta_{b,PAO}$	1.029	
	PAO-specific endogenous mass loss rate constant at temperature T	$b_{PAO,T}$	0.034	gVSS/gVSS.d
Stoichiometric	*OHO*			
	Biomass yield of OHOs	Y_{PAOv}	0.45	gVSS/gCOD
	Fraction of endogenous residue of the OHOs	$f_{XE,OHO}$	0.20	gVSS/gVSS
	Fraction of P in the active OHO mass	$f_{P,OHO}$	0.03	gP/gVSS
	Fraction of P in the endogenous mass (OHO and PAO)	f_p	0.03	gP/gVSS
	Fraction of fixed (inorganic) suspended solids of OHOs	$f_{FSS,OHO}$	0.15	gFSS/gVSS
	PAO			
	Biomass yield of PAOs	Y_{PAOv}	0.45	gVSS/gCOD
	Fraction of endogenous residue of the PAOs	$f_{XE,PAO}$	0.25	gVSS/gVSS
	Fraction of P in the active PAO mass	$f_{P,PAO}$	0.38	gP/gVSS
	Fraction of P in the endogenous mass (OHO and PAO)	f_p	0.03	gP/gVSS
	VSS/TSS ratio for PAO active mass	$f_{VT,PAO}$	0.46[(b)]	gVSS/gTSS
	Ratio of P release/VFA uptake	$f_{PO4,REL}$	0.50	gP/gCOD
	Fraction of fixed (inorganic) suspended solids of PAOs	$f_{FSS,PAO}$	1.30	gFSS/gVSS
	Inerts			
	Fraction of P in the inert mass	f_p	0.03	gP/gIVSS
	General			
	COD/VSS ratio of the sludge	f_{cv}	1.48	gCOD/gVSS
	VSS/TSS ratio for OHO active and endogenous masses PAO endogenous mass, and inert mass	f_{VT}	0.80[(b)]	gVSS/gTSS
	Nitrogen content of active biomass	f_n	0.10	gN/gVSS

[(a)] $k_T = k_{20} \cdot \theta^{(T-20)}$; example: $k_{F,14} = 0.060 \cdot 1.029^{(14-20)} = 0.051$

[(b)] These values are not required if the FSS is calculated from Eq. 6.24c

Table 6.5 Biological system characteristics for the EBPR design example (Johannesburg configuration).

Description	Symbol	Value	Units
Temperature	T	14	ºC
Number of anaerobic zones	n	2	reactors
Sludge retention time	SRT	20	d
Anaerobic mass fraction	f_{xa}	0.10	gVSS/gVSS
Sludge recycle ratio based on influent flow	s	0.75	$m^3.d/m^3.d$
Aerobic to anoxic recycle ratio	a	1.5	$m^3.d/m^3.d$
Dissolved O_2 in the sludge recycle	$S_{O2,s}$	0	gO_2/m^3
Nitrate concentration in the sludge recycle	$S_{NO3,s}$	0.5	$gNO_3\text{-}N/m^3$
Total suspended solids in the effluent	TSS_e	5	$gTSS/m^3$
Design aerobic TSS concentration	$X_{TSS,OX}$	4,000	$gTSS/m^3$

6.9.3 Calculations

Following the same procedure as presented in Section 6.6, the detailed calculations are shown in Table 6.6 over the following pages. Each step is presented with symbols, values, units, symbol definition, equations used to calculate a given parameter, and the detailed calculation with the numerical values for each parameter. At the end, a COD mass balance is made as a validation check of the calculations.

Note that in step 3.2, the fermentable COD leaving in the effluent of the last anaerobic reactor is calculated by iteration.

Table 6.6 Detailed calculations for the EBPR design example.

1. System configuration			
Johannesburg configuration operated at 14 ºC			
2. Influent and sludge recycle composition (from previous tables)			
Q_i	15	MLD	influent flow rate
2.1 Influent concentrations			
Influent and bioreactor data			
COD_i	750	$gCOD/m^3$	influent concentration of total COD
$S_{S,i}$	146	$gCOD/m^3$	influent concentration of RBCOD
$S_{VFA,i}$	22	$gCOD/m^3$	influent concentration of VFAs
$S_{F,i}$	124	$gCOD/m^3$	influent concentration of fermentable COD
$X_{S,i}$	439	$gCOD/m^3$	influent concentration of slowly biodegradable COD
$COD_{b,i}$	585	$gCOD/m^3$	influent concentration of biodegradable COD ($S_{S,i} + X_{S,i}$)
$S_{U,i}$	53	$gCOD/m^3$	influent concentration of soluble inert COD
$X_{U,i}$	113	$gCOD/m^3$	influent concentration of particulate inert COD
$S_{NO3,i}$	0	$gNO_3\text{-}N/m^3$	influent concentration of nitrate
$S_{O2,i}$	0	gO_2/m^3	influent concentration of dissolved oxygen

$X_{FSS,i}$	49	$gFSS/m^3$	influent concentration of fixed (inorganic) suspended solids
P_i	17	gP/m^3	influent concentration of total P
2.2 Influent fluxes used for calculations (= Qi · influent concentration of component)			
$FCOD_i$	11,250	kgCOD/d	influent daily flux of total COD
$FS_{S,i}$	2,190	kgCOD/d	influent daily flux of RBCOD
$FS_{VFA,i}$	330	kgCOD/d	influent daily flux of VFAs
$FS_{F,i}$	1,860	kgCOD/d	influent daily flux of fermentable COD
$FCOD_{b,i}$	8,770	kgCOD/d	influent daily flux of biodegradable COD ($S_{S,i} + X_{S,i}$)
$FX_{U,i}$	1,688	kgCOD/d	influent daily flux of particulate unbiodegradable COD
$FS_{U,i}$	795	kgCOD/d	influent daily flux of soluble unbiodegradable COD
$FX_{FSS,i}$	735	kgFSS/d	influent daily flux of fixed (inorganic) suspended solids
2.3 Sludge recycle characteristics			
s	0.75	$m^3.d/ m^3.d$	sludge recycle ratio based on influent flow
$S_{O2,s}$	0	gO_2/m^3	dissolved O_2 in the sludge recycle
$S_{NO3,s}$	0.5	$gNO_3\text{-}N/m^3$	nitrate concentration in the sludge recycle
3. Division of $S_{S,i}$ between PAOs and OHOs			
3.1 Fermentable COD available for conversion into VFAs after denitrification reactor (and O_2 consumption) in AN reactor (in units of $gCOD/m^3$ of influent)			
$S_{F,i,conv}$	$= S_{F,i} – 8.6 \cdot (s \cdot S_{NO3,s} + S_{NO3,i}) – 3 \cdot (s \cdot S_{O2,s} + S_{O2,i})$		
	$= S_{F,i}$ – COD for denitrification - COD for D.O.		
	$= 124 – 8.6 \cdot (0.75 \cdot 0.5 + 0) – 3 \cdot (0.75 \cdot 0 + 0)$		
COD for denit.	3.2	$gCOD/m^3$	
COD for D.O.	0.0	$gCOD/m^3$	
$S_{F,i,conv}$	121	$gCOD/m^3$	
3.2 Fermentable COD lost in the effluent of the last anaerobic reactor			
N	2		the 2nd AN reactor
calculation done by iterations			
a- suppose a seed1 $S_{F,ANn}$ value of 0. This value is used to calculate MX_{OHOv}			
b- type the calculated MX_{OHOv} calculated value as seed2 value			
c- repeat steps a and b until the seed2 $S_{F,ANn}$ equals the calculated $S_{F,ANn}$			
$S_{F,ANn}$	$= S_{F,i,conv} / (1+s) / (1 + (k_{F,T} \cdot (f_{xa} \cdot MX_{OHOv} / (N \cdot Q_i \cdot (1 + s)))))^n$		
	$=121 / (1 + 0.75) / (1 + (0.051 \cdot (0.10 \cdot 12{,}500 / (2 \cdot 15 \cdot (1 + 0.75)))))^2$		
	seed1:		
$S_{F,ANn}$	14.2	14.2	$gCOD/m^3$
	↓	↑	
		seed2:	
MX_{OHOv}	12,490	12,490	kgCOD
	$= Y_{OHOv} / (1 + b_{OHO,T} \cdot SRT) \cdot FCOD_{b,OHO} \cdot SRT$ (note that $FCOD_{b,OHO}$ is calculated in step 3.4)		
	$= 0.45 / (1 + 0.202 \cdot 20) \cdot 7{,}000 \cdot 20$		

3.3 VFAs stored by PAOs			
$FS_{S,PAO}$	$= Q_i \cdot (S_{F,i,conv} - (1 + s) \cdot S_{F,ANn}) + Q_i \cdot S_{VFA,i}$		
	$= 1 \cdot (121- (1 + 0.75) \cdot 14.3) + 1 \cdot 22$		
$FS_{S,PAO}$	1,770	kgCOD/d	
3.4 Remaining biodegradable COD available to OHOs			
$FCOD_{b,OHO}$	$= FCOD_{b,i} - FS_{S,PAO}$		
	$= 8,770 - 1,770$		
$FCOD_{b,OHO}$	7,000	kgCOD/d	
4. Biomass (VSS) equations			

Corresponds to the biological mass present in the system as synthesized from the influent COD (in g/d) taking into account the cumulative effect of SRT [(g/d) · d = g in the system]

4.1 PAOs			
Active mass			
Y_{PAOv}	0.45	gVSS/gCOD	
$Y_{PAOv,obs}$	$= Y_{PAOv} / (1 + b_{PAO,T} \cdot SRT)$		
	$= 0.45 / (1 + 0.034 \cdot 20)$		
$Y_{PAO,vobs}$	0.269 gVSS / gCOD		
MX_{PAOv}	$= Y_{PAOv,obs} \cdot FS_{S,PAO} \cdot SRT$		
	$= 0.269 \cdot 1,770 \cdot 20$		
MX_{PAOv}	9,511	kgVSS in the system	
Endogenous mass			
$MX_{E,PAOv}$	$= f_{XE,PAO} \cdot b_{PAO,T} \cdot MX_{PAOv} \cdot SRT$		
	$= 0.25 \cdot 0.0337 \cdot 9511 \cdot 20$		
$MX_{E,PAOv}$	1,603	kgVSS	
4.2 OHOs			
Active mass			
Y_{OHOv}	0.45	gVSS/gCOD	
$Y_{OHOv,obs}$	$= Y_{OHOv} / (1 + b_{OHO,T} \cdot SRT)$		
	$= 0.45 / (1 + 0.202 \cdot 20)$		
$Y_{OHOv,obs}$	0.089	gVSS/gCOD	
MX_{OHOv}	$= Y_{OHOv,obs} \cdot FCOD_{b,OHO} \cdot SRT$		
	$= 0.089 \cdot 7,000 \cdot 20$		
MX_{OHOv}	12,490	kgVSS	(this value is the calculated MX_{OHOv} value of step 3.2)
Endogenous mass			
$MX_{E,OHOv}$	$= f_{XE,OHO} \cdot b_{OHO,T} \cdot MX_{OHOv} \cdot SRT$		
	$= 0.20 \cdot 0.202 \cdot 12,490 \cdot 20$		
$MX_{E,OHOv}$	10,100	kgVSS	

4.3 Inert mass			
MX_{Uv}	$= f_{XU,COD,i} \cdot FCOD_i \cdot SRT / f_{cv}$		
	$= 0.15 \cdot 11{,}250 \cdot 20 / 1.48$		
MX_{Uv}	22,804	kgVSS	
5. P removal			
5.0 P release			
$S_{PO4,rel}$	$= f_{PO4,rel} \cdot FS_{S,PAO} / Q_i$		
	$= 0.5 \cdot 1{,}770 / 15$		
$S_{PO4,rel}$	59	gP/m^3	gP/m^3 of influent, not gP/m^3 of AN reactor
5.1 ΔP by PAOs			
ΔP_{PAO}	$= f_{P,PAO} \cdot MX_{PAOv} / (SRT \cdot Q_i)$		
	$= 0.38 \cdot 9{,}511 / (20 \cdot 15)$		
ΔP_{PAO}	12.05	gP/m^3	
5.2 ΔP by OHOs			
ΔP_{OHO}	$= f_p \cdot MX_{OHOv} / (SRT \cdot Q_i)$		
	$= 0.03 \cdot 12{,}500 / (20 \cdot 15)$		
ΔP_{OHO}	1.25	gP/m^3	
5.3 ΔP by endogenous mass			
ΔP_{XE}	$= \Delta P_{XE,PAO} + \Delta P_{XE,OHO}$		
$\Delta P_{XE,PAO}$	$= f_p \cdot MX_{E,PAOv} / (SRT \cdot Q_i)$		
	$= 0.03 \cdot 1{,}603 / (20 \cdot 15)$		
$\Delta P_{XE,PAO}$	0.16	gP/m^3	
$\Delta P_{XE,OHO}$	$= f_p \cdot MX_{E,OHOv} / (SRT \cdot Q_i)$		
	$= 0.03 \cdot 10{,}100 / (20 \cdot 15)$		
$\Delta P_{XE.OHO}$	1.01	gP/m^3	
ΔP_{XE}	1.17	gP/m^3	
5.4 ΔP by influent inert mass			
ΔP_{XU}	$= f_p \cdot MX_{Uv} / (SRT \cdot Q_i)$		
	$= 0.03 \cdot 22{,}804 / (20 \cdot 15)$		
ΔP_{XU}	2.28	gP/m^3	
5.5 ΔP by chemical P precipitation due to salts present in the influent or added to the system			
Not considered			
5.6 Potential total P removal			
$\Delta P_{SYS,pot}$	$= \Delta P_{PAO} + \Delta P_{OHO} + \Delta P_{XE} + \Delta P_{XU}$		
	$= 12.05 + 1.25 + 1.17 + 2.28$		
$\Delta P_{SYS,pot}$	16.8	gP/m^3	

5.7 Actual total P removal		
P_i	17.0	gP/m^3
$\Delta P_{SYS,actual}$	$= \min(\Delta P_{SYS,pot}; P_i) = \min(16.8; 17.0)$	
$\Delta P_{SYS,actual}$	16.8	gP/m^3
5.8 Particulate P in the effluent		
To calculate after step 6.5 where the P content of TSS is calculated		
$X_{P,e}$	$= f_{P,TSS} \cdot TSS_e$	
	$= 0.124 \cdot 5$	
$X_{P,e}$	0.6	gP/m^3
5.9 Effluent total P		
P_e	$= P_i - \Delta P_{SYS,actual} + X_{P,e}$	
	$= 17.0 – 16.8 + 0.6$	
P_e	0.8	gP/m^3
6. VSS and TSS		
6.1 VSS and active fraction		
MX_{bio}	$= MX_{PAOv} + MX_{OHOv}$	
	$= 9,511 + 12,490$	
MX_{bio}	22,000	kgVSS
MX_{VSS}	$= MX_{PAOv} + MX_{OHOv} + MX_{E,PAOv} + MX_{E,OHOv} + MX_{Uv}$	
	$= 9,511 + 12,490 + 1,603 + 10,100 + 22,804$	
MX_{VSS}	56,506	kgVSS
$f_{bio,VSS}$	$= MX_{bio} / MX_{VSS}$	
	$= 22,000 / 56,506$	
$f_{bio,VSS}$	39%	
6.2 FSS		
$f_{PAO,act}$	$= [(Q_i \cdot SRT \cdot \Delta P_{SYS,actual}) – (f_p \cdot (MXvss – MX_{PAOv})] / MX_{PAOv}$	
	$= [(15 \cdot 20 \cdot 16.8) – (0.03 \cdot (56,506 – 9,511)] / 9,511$	
$f_{PAO,act}$	0.38	gP/gVSS
MX_{FSS}	$= f_{FSS,OHO} \cdot MX_{OHOv} + f_{FSS,PAO} \cdot (f_{P,PAO,act}/f_{P,PAO}) \cdot MX_{PAOv} + FX_{FSS,i} \cdot SRT$	
	$= 0.15 \cdot 12,490 + 1.3 \cdot (0.38/0.38) \cdot 9,511 + 735 \cdot 20$	
MX_{FSS}	28,938	kgFSS
6.3 TSS		
MX_{TSS}	$= MX_{VSS} + MX_{FSS}$	
	$= 56,506 + 28,938$	
MX_{TSS}	85,443	kgTSS
6.4 f_{VT}		
f_{VT}	$= MX_{VSS} / MX_{TSS}$	
	$= 56,506 / 85,443$	
f_{VT}	0.66	gVSS/gTSS

6.5 P content of TSS

$f_{P,TSS}$ $= [(f_{P,OHO} \cdot MX_{OHOv} + f_p \cdot (MX_{E,OHOv} + MX_{E,PAOv}) + f_p \cdot MX_{Uv}) / f_{VT}$

$+ (f_{P,PAO} \cdot MX_{PAOv}) / f_{VT,PAO} + f_{P,FSS,i} \cdot MX_{FSS})] / MX_{TSS}$

$= [(0.03 \cdot 12,489 + 0.03 \cdot (10,109 + 1,603) + 0.03 \cdot 22,804) / 0.66 + (0.38 \cdot 9,517) / 0.46 + 0.02 \cdot 28,947)]/ 85,443$

$f_{P,TSS}$ 0.124 gP/gTSS

7. Process volume (based on TSS; may also be based on VSS)

Note that the influent flow rate needs to be appropriate

$X_{TSS,OX}$ 4,000 gTSS / m^3

V_R $= MX_{TSS} / X_{TSS,OX}$

$= 85,443/ 4,000$

V_R 21,361 m^3

The volume of the anaerobic zone (divided in two sections) depends on the anaerobic mass fraction.

$V_{R,AN}$ $= f_{xa} V_R$

$= 0.10 \cdot 21,361$

$V_{R,AN}$ 2,136 m^3

The anoxic and aerobic mass fractions, and thus the volume of these zones, should be estimated according to the procedure presented in Chapter 5 on nitrogen removal and in Ramphao *et al.* (2005) where the equations that relate the volume fractions to the mass fractions according to recycle ratios are given for various types of reactor configurations, including the JHB. Using an estimate of an aerobic and a total anoxic mass fraction of 0.45 each, the volume (m^3) for each zone would be approximately: AN1: 1,060, AN2: 1,060, AX: 7,000, OX: 10,500, AX-RAS: 1,750, for a total volume of 21,370 m^3. Note that this preliminary approximation does not take into consideration that sludge concentration in the RAS-anoxic zone is 2.3 times more concentrated than in the mainstream zones ((1+r)/r) which results in approximately one third of the anoxic mass being in the RAS-anoxic zone and a lower total process volume requirement.

8. Nitrogen requirement

FN_s $= f_n \cdot MX_{VSS} / SRT$

$= 0.10 \cdot 56,506 / 20$

FN_s 283 kgN/d

$TKN_{i,s}$ $= FN_s / Q_i$

$= 283 / 15$

$TKN_{i,s}$ 18.8 gN/m^3

9. Oxygen demand (OD)

OD by PAOs: for synthesis and endogenous respiration

FO_{PAO} $= FO_{PAO,s} + FO_{PAO,endo}$

$FO_{PAO,s}$ $= FS_{S,PAO} \cdot (1 – f_{cv} \cdot Y_{PAOv})$

$= 1,770 \cdot (1 – 1.48 \cdot 0.45)$

$FO_{PAO,s}$ 591 kgO_2/d

$FO_{PAO,endo}$ $= FS_{S,PAO} \cdot f_{cv} \cdot (1 – f_{XE,PAO}) \cdot b_{PAO,T} \cdot Y_{PAOv,obs} \cdot SRT)$

$= 1,770 \cdot 1.48 \cdot (1 – 0.25) \cdot 0.0337 \cdot 0.268 \cdot 20$

$FO_{PAO,endo}$ 356 kgO_2/d

FO_{PAO} 947 kgO_2/d

OD by OHOs: for synthesis and endogenous respiration

FO_{OHO}	$= FO_{OHO,s} + FO_{OHO,endo}$		
$FO_{OHO,s}$	$= FCOD_{b,OHO} \cdot (1 - f_{cv} \cdot Y_{OHOv})$		
	$= 7{,}000 \cdot (1 - 1.48 \cdot 0.45)$		
$FO_{OHO,s}$	2,338		
$FO_{OHO,endo}$	$= FCOD_{b,OHO} \cdot f_{cv} \cdot (1 - f_{XE,OHO}) \cdot b_{OHO,T} \cdot Y_{OHOv,obs} \cdot SRT$		
	$= 7{,}000 \cdot 1.48 \cdot (1 - 0.20) \cdot 0.202 \cdot 0.0892 \cdot 20$		
$FO_{OHO,endo}$	2,990	kgO_2/d	
FO_{OHO}	5,327	kgO_2/d	

OD total (carbonaceous)

FO_c	$= FO_{PAO} + FO_{OHO}$		
	$= 947 + 5{,}327$		
FO_c	6,274	kgO_2/d	

or in a simplified form:

FO_c	$= (1 - f_{cv} \cdot Y_{OHOv}) \cdot FCOD_{b,i} + f_{cv} \cdot (1 - f_{XE,OHO}) \cdot (b_{PAO,T} \cdot MX_{PAOv} + b_{OHO,T} \cdot MX_{OHOv})$		
	$= (1 - 1.48 \cdot 0.45) \cdot 8{,}770 + 1.48 \cdot (1 - 0.20) \cdot (0.0337 \cdot 9{,}511 + 0.202 \cdot 12{,}490)$		
FO_c	6,288	kgO_2/d	

COD mass balance verification					
Input					
$FCOD_i$		11,250	kgCOD/d	100%	IN
Output					
O_2 demand for synthesis and endogenous respiration					
FO_c		6,274	kgCOD/d	55.8%	
Soluble inerts leaving by the effluent					
$FS_{U,i}$		795	kgCOD/d	7.1%	
Sludge	gVSS	gCOD/d			
		(= gVSS · f_{cv} / SRT = gVSS · 1.48 / 20 = gVSS · 0.0740)			
MX_{PAOv}	9,511	704	kgCOD/d	6.3%	
MX_{OHOv}	12,490	925	kgCOD/d	8.2%	
MX_{bio}	22,000	1,628		14.5%	
$MX_{E,PAOv}$	1,603	119	kgCOD/d	1.1%	
$MX_{E,OHOv}$	10,100	747	kgCOD/d	6.6%	
MX_{Uv}	22,804	1,688	kgCOD/d	15.0%	
$MX_{endo+inert}$	34,506	2,553		22.7%	
MX_{VSS}	56,506	4,181	kgCOD/d	37.2%	
	Sum:	11,250	kgCOD/d	100%	OUT
	Delta (OUT-IN):	0	kgCOD/d	0%	

The 100% mass balance for COD indicates that all the influent COD is accounted for in the calculated values of oxygen demand and sludge production. From the COD mass balance, and for the conditions of the design example, the fate of the influent COD is as follows: 56% is oxidized with oxygen, 7% escapes in the effluent as soluble unbiodegradable organics and 37% becomes activated sludge. The sludge is composed of 39% (1,628/4,181) active biomass and 61% (2,553/4,181) inactive particulate matter of which 40% (1,688/4,181) are influent inerts and 21% ((119+747)/4,181) endogenous residue) on a COD basis. A summary of the EBPR system design results is presented in Table 6.7.

Table 6.7 Summary of EBPR system design results (Johannesburg configuration).

Description	Parameter	Units	Value
1. Influent and bioreactor			
Type of wastewater	raw/settled		raw
Temperature	T	ºC	14
Influent flow rate	Q_i	MLD	15
Influent total COD	COD_i	gCOD/m^3	750
Influent rapidly biodegradable COD	$S_{S,i}$	gCOD/m^3	146
Influent biodegradable COD	$COD_{b,i}$	gCOD/m^3	585
Influent total P	P_i	gP/m^3	17
Sludge retention time	SRT	d	20
Sludge recycle ratio	s	m^3.d /m^3.d	0.75
Aerobic recycle ratio	a	m^3.d /m^3.d	1.5
Nitrate concentration in sludge recycle	$S_{NO3,s}$	gN/m^3	0.5
2. Portion of $S_{S,i}$ for PAOs and of $COD_{b,i}$ for OHOs			
Concentration of fermentable COD in the last AN reactor	$S_{F,ANn}$	gCOD/m^3	14.3
Flux of $S_{S,i}$ for PAOs	$FS_{S,PAO}$	kgCOD/d	1,770
Flux of $COD_{b,i}$ for OHOs	$FCOD_{b,OHO}$	kgCOD/d	7,000
3. System biomass (VSS) equations			
Mass of PAOs	MX_{PAOv}	kgVSS	9,511
Mass of endogenous residue from PAOs	$MX_{E,PAOv}$	kgVSS	1,603
Mass of OHOs	MX_{OHOv}	kgVSS	12,490
Mass of endogenous residue from OHOs	$MX_{E,OHOv}$	kgVSS	10,110
Mass of unbiodegradable organics from influent	MX_{Uv}	kgVSS	22,804
4. P removal			
PO_4 release	S_{PO4_rel}	gP/m^3	59.0
P removal by PAOs	ΔP_{PAO}	gP/m^3	12.1
P removal by OHOs	ΔP_{OHO}	gP/m^3	1.3
P removal by endogenous residue	ΔP_{XE}	gP/m^3	1.2
P removal by X_U	ΔP_{XU}	gP/m^3	2.3

Potential P removal by system	$\Delta P_{SYS,pot}$	gP/m^3	16.8
Actual P removal by system	$\Delta P_{SYS,actual}$	gP/m^3	16.8
Effluent particulate P (from TSS_e)	$X_{P,e}$	gP/m^3	0.6
Influent total P	P_i	gP/m^3	17.0
Effluent total P	P_e	gP/m^3	0.9
5. Volatile and total suspended solids (VSS and TSS) in system			
Mass of active biomass	MX_{bio}	kgVSS	22,000
Mass of VSS	MX_{VSS}	kgVSS	56,506
Ratio of AVSS/VSS	$f_{bio,VSS}$	gAVSS/gVSS	0
Mass of fixed SS	MX_{FSS}	kgFSS	28,938
Mass of TSS	MX_{TSS}	kgTSS	85,443
Ratio of VSS/TSS	f_{VT}	gVSS/gTSS	0.66
Fraction of P in TSS	$f_{P,TSS}$	gP/gTSS	0.12
6. Bioreactor total volume			
Bioreactor volume	V_R	m^3	21,361
7. N requirement			
N requirement for synthesis	$TKN_{i,s}$	kgN/d	18.8
8. Oxygen demand			
Flux of O_2 demand by PAOs	FO_{PAO}	kgO_2/d	947
Flux of O2 demand by PAOs	FO_{OHO}	kgO_2/d	5,327
Flux of carbonaceous O_2 demand	FO_c	kgO_2/d	6,274
COD output/COD input	COD mass balance	gCOD/gCOD	100 %

Flow rate is in m^3/d and mass fluxes in g/d
For a flow rate 1,000 or greater mass fluxes can be read in kg/d

6.10 INFLUENCE OF OPERATIONAL FACTORS ON FULL-SCALE EBPR WWTP

6.10.1 Influence on volatile and total suspended solids and oxygen demand

The model for EBPR systems presented above enables the volatile suspended solids (VSS) and total suspended solids (TSS) of the mixed liquor (eqs. 6.23 and 6.24, respectively) and the carbonaceous oxygen demand (Eq. 6.31) to be calculated. A comparison of the mass of VSS and TSS generated and carbonaceous oxygen demand with and without EBPR per kg COD load on the bioreactor *versus* sludge age are shown in Figure 6.31 and Figure 6.32 for raw and settled wastewaters, respectively, with characteristics as shown. These characteristics were an EBPR system with two anaerobic reactors in-series with a total anaerobic mass fraction (f_{xa}) of 15% and no nitrate recycled to the anaerobic reactor operated at 20°C. From this comparison it appears that including EBPR in the activated sludge system increases the VSS only slightly, by about 5 to 12% and 15-25% for raw and settled wastewaters, respectively (depending on sludge age). This increase in VSS is due to the lower endogenous mass loss/death rate of the PAOs (0.04 d^{-1} at 20 °C) compared to the OHOs (0.24 d^{-1} at 20 °C). However, the TSS is increased substantially, by about 20 to 25% and 45 to 55% for raw and settled wastewaters,

respectively (depending on sludge age). This higher TSS production is due to the large quantities of stored inorganic polyphosphate and the associated inorganic cations necessary to stabilize the polyphosphate chains - principally Mg^{2+} and K^{+} (Fukase *et al.,* 1982; Arvin *et al.,* 1985; Comeau *et al.,* 1986; Wentzel *et al.,* 1989a; Ekama and Wentzel, 2004). The high inorganic content of the PAO biomass causes the VSS/TSS to be much lower than that of the OHOs, 0.46 mgVSS/mgTSS compared to 0.75 to 0.85 mgVSS/mgTSS. Thus, the higher the PAO fraction of the mixed liquor, the higher the EBPR and the lower the VSS/TSS ratio of the mixed liquor.

The increase in TSS with the inclusion of EBPR needs to be taken into account in the design of the bioreactor volume (Eq. 6.27) and daily sludge production. Also, since the inorganic cations that stabilize the polyphosphate are derived from the influent wastewater, there must be sufficient concentrations of these cations in the influent; otherwise the EBPR may be adversely affected (Wentzel *et al.,* 1988; Lindrea *et al.,* 1994). Further, because the VSS mass generated per kg COD load is greater with EBPR than without, the oxygen demand with EBPR is correspondingly reduced, by approximately 5-6% and 8-9% for raw and settled wastewaters, respectively (depending on sludge age, Figure 6.32).

Although there is only a small difference in VSS production between an EBPR and a non-EBPR system, the constituent sludge fractions for the two systems differ markedly. This can be readily demonstrated by comparing the percentage composition of the VSS mass generated in systems exhibiting EBPR to those that are not: to illustrate, percentage composition of the VSS mass are shown in Figure 6.33 for systems at 20 °C with no EBPR and with EBPR, respectively treating wastewater with characteristics as shown. Note that the EBPR system has a smaller OHO active mass than the non-EBPR system, but that the EBPR system has a significant concentration of PAO biological active mass.

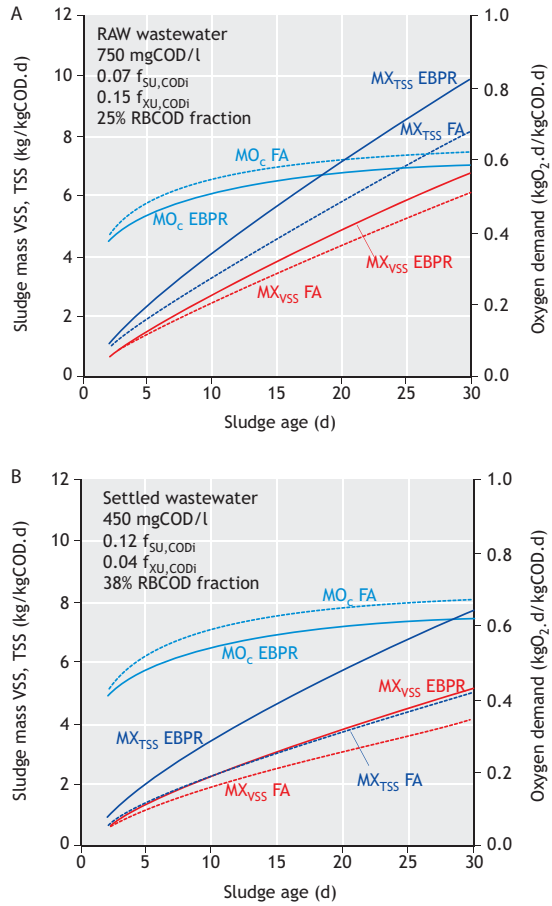


Figure 6.31 and 6.32 Approximate masses of volatile solids (MX_{VSS}) and total solids (MX_{TSS}) and daily carbonaceous oxygen demand (MO_c) per kg COD load on the biological reactor in fully aerobic (FA) and enhanced biological P-removal activated sludge systems treating (A, Fig 6.31) raw and (B, Fig 6.32) settled wastewater.

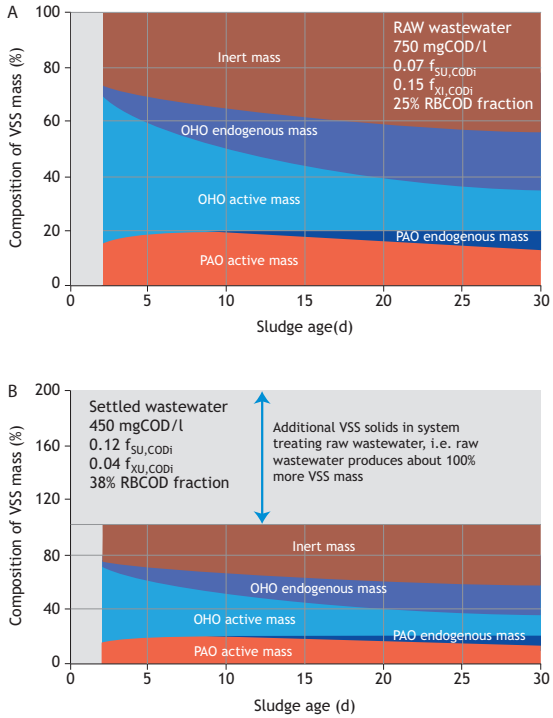


Figure 6.33 Percentage composition of VSS mass for EBPR systems treating (A) raw and (B) settled wastewater.

6.10.2 P/VSS ratio

A parameter often used to evaluate the EBPR performance of an activated sludge system is the P/VSS (or P/TSS) ratio of the mixed liquor. In Figure 6.34, calculated P/VSS ratios for a system with two-in-series anaerobic reactors and wastewater characteristics as shown are plotted *versus* sludge age. A zero discharge of nitrate to the anaerobic reactor is assumed.

From Figure 6.34, as the system sludge age increases, the P/VSS ratio increases up to a sludge age of approximately 10 days. Further increase in sludge age causes a decrease in P/VSS ratio. The initial increase in P/VSS with sludge age can be ascribed to increasing OHO active mass with sludge age. This produces an increased fermentable COD to VFA conversion efficiency in the anaerobic reactor and accordingly an increased PAO active mass (with associated P content of 0.38 mgP/mgVSS). The decrease in P/VSS can be ascribed to the endogenous respiration effect on PAOs.

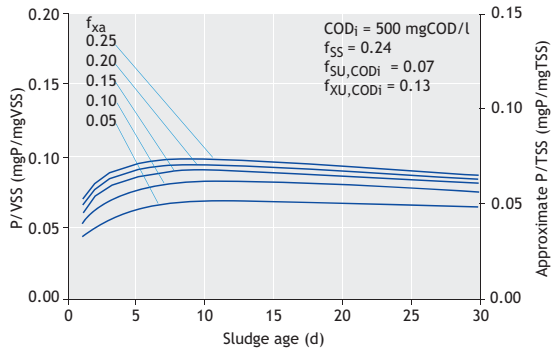


Figure 6.34 Predicted phosphorus to volatile (P/VSS) and total (P/TSS) suspended solids ratios *versus* sludge age for mixed liquor in a biological enhanced P-removal system with various anaerobic mass fractions (f_{xa}) treating wastewater with the characteristics shown.

It appears that the P/VSS ratio is a consequence of the selection of the fundamental design parameters that are sludge age and anaerobic mass fraction. Also, the P/VSS ratio is a function of the wastewater characteristics (*e.g.* RBCOD fraction). Accordingly, the parameter P/VSS ratio can fulfil a function in design only if a prior experimental relationship between the ratio and the design parameters has been established for the wastewater to be treated. It cannot be used reliably as a basic design parameter.

6.11 INTEGRATED DESIGN OF NDEBPR SYSTEMS

6.11.1 Background

In some countries legislation on permissible effluent ammonia concentrations necessitates that nitrification be incorporated in an EBPR removal activated sludge system. In the steady mixed-culture EBPR model, the nitrate recycled to the anaerobic reactor needs to be known due to the adverse influence of recycling nitrate to the anaerobic reactor

on P removal. Indeed, one of the principal orientations in any design procedure for P removal is to prevent nitrate recycling. This can be achieved by preventing nitrification in a simple configuration such as the Phoredox or the A/O systems but this option is not available in some countries. Accordingly, reliable and accurate quantification of denitrification in NDEBPR systems is essential for P removal design, in addition to N removal design. One approach that has been used to quantify denitrification in NDEBPR systems was to estimate the denitrification using the theory and procedures for nitrification-denitrification (ND) systems, as set out in Chapter 5 (WRC, 1984). Experimental data indicated that this approach appeared to predict the observed denitrification quite closely (Nicholls, 1982). However, from the mechanisms for EBPR and the development of EBPR kinetic theory, an inconsistency in this approach became evident: the RBCOD appeared to be used twice; in the anaerobic reactor where it is converted to VFAs which are sequestered and stored as PHA by the PAO, and in the primary anoxic reactor for denitrification. This situation would be possible only if the PAOs denitrified significantly using most of the VFAs internally stored as PHA in the upstream anaerobic reactor as electron donor in the downstream anoxic reactor, which implies that the principal P uptake should be in the primary anoxic reactor, not the aerobic reactor. Although this behaviour was not observed in some earlier lab-scale NDEBPR systems and enhanced culture work conducted at the University of Cape Town (Wentzel *et al.,* 1989a), it was clearly shown by Vlekke *et al.* (1988, Kuba *et al.* (1996), Hu *et al.* (2007) and integrated in the Activated Sludge Model 2d: ASM2d (Henze *et al.,* 1999). While ASM2d models PAO PHA utilization under anoxic conditions, it does not address the changes in EBPR behaviour with anoxic P uptake; EBPR P removal declines by as much as a third (Ekama and Wentzel, 1999). ASM2d allows P uptake to commence in the anoxic reactor, but the predicted P removal is the same as if the uptake had taken place only in the aerobic reactor. Subsequent model modifications have sought to address this, *e.g.* Hu *et al.* (2007).

Clearly for completeness, denitrification had to be incorporated into the steady-state mixed-culture model, an aspect omitted up to this stage. Using plug flow anoxic reactors and batch tests, Clayton *et al.* (1991) undertook an experimental investigation into the kinetics of denitrification in NDEBPR systems. They found that in NDEBPR systems:

- In the primary anoxic reactor, (*i*) the rapid rate of denitrification associated with RBCOD was much reduced or absent, and (*ii*) the slower rate of denitrification associated with SBCOD was approximately 2.5 times the rate measured in primary anoxic reactors of ND systems.
- In the secondary anoxic reactor, the denitrification rate was approximately 1.5 times the rate measured in secondary anoxic reactors of ND systems.

From an extensive enquiry into the causes, Clayton *et al.* (1991) concluded that the increased denitrification rates were not due to:

- Denitrification by PAOs; for the systems investigated, PHA and P measurements indicated that the PAOs did not denitrify, which remains controversial.
- Modification of the sewage in the anaerobic zone; wastewater that had not passed through an anaerobic zone induced the same denitrification response as sewage that had passed through the anaerobic zone.

The above observations led Clayton *et al.* (1991) to conclude that the increased rate was due to a stimulation in the active sludge mass of an increased rate of hydrolysis of SBCOD in the anoxic reactors of the NDEBPR systems, apparently induced by the presence of the anaerobic reactor in these systems.

6.11.2 Denitrification potential in NDEBPR systems

The denitrification potential is the maximum amount of nitrate that can be removed by biological means in the anoxic reactors. Since the experimental investigation into denitrification kinetics in NDEBPR

systems indicated that the formulation developed for ND systems can be applied to NDEBPR systems, the techniques set out in Chapter 5 to develop equations for denitrification potentials in ND systems can also be followed for NDEBPR systems. Note that a prime (') symbol is added to specific denitrification rate constants to indicate that the parameter value is different between a ND system (without prime) and a NDEPBR system (with a prime) (Clayton *et al.,* 1991; Ekama and Wentzel, 1999).

$$dS_{NO3} / dt = K_T \cdot X_{OHO} \qquad (mgNO_3^-\text{-N/l.d}) \quad (6.32)$$

dS_{NO3}/dt rate of denitrification ($mgNO_3^-$-N/l.d)
K_T specific denitrification rate at temperature T for a NDEBPR system ($mgNO_3^-$-N/mg AVSS.d)

6.11.2.1 Denitrification potential of the primary anoxic reactor

Denitrification in the primary anoxic reactor is via utilization of any RBCOD leaking through the anaerobic reactor, and SBCOD. Procedures to determine the amount of RBCOD leaking through the anaerobic reactor to the primary anoxic reactor are set out in Section 6.8.3.3, where $S_{F,ANn}$ is the concentration of fermentable COD in the anaerobic reactor outflow, and $S_{F,Ann}$ (1+recycle ratio) the mass per litre influent flow. These procedures take into account the utilization of RBCOD in the anaerobic reactor due to storage by PAOs (either directly or following conversion) or to denitrification / aerobic respiration by OHOs. Accordingly, the denitrification potential in the primary anoxic reactor (D_{p1}) can be expressed as:

$$D_{p1} = S_{F,ANn}(1+r)(1-f_{cv}Y_{OHOv}) / 2.86 + K_{2,T}X_{OHO}HRT_{np} \qquad (mgN/l_{inf}) \quad (6.33)$$

where:
D_{p1} denitrification potential in the primary anoxic reactor (mgN/l_{inf})
$K_{2,T}$ specific denitrification rate in the primary anoxic reactor of a NDEBPR system on SBCOD at temperature T and ~ 0.23 $mgNO_3^-$-N/mgAVSS.d (Clayton *et al.,* 1991; Ekama and Wentzel, 1999) *i.e.* ~ 2.5 times higher than in ND systems (K_{2T})
HRT_{np} nominal hydraulic retention time of the process (d)

Following the procedures set out in Chapter 5, Eq. 6.33 can be modified and simplified to give:

$$D_{p1} = S_{F,ANn}(1+r)(1-f_{cv}Y_{OHOv}) / 2.86 + \frac{f_{x1}K_{2,T}(COD_{b,i} - S_{s,PAO})Y_{OHO}\,SRT}{(1+b_{OHO,T}\,SRT)} \qquad (6.34a)$$

or,

$$D_{p1} = \alpha + f_{x1}K_{2,T}\beta \qquad (6.34b)$$

where:
f_{x1} primary anoxic reactor mass fraction

$$\alpha = S_{F,ANn}(1+r)(1-f_{cv}Y_{OHOv}) / 2.86 \qquad (6.35a)$$

$$\beta = \frac{(COD_{b,i} - S_{S,PAO})\,Y_{OHO}\,SRT}{(1+b_{OHO,T}\,SRT)} \qquad (6.35b)$$

In Eq. 6.34 it is assumed that the initial rapid rate of denitrification ($K_{2,T}$) on RBCOD leaking through the anaerobic reactor $S_{F,Ann}(1+r)$ is always complete, *i.e.* the actual retention time in the primary anoxic reactor is longer than the time required to utilize this RBCOD. As with ND systems, an equation can be developed to determine the minimum primary anoxic mass fraction $f_{x1,min}$ to deplete this RBCOD:

$$f_{x1,min} = \frac{S_{F,ANn}(1+r)(1-f_{cv}\,Y_{OHOv})\,(1+b_{OHO,T}\,SRT)}{(COD_{b,i} - S_{S,PAO})\,2.86\,K_{1,T}\,Y_{OHO}\,SRT} \qquad (6.36a)$$

$$f_{x1,min} = \alpha / (\beta \cdot K_{1,T}) \qquad (6.36b)$$

Where $K_{1,T}$ is the initial rapid rate of denitrification in the primary anoxic reactor of a NDEBPR system on RBCOD at T °C and equal to that in a ND system K_{1T}.

Substituting the values for the constants into Eq. 6.36 and assuming 80 per cent of the influent RBCOD is sequestered by the PAOs in the anaerobic reactor, $f_{x1,min} < 0.02$ for SRT > 10 days at 14 °C with $COD_{b,i}$ = 800 mgCOD/l and $f_{SS} = 0.24$. This value of 2% of anoxic mass fraction is much lower than actual primary anoxic reactors mass fraction so that for nearly all cases eqs. 6.34 and 6.35 will be valid.

However, eqs. 6.34a and 6.34b are not without complication. To calculate the primary anoxic denitrification potential (D_{p1}), the concentration of RBCOD in the outflow from the anaerobic reactor ($S_{F,ANn}$) is required. To calculate $S_{F,ANn}$, the concentration of nitrate recycled to the anaerobic reactor is required which in turn requires D_{p1} to be known. This aspect will be dealt with in more detail in Section 6.11.3.2 below.

6.11.3.2 Denitrification potential of the secondary anoxic reactor

The denitrification potential of the secondary anoxic reactor (D_{p3}) is found by following the principles set out in Chapter 5, and is given by:

$$D_{p3} = \frac{f_{x3} K_{3,T} (COD_{b,i} - S_{S,PAO})\, Y_{OHO}\, SRT}{(1 + b_{OHO,T}\, SRT)} \qquad (6.37a)$$

$$D_{p3} = f_{x3} K_{3,T} \beta \qquad (6.37b)$$

where:

f_{x3} secondary anoxic reactor mass fraction

$K_{3,T}$ specific denitrification rate in the secondary anoxic reactor at temperature T and ~ 0.10 $mgNO_3$-N/mgAVSS.d (Clayton *et al.,* 1991; Ekama and Wentzel, 1999) *i.e.* ~ 1.5 times higher than in ND systems ($K_{3,T}$).

Eq. 6.37 applies to secondary anoxic reactors situated both in the mainstream (*e.g.* 5-stage Modified Bardenpho) and in the underflow recycle (*e.g.* JHB system). However, in applying Eq. 6.37 to secondary anoxic reactors situated in the underflow recycle, care must be taken in evaluating f_{x3}, because the mixed-liquor concentration is increased by a factor (1+s)/s in the underflow anoxic reactor compared to the mainstream reactors.

The higher $K_{2,T}$ and $K_{3,T}$ denitrification rates in a NDEBPR system compared with ND systems require the case of higher η values on the OHO hydrolysis/growth processes of SBCOD under anoxic conditions in ASM2 and ASM2d.

6.11.3 Principles of denitrification design procedures for NDEBPR systems

In NDEBPR systems, design is oriented to achieve the following in a single sludge system:

1. COD removal,
2. N removal (nitrification/denitrification), and
3. P removal (EBPR).

Conflict between these objectives may arise, in particular between N and P removal, *e.g.* unaerated mass required for anoxic reactors (N removal) and anaerobic reactors (P removal). For each design, the priorities for treatment need to be assessed and a compromise reached to optimize the system.

In some countries, the design of NDEBPR systems usually focuses on EBPR with denitrification as a secondary design priority, because legislation limits effluent P concentrations, and only in selected cases are effluent nitrate concentrations limited. Accordingly, in such situations the fundamental principle in denitrification design for NDEBPR systems is to ensure that the anaerobic reactor is protected from recycling of nitrate. This fundamental principle will determine the selection of the system configuration (5-stage Modified Bardenpho, JHB and UCT/MUCT considered in this chapter) and provides procedures for sizing the anoxic reactors.

When selecting a system configuration for EBPR, it is necessary to establish whether complete

denitrification can be achieved. For the wastewater characteristics, *i.e.* influent TKN and COD concentrations (TKN_i and COD_i), maximum specific growth rate of the nitrifiers at 20 °C ($\mu_{ANO,max,20}$) and the average minimum water temperature, the maximum unaerated sludge mass fraction ($f_{x,max}$) and the nitrification capacity (NIT_c) can be calculated for a selected sludge age (SRT), see Chapter 5. This $f_{x,max}$ needs to be divided between anaerobic (for EBPR) and anoxic (for denitrification) mass fractions. Consequently, the maximum anoxic sludge mass fraction ($f_{xd,max}$) is the difference between the maximum unaerated mass fraction ($f_{x,max}$) and the selected anaerobic sludge mass fraction (f_{xa}), *i.e.*

$$f_{xd,max} = f_{x,max} - f_{xa} \qquad (6.38)$$

where:

$f_{xd,max}$	maximum anoxic mass fraction
$f_{x,max}$	maximum unaerated mass fraction

The value of $f_{x,max}$ is given by Eq (5.19), Chapter 5 for a selected SRT, $\mu_{ANO,max,20}$, S_f and T_{min}.

The value of $f_{xd,max}$ can then be subdivided between primary and secondary anoxic sludge mass fractions (f_{x1} and f_{x3}) and this division fixes the denitrification potential of these two reactors (D_{p1} and D_{p3}) and hence also of the system. If the denitrification potential of the system exceeds the nitrification capacity (*i.e.* $D_{p1} + D_{p3} > NIT_c$) then complete denitrification is possible and the secondary anoxic reactor is situated in the mainstream, the 5-stage Modified Bardenpho. If complete denitrification is not possible, with the 5-stage Modified Bardenpho nitrate will appear in the effluent and be recycled via the s-recycle to the anaerobic reactor. Accordingly, the secondary anoxic reactor is moved to the underflow recycle, the JHB system, in which event the denitrification potential of the secondary anoxic reactor (D_{p3}) must exceed the nitrate and oxygen loads via the underflow s-recycle. If this requirement is not met, nitrate will 'leak' through the underflow secondary anoxic reactor into the anaerobic reactor. In this event, since the denitrification potential of the primary anoxic reactor (D_{p1}) is greater than that of the secondary anoxic reactor (D_{p3}) for equal anoxic mass fractions, incorporation of a secondary anoxic reactor becomes an inefficient utilization of anoxic mass fraction and the secondary anoxic mass fraction is added to the primary anoxic reactor, the UCT/MUCT system. Alternatively, if very low effluent nitrate concentrations are required, the secondary anoxic reactor can be retained and methanol can be added to it.

6.11.4 Analysis of denitrification in NDEBPR systems

Analysis of the denitrification behaviour in the NDEBPR system is essentially the same as for the ND system (Chapter 5) except that:

- The mass fraction for denitrification ($f_{xd,max}$) for the NDEBPR system is given by Eq. 6.38, whereas $f_{xd,max}$ for the ND system is given by Eq. 5.56. Hence, for the same maximum unaerated sludge mass fraction ($f_{x,max}$), the NDEBPR system has a lower mass fraction than the ND system, by an amount equal to f_{xa}.
- The specific denitrification rates for ND systems (K_2 and K_3, Chapter 5) are substituted with the rates measured for NDEBPR systems ($K_{2,T}$ and $K_{3,T}$, Section 6.11.2).
- The denitrification potentials for the primary and secondary anoxic reactors are modified from Chapter 5 for the ND system to those given by eqs. 6.34 and 6.37 for the NDEBPR system to take account of the storage of COD by the PAOs in the anaerobic reactor, and the non-participation of the PAOs in denitrification.

The objective of the simplified steady state model presented below is to obtain an estimate of the a-recycle ratio to load the anoxic reactor to its denitrification potential. A detailed analysis of EBPR systems can be realized with simulation programs. Taking account of the above, denitrification equations are developed below for the UCT system.

6.11.4.1 UCT System

In the UCT system the denitrification behaviour is very similar to that in the MLE system, so that, taking due account of the effect of incorporating the anaerobic reactor, the design equations and procedures developed for the MLE system can be readily adapted for application to the UCT system.

In this application, the following principles are of importance:

- Since complete denitrification is not possible, the entire anoxic mass fraction available is used, in the form of a primary anoxic reactor.
- The a-recycle ratio determines the split of nitrate between the primary anoxic reactor and the effluent. The a-recycle ratio is selected so that the equivalent nitrate loads to the primary anoxic reactor via the a and s recycles just load the reactor to its denitrification potential.

Taking account of the above, design equations are developed below for the UCT system.

- Denitrification potential (D_{p1}): The denitrification potential of the primary anoxic reactor (D_{p1}) is found from Eq. 6.34 with $f_{x1} = f_{xd,max}$, *i.e.*:

$$D_{p1} = \alpha + f_{xd,max} K_{2,T} \beta \qquad (6.39)$$

- Effluent nitrate concentration ($S_{NO3,e}$): If the nitrate concentration in the outflow of the primary anoxic reactor is zero, then:

$$S_{NO3,e} = NIT_c / (a + s + 1) \qquad (6.40)$$

- Optimum a-recycle ratio (a_{opt}): Due to the similarities between the MLE and UCT systems, an equation for a_{opt} for the UCT system can be developed by following the procedure for the MLE system: *i.e.* a_{opt} is the a-recycle that just loads the primary anoxic to its denitrification potential (D_{p1}). From a mass balance around the primary anoxic reactor, the equivalent nitrate load on this reactor ($FS_{NO3,x1}/Q_i$) is given by:

$$\frac{FS_{NO3,x1}}{Q_i} = s\left[S_{NO3,e} + \frac{S_{O2,s}}{2.86}\right] + a\left[S_{NO3,e} + \frac{S_{O2,a}}{2.86}\right] \qquad (6.41)$$

where:
$S_{O2,s}$ and $S_{O2,a}$ are the dissolved O_2 concentration in the s and the a recycles, respectively.

Equating Eq. 6.41 to the denitrification potential given by Eq. 6.39, recognising the a = a_{opt} and solving for a_{opt} gives:

$$a_{opt} = [\ -B + \sqrt{B^2 - 4AC}\ ] / (2A) \qquad (6.42)$$

where:

A	$S_{O2,a} / 2.86$
B	$NIT_c - D_{p1} + \{(s+1)\ S_{O2,a} + s\ S_{O2,s}\} / 2.86$
C	$s\ NIT_c - (s + 1)\ (D_{p1} - s\ S_{O2,s}/2.86)$

At a = a_{opt}, Eq. 6.42 will give the minimum $S_{NO3,e}$ achievable. Eq. 6.42 is valid for all a ≤ a_{opt} because for all a ≤ a_{opt} the assumption on which Eq. 6.42 is based is valid, *i.e.* zero nitrate concentration in the outflow from the primary anoxic reactor. If the system is operated with a > a_{opt}, the equivalent nitrate load on the primary anoxic reactor via the a- and s-recycles exceeds the denitrification potential, and nitrate will also be recycled via the r-recycle to the anaerobic reactor, to the detriment of EBPR. Furthermore, if nitrate does 'leak' through the primary anoxic reactor then the nitrate concentration in the outflow from the primary anoxic reactor is no longer zero, and consequently, Eq. 6.40 for the effluent nitrate concentration ($S_{NO3,e}$) is not valid.

6.11.5 Maximum nitrate recycled to anaerobic reactor

The design procedures for denitrification in the previous section have been developed assuming that the increased denitrification rates ($K_{2,T}$ and $K_{3,T}$) apply, *i.e.* that the system is exhibiting EBPR. However, recycling nitrate or oxygen to the anaerobic reactor has a detrimental effect on EBPR.

In a case where so much nitrate or oxygen is recycled that all the fermentable COD is consumed for denitrification, none would remain available for conversion to VFAs. In this case, in Eq. 6.8 setting $S_{F,i,conv} = 0$ and solving for $S_{NO3,s}$ gives:

$$S_{NO3,s} = \left[\left\{ \frac{S_{F,i}}{8.6} - \frac{(sS_{O2,s} + S_{O2,i})}{2.86} \right\} - S_{NO3,i} \right] / s \qquad (6.43)$$

This nitrate concentration effectively sets the maximum amount of nitrate that can be recycled to the anaerobic reactor with the equations in this chapter remaining valid. At this $S_{NO3,s}$ concentration if there is any VFAs present in the influent, EBPR will still be obtained.

Should $S_{NO3,s}$ be exceeded, a competition between the PAOs and the OHOs for the VFAs develops (for storage and denitrification, respectively) and a kinetic model will be required to determine system performance, and the equations developed in this chapter are not valid for this situation.

6.12 CONCLUSIONS

Enhanced biological phosphorus removal (EBPR) has been developed to assist in the control of eutrophication by removing phosphorus from wastewaters without the use of chemicals. The high phosphorus content of the biomass wasted from EBPR processes makes it amenable to phosphorus recovery by struvite formation (magnesium ammonium phosphate: $MgNH_4PO_4$) especially when an anaerobic digester is used, or as hydroxyapatite [$Ca_{10}(PO4)_5(OH)_2$] when little ammonia is available.

In some sensitive water bodies, very low phosphorus (and nitrogen) discharge limits have been promulgated, sometimes below 0.1 g total P per m3. To consistently achieve such low levels, coagulants and filtration or ultrafiltration systems need to be used.

Phosphorus-accumulating organisms (PAOs) have been studied in order to understand the biochemical mechanisms of their anaerobic, anoxic and aerobic metabolism. From these studies, process optimisation principles have been derived and mathematical models have been developed for steady-state design analysis and incorporated into software programs to study various scenarios and facilitate the design, optimisation and development of EBPR systems. The effect of nitrate in sludge or internal recycles, and the effect of dynamic changes in loadings (*e.g.* organic surcharges after a weekend or the addition of industrial wastes) can best be quantified with such software programs.

Future developments in the field should come from improved understanding of the biochemical mechanisms of different groups of PAOs, GAOs and filamentous organisms to propose practical control strategies to favour the dominance of PAOs. Some PAO groups, such as *Tetrasphaera*, appear to be of high importance in many EBPR processes, where much remains to be learned about how they function and how to best exploit their activity to benefit P removal. With a better fundamental understanding of biochemical processes, improved parametric and metabolic models could then be developed that would lead to more accurate models and more robust EBPR processes.

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NOMENCLATURE

Symbol	Description	Unit
a	Mixed liquor recycle ratio based on influent flow	$m^3.d/m^3.d$
a_{opt}	A-recycle ratio that gives a minimum N_{ne}	$m^3.d/m^3.d$
b_{OHO}	Specific endogenous mass loss rate of the OHOs	gEVSS/gVSS.d
$b_{OHO,T}$	OHO specific endogenous mass loss rate at temperature T	gEVSS/gVSS.d
b_{PAO}	Specific endogenous mass loss rate of the PAOs	gEVSS/gVSS.d
$b_{PAO,T}$	PAO specific endogenous mass loss rate at temperature T	gEVSS/gVSS.d
COD_b	Concentration of biodegradable COD	$gCOD/m^3$
$COD_{b,i}$	Concentration of biodegradable COD in the influent	$gCOD/m^3$
$COD_{b,OHO}$	Concentration of biodegradable COD available to the OHOs	$gCOD/m^3$
D_{P1}	Denitrification potential of the primary anoxic reactor	$gNO_3\text{-}N/m^3$ influent
D_{P3}	Denitrification potential of the secondary anoxic reactor	$gNO_3\text{-}N/m^3$ influent

f_{xa}	Anaerobic mass fraction	gVSS/gVSS
f_{x1}	Primary anoxic reactor mass fraction	gVSS/gVSS
$f_{x1,min}$	Minimum primary anoxic mass fraction	gVSS/gVSS
f_{x3}	Secondary anoxic reactor mass fraction	gVSS/gVSS
$FCOD_{b,i}$	Daily mass of influent biodegradable organics	gCOD/d
$FCOD_{b,OHO}$	Daily mass of biodegradable substrate available to OHOs	gCOD/gCOD
$FCOD_i$	Daily mass of influent COD	gCOD/d
f_{CV}	COD/VSS ratio of the sludge	gCOD/gVSS
$f_{FSS,OHO}$	Inorganic content of OHOs	gFSS/gTSS
$f_{FSS,PAO}$	Inorganic content of PAOs	gFSS/gTSS
f_n	N content of the sludge	gN/gVSS
FN_s	Daily mass of nitrogen required for sludge production	gN/d
FO_c	Daily mass of carbonaceous oxygen demand	gO_2/d
FO_{OHO}	Daily mass of oxygen consumed by OHOs	gO_2/d
FO_{PAO}	Daily mass of oxygen consumed by PAOs	gO_2/d
FO_t	Daily mass of total oxygen demand	gO_2/d
$f_{P,FSS}$	Fraction of P in the fixed (inorganic) suspended solids	gP/gFSS
$f_{P,FSS,i}$	Fraction of P in the influent FSS	gP/gFSS
$f_{P,OHO}$	Fraction of P in the active OHO mass	gP/gAVSS
$f_{P,PAO}$	Fraction of P in the active PAO mass	gP/gAVSS
$f_{P,TSS}$	P content with respect to TSS	gP/gTSS
f_p	P content with respect to VSS	gP/gVSS
f_p	Fraction of P in the OHO endogenous mass	gP/gEVSS
f_p	Fraction of P in the PAO endogenous mass	gP/gEVSS
f_p	Fraction of P in the inert mass	gP/gIVSS
$f_{PO4,rel}$	Ratio of P release/VFA uptake	gP/gCOD
$FS_{F,CONV}$	Daily mass of fermentable COD converted into VFAs in the anaerobic reactors	gCOD/d
$f_{SU,CODi}$	Influent unbiodegradable soluble COD fraction	gCOD/gCOD
$FS_{PO4,rel}$	Daily mass of P released by PAOs	gP/d
$f_{SS,CODi}$	Influent readily biodegradable fraction of influent total COD	gCOD/gCOD
f_{SS}	Influent readily biodegradable fraction of the influent biodegradable COD	gCOD/gCOD
$FS_{S,PAO}$	Daily mass of S_S stored by PAOs in the anaerobic reactor	gCOD/d
$FS_{VFA,i}$	Daily mass of influent VFAs	gCOD/d
$f_{SVFA,SSi}$	Fraction of VFAs of the readidly biodegradable COD	gCOD/g COD
f_{VT}	VSS/TSS ratio for OHO active and endogenous masses, PAO endogenous mass and inert mass	gVSS/gTSS
$f_{VT,PAO}$	VSS/TSS ratio for PAO active mass	gVSS/gTSS
$f_{xd,max}$	Maximum anoxic mass fraction	gVSS/gVSS
$f_{XE,OHO}$	Fraction of endogenous residue of the OHOs	gEVSS/gAVSS
$f_{XE,PAO}$	Fraction of endogenous residue of the PAOs	gEVSS/gAVSS
$FX_{FSS,i}$	Daily mass of influent inorganics	gFSS/d

$f_{XU,CODi}$	Fraction of influent unbiodegradable particulate COD	g COD/gCOD
$f_{x,max}$	Maximum unaerated mass fraction	g VSS/gVSS
$FX_{S,i}$	Daily mass of influent slowly-biodegradable COD	g COD/d
HRT_{np}	Average nominal hydraulic retention time of the process	d
$K_{1,T}$	Specific denitrification rate in primary anoxic reactor of NDEBPR system on RBCOD at temperature T	gNO_3^--N/gOHOVSS.d
$K_{2,T}$	Specific denitrification rate in primary anoxic reactor of NDEBPR system on SBCOD at temperature T	gNO_3^--N/gOHOVSS.d
$K_{3,T}$	Specific denitrification rate in secondary anoxic reactor of NDEBPR system on SBCOD at temperature T	gNO_3^--N/gOHOVSS.d
$k_{F,T}$	First-order fermentation rate constant at temperature T	m^3/gOHOVSS.d
K_T	Specific denitrification rate of OHOs for an NDEBPR system (') at temperature T	gNO_3^--N/gOHOVSS.d
$MX_{E,OHOv}$	Mass of OHO endogenous residue in the system	gEVSS
$MX_{E,PAOv}$	Mass of PAO endogenous residue in the system	gEVSS
MX_{FSS}	Mass of fixed (inorganic) suspended solids in the system	gFSS
MX_{OHOv}	Mass of OHOs in the system	gAVSS
MX_{PAOv}	Mass of PAO in the system	gAVSS
MX_{TSS}	TSS mass in the system	gTSS
MX_{Uv}	Mass of inert organic matter in the system, coming from the influent	gVSS (or gIVSS)
MX_{VSS}	Mass of volatile suspended solids in the system	gTSS
n	Number of the anaerobic reactor from a series	-
N	Total number of anaerobic reactors of equal volume in the series n = 1,2.....N	-
NIT_c	Nitrification capacity of the bioreactor	gNO_3-N/m^3
Q_i	Daily average influent flow rate	m^3/d
$Q_{i,ADWF}$	Average dry weather flow	Ml/d
r	Mixed-liquor recycle ratio from the aerobic to anoxic (or anaerobic) reactor based on influent flow	m^3.d/m^3.d
s	Return activated sludge recycle ratio based on influent flow	m^3.d/m^3.d
S_{Alk}	Alkalinity concentration	mg$CaCO_3$/l
S_F	Fermentable organic matter concentration	gCOD/m^3
$S_{F,ANn}$	Fermentable organic matter conc. in the n^{th} AN reactor	gCOD/m^3
$S_{F,conv}$	Fermentable organic matter converted into VFAs per volume of influent	gCOD/m^3
$S_{F,DENIT}$	Fermentable substrate consumed by denitrification in the anaerobic reactor	gCOD/m^3
$S_{F,i}$	Fermentable organic matter concentration in the influent	gCOD/m^3
$S_{F,i,conv}$	$S_{F,i}$ available for conversion into VFAs per volume of influent	gCOD/m^3
$S_{F,OXID}$	Fermentable substrate consumed by aerobic oxidation in the anaerobic reactor	gCOD/m^3
$S_{NO3,e}$	Effluent nitrate concentration	gNO_3-N/m^3
$S_{NO3,i}$	Influent nitrate concentration (to the AN reactor)	gNO_3-N/m^3
$S_{NO3,s}$	Nitrate conc. in the sludge recycle to the AN reactor	gNO_3-N/m^3

S_{O2}	Dissolved oxygen concentration	gO_2/m^3
$S_{O2,a}$	Oxygen conc. in the anoxic recycle to the AN reactor	gO_2/m^3
$S_{O2,i}$	Influent oxygen concentration	gO_2/m^3
$S_{O2,s}$	Oxygen concentration in the sludge recycle to the AN reactor	gO_2/m^3
$S_{PO4,rel}$	Concentration of P released	gP/m^3
SRT	Sludge age	d
$S_{S,i}$	Influent readily biodegradable COD concentration	$gCOD/m^3$
$S_{S,PAO}$	Concentration of S_S stored by PAOs	$gCOD/m^3$
$S_{U,i}$	Influent inert soluble organic matter concentration	$gCOD/m^3$
S_{VFA}	Volatile fatty acids concentration	$gCOD/m^3$
$S_{VFA,i}$	VFA concentration in the influent	$gCOD/m^3$
t	Time	h
T	Temperature	^oC
TKN	Total Kjeldahl nitrogen concentration	gN/m^3
$TKN_{i,s}$	Influent TKN required for biomass synthesis	gN/m^3
T_{min}	Minimum temperature	^oC
P_e	Effluent total phosphorus concentration	gP/m^3
P_i	Influent total phosphorus concentration	gP/m^3
TSS	Total suspended solids	$gTSS/m^3$
V_R	Volume of biological process (bioreactor)	l
VSS	VSS concentration	$gVSS/m^3$
$X_{FSS,i}$	Influent fixed suspended solids (FSS) concentration	g FSS/m^3
X_{OHO}	Ordinary heterotrophic organism concentration	$gCOD/m^3$
$X_{OHO,AN}$	Concentration of OHOs in the anaerobic reactor	$gCOD/m^3$
X_{PAO}	Phosphorus-accumulating organisms	$gCOD/m^3$
X_S	Slowly biodegradable organics concentration	g COD/m^3
$X_{S,i}$	Influent slowly biodegradable organics concentration	g COD/m^3
X_{TSS}	Reactor total suspended-solids concentration	g TSS/m^3
$X_{TSS.OX}$	Selected required TSS concentration in the aerobic reactor	$gTSS/m^3$
$X_{U,i}$	Influent inert particulate matter concentration	$gCOD/m^3$
X_{VSS}	Reactor volatile suspended solids concentration	$gVSS/m^3$
$X_{VSS.OX}$	Selected desired TSS concentration in the aerobic reactor	$gVSS/m^3$
Y_{OHOv}	OHO biomass yield	gAVSS/gCOD
ΔP_{OHO}	P removal due to OHOs	gP/m^3 influent
ΔP_{PAO}	P removal due to PAOs	gP/m^3 influent
ΔP_{SYS}	Total P removal by the system	gP/m^3 influent
$\Delta P_{SYS.actual}$	Total P actual removal by the system	gP/m^3 influent
$\Delta P_{SYS,pot}$	Total P potential removal by the system	gP/m^3 influent
ΔP_{XE}	P removal due to endogenous residue mass	gP/m^3 influent
ΔP_{XU}	P removal due to inert mass	gP/m^3 influent

Abbreviation	Description
A/O	Anaerobic/oxic process
A^2O	Anaerobic, anoxic, aerobic process
AN	Anaerobic
AX	Anoxic
AVSS	Active volatile suspended solids
BNR	Biological nitrogen removal
DDGGE	Dry denaturing gradient gel electrophoresis
e	Effluent
EBPR	Enhanced biological phosphorus removal
EM	Electron microscopy
EVSS	Endogenous residue as volatile suspended solids
FISH	Fluorescence *in situ* hybridisation
FSS	Fixed (inorganic) suspended solids
HRT	Hydraulic retention time
IVSS	Inert volatile suspended solids
i	Influent
JHB	Johannesburg process
MLE	Modified Ludzack-Ettinger process
MLSS	Mixed-liquor suspended solids
MLVSS	Mixed-liquor volatile suspended solids
MUCT	Modified UCT process
NIT	Nitrifying organisms
ND	Nitrification-denitrification
NDEBPR	Nitrification-denitrification EBPR
OHO	Ordinary heterotrophic organism
OUR	Oxygen uptake rate
OX	Aerobic
PAO	Phosphate-accumulating organism
PHA	Poly-β-hydroxyalkanoates
PHB	Poly-β-hydroxybutyrate
PHV	Poly-β-hydroxyvalerate
PO_4	Phosphate
RAS	Return activated sludge
RBCOD	Readily biodegradable COD
SBCOD	Slowly biodegradable particulate organic matter
SBR	Sequencing batch reactor
SRT	Sludge retention time
SST	Secondary settling tank
TCA	Tricarboxylic acid cycle
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus

TSS	Total suspended solids
UCT	University of Cape Town process
VFA	Volatile fatty acid
VSS	Volatile suspended solids
VIP	Virginia initiative plant process
w	Sludge wastage from the aerobic reactor
ws	Sludge wastage from the sludge recycle line

Greek symbols	Description	Units
α	Constant alpha	
β	Constant beta	
$\mu_{ANO,max,20}$	Maximum specific growth rate of nitrifiers at 20 $^{\circ}$C	d^{-1}
$\theta_{k,F}$	Arrhenius temperature coefficient for k_F	-
η	Reduction factor for aerobic hydrolysis/growth process rates on SBCOD for anoxic conditions	
$\theta_{b,OHO}$	Arrhenius temperature coefficient for b_{OHO}	-
$\theta_{b,PAO}$	Arrhenius temperature coefficient for b_{PAO}	-

Figure 6.35 Fundamental research using PAO cultures enriched in laboratory-scale sequencing batch reactors (SBRs) have contributed significantly to development of metabolic models. The photo is a screenshot of the online course on EBPR (https://experimentalmethods.org/courses/activated-sludge-activity-tests/) based on Van Loosdrecht *et al.*, 2016.