# BIOLOGICAL PHOSPHORUS REMOVAL PROCESSES FOR WASTEWATER TREATMENT

**A. Sathasivan**
*Department of Civil and Construction Engineering, Curtin University of Technology, Perth WA 6845, Australia.*

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**Summary**

Presence of nutrients, especially nitrogen and phosphorus in wastewater effluents and their impacts on natural water bodies are of major concern. With the recent evidence that anthropogenic phosphorus (not nitrogen) addition in micrograms per litre level can trigger algal growth, phosphorus removal to lowest level would become increasingly important. Chemical and biological means are adopted to remove them. While phosphorus could be removed chemically, nitrogen removal is mostly carried out by biological means. Biological phosphorus removal process is popular over chemical means for it's simplicity, economy and various environmental benefits. Biological phosphorus removal process relies on enhancing the ability of microorganisms to uptake

more phosphorus into their cell. Therefore, these processes are often referred to as enhanced biological phosphorus removal (EBPR) processes. EBPR has been implemented worldwide in many wastewater treatment plants. Despite its promise to provide efficient phosphorus removal performance, at times unreliable performance has been reported. In search for answers to such questions many researchers have contributed to the understanding of these processes in the past fifty years. Although uncertainty still remains to some extent, vast knowledge has been created and put into practice. Fundamentally, proposal of anaerobic/aerobic biochemical pathways and identifying bacteria possibly responsible for EBPR (Candidus *Accumlibacter phosphatis*) and that could destabilize EBPR (*Candidatus* Competibacter phosphatis) are major outcomes. Practically, adopting simultaneous nitrification/denitrification and phosphorus accumulation by supplying very low level of dissolved oxygen in the aerobic zone has been very significant. This has the potential to cut down the cost of operation, while markedly improving the nutrient and carbonaceous matter removal. Biological means can achieve effluent phosphorus concentration up to 0.1 mg/L with average around 0.5 mg/L. The new requirement will need additional chemical treatment to further remove phosphorus to micrograms per litre levels. This paper summarizes the existing knowledge base and provide insight into how they are adopted in practice.

## 1.Introduction

Eutrophication (i.e., nutrient enrichment due to human activities) in surface waters are primarily due to nitrogen and phosphorus. The most recognizable manifestations of this phenomena are algal blooms that occur during summer. Over-nutrient enrichment results in low dissolved oxygen (DO), fish kills, murky water and depletion of desirable flora and fauna. In some cases toxic algae such as *microsystis* was found in algal blooms. In addition, the increase in algae increases the need to increase chlorine doses of drinking water, which in turn, leads to higher levels of disinfection by-products (Fisher et al, 2004; Jack et al., 2002) that have been shown to increase the risk of cancer (Wang et al, 2007). Excessive amounts of nutrients can also stimulate the activity of harmful microbes, such as *Pfisteria* (Hasselgren *et al.*, 2008).

Approximately 25% of all water body impairments are due to nutrient-related causes (U.S. EPA, 2007). In efforts to reduce the number of nutrient impairments, many point source dischargers have received more stringent effluent limits for nitrogen and phosphorus. To achieve these new, lower effluent limits, facilities have begun to look beyond traditional treatment technologies. There are physical, chemical, and microbiological means of removing nutrients. Biological nutrient removal processes remove nitrogen and phosphorus from wastewater through the proper use of microorganisms under different environmental conditions. Biological uptake for growth of biomass also removes nitrogen and phosphorus, but they are not significant. In domestic wastewaters removal more than this is required and hence other means are needed. The biological processes that primarily remove nitrogen are ammonification (conversion of organic nitrogen to ammoniacal nitrogen), nitrification (conversion of ammonia to nitrate) and denitrification (conversion of nitrate to nitrogen gas ($N_2$) which escapes to atmosphere).

Despite the traditional belief that algal blooms could be controlled by both nitrogen and phosphorus, it was clearly shown that phosphorus is the key and controlling nutrient and that nitrogen control could show negative effect such as encouragement of some group of algae (Schindler, 2006; Chemistry times, 2008). Bowman et al., (2007) showed phosphorus addition in the range of even 0.1-5.6 µg/L over a long period could trigger algal blooms in part of a natural lake.

Therefore, phosphorus removal from wastewaters would become increasingly important. To remove phosphorous, it must either be converted into a particulate form and removed as a particulate by sedimentation, filtration, or some other solids removal process or be concentrated into a side-stream using membrane treatment.

Figure 1 summarizes various options for removing or converting phosphorous species. Three options are available to remove phosphorous from the system: Convert phosphorous to chemical species by adding a metal salt or lime (precipitation); remove with membrane treatments; and incorporate the phosphorous into biomass.

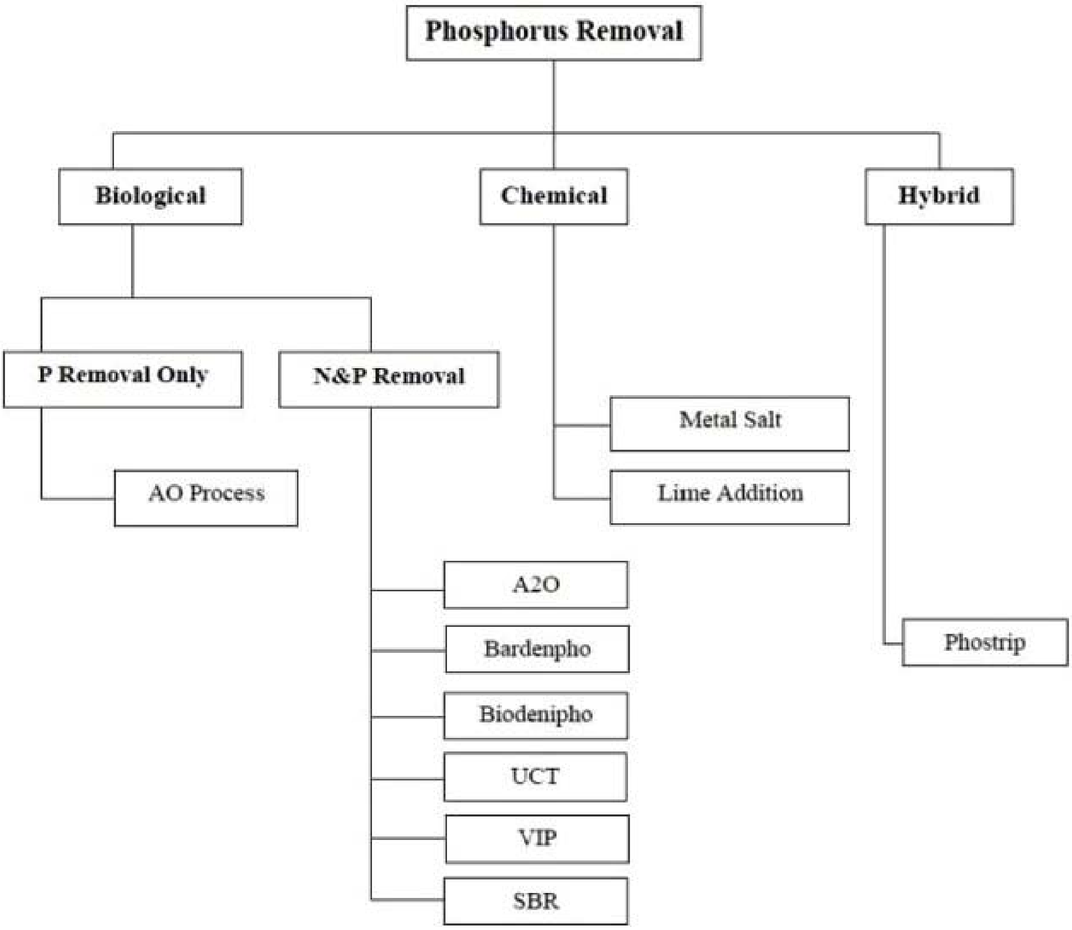


Figure 1: Overview of phosphorus removal processes.

Adopted from Department of the Army, 2001, Biological Nutrient Removal, Public Works Technical Bulletin 420-49-39. www.wbdg.org/ccb/ARMYCOE/PWTB/pwtb_420_49_39.pdf

The efficiency of phosphorus removal by chemical precipitation depends on two factors: the chemical equilibrium between the phosphorus containing water and solid, and the efficiency of the solids removal process. Usually the later process controls the removal efficiency.

Processes that will remove essentially all pollutants from wastewater, such as reverse osmosis or nano-filtration membranes, can be used to remove phosphorous (Ratanatamskul et al., 1996). Membrane treatment is expensive and not currently used for mainstream phosphorous removal; however, membranes used for another objective (e.g., total dissolved solids removal) can also remove phosphorous.

Typically, biomass contains 1.5 to 2.5 % (w/w) phosphorous per volatile solids. Under certain conditions, the biomass will accumulate phosphorous levels of 6 to 8 %, far in excess of the nutritional requirements. Phosphorus accumulated in sludge or biomass is removed by sedimentation (solid separation).

Biomass containing biologically removed phosphorus could be used as fertiliser. In some plants, effluent-P concentration of <1.0 or even less than 0.3 mg/l had been achieved successfully. The process of removing phosphorus by accumulating it with biomass is referred to as enhanced biological phosphorous removal (EBPR) process.

The phosphorous removal efficiency for biological systems depends on the phosphorus content of the sludge removed and the efficiency of the solids separation process. While this process has been shown to be economical and feasible in many cases, at times phosphorus removal was found to be fluctuating for unknown reasons. The uncertainty has led to intensive research in this field in the past few decades.

Much have been understood since Srinath et al., (1959) reported the observation of biological phosphorus removal in an activated sludge plant. Despite the fact that wastewater treatment processes routinely adopts EBPR, the process is still not completely understood and the uncertainty still remains to an extent (Oehmen et al, 2007). This article summarises the essential outcomes of the past research, and how they are operationally utilised.

## 2. The EBPR Process Description

The EBPR process basically consists of consequent anaerobic and aerobic zone, the former zone followed by the later, instead of an aeration tank in a conventional activated sludge process (Figure 2a).

The major feature of this process is that organic matter uptake and phosphorus release take place under anaerobic condition and phosphorus uptake takes place under subsequent aerobic zone.

In most cases, it results in lower than influent phosphorus concentration (Figure 2b). Phosphorus is accumulated in the sludge and is removed by sedimentation. Organisms that help achieve this process by accumulating poly-P reserves are called polyphosphate accumulating organisms (PAOs).

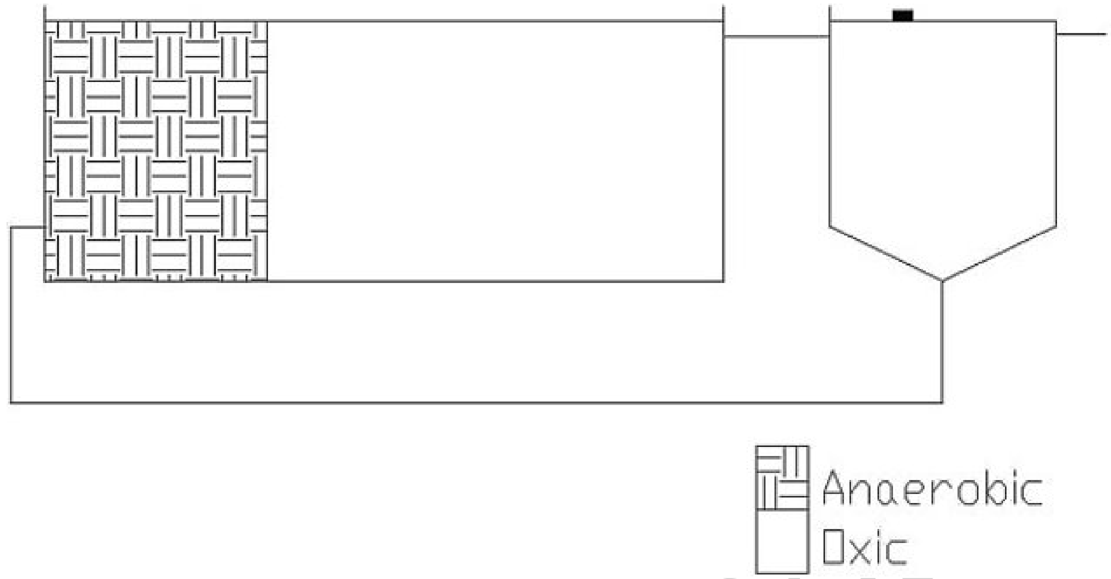


Figure 2a: Enhanced biological phosphorus removal process: simple modification of activated sludge process

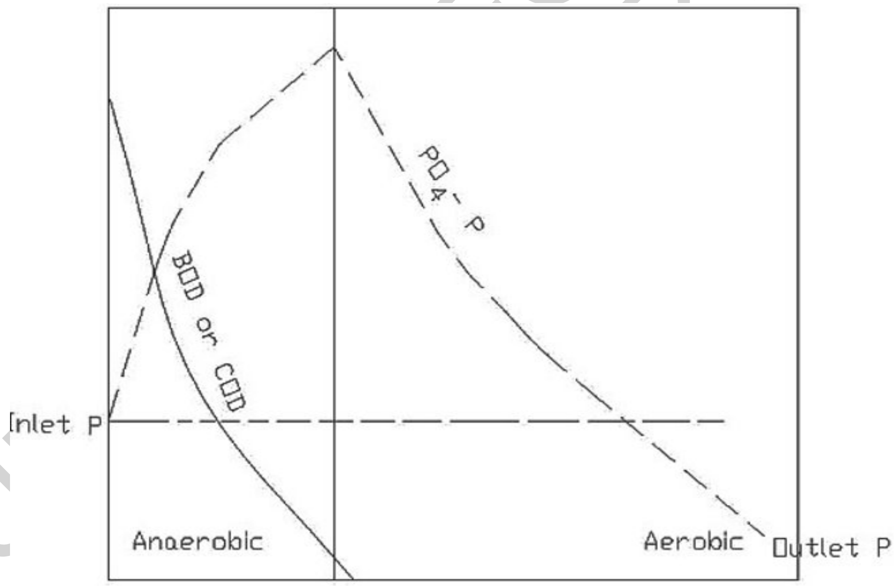


Figure 2b: Typical changes occurring in dissolved contents in an EBPR process

## 3. Microbiological characteristics of PAOs

Microbiological understanding in the initial years were hampered by lack of available techniques to isolate or to identify PAOs. Later in early 2000s availability of molecular microbiological tools have helped in identifying some of the major organisms and these proved that earlier description of the organisms were not correct. Proper isolation was still not possible and identification of multiple organisms that could play role in EBPR process made it difficult to assign the major changes observed to single organisms.

The first major morphological description of PAOs were made by Fuhs and Chen, (1975) based on microscopic observations of PAO-enriched sludge. They described them as non-motile rods or cocci, usually exist in clusters, are PHB (poly-β-hydroxybutyrate) staining positive, and contain Neisser positive granules in the cell.

They also reported that *Acinetobacter spp*. as the predominant microrognisms in EBPR process. When similar culture dependent identification methods were used, several others also reported the predominance of *Acinetobacter spp*. in EBPR processes (Buchan, 1983; Lotter, 1985; Wentzel et al., 1986). In these methods only those bacteria that are culturable on the artificial media used under the defined conditions can be isolated and identified.

Wagner et al., (1994) using molecular microbiological techniques showed that classical culture-dependent methods for bacterial counting are strongly selective for *Acinetobacter spp*. Further research also has demonstrated that no pure cultures of *Acinetobacter spp* have shown typical characteristics of EBPR sludges with high phosphorous removal capability (Jenkins and Tandoi, 1991; van Loosdrecht et al., 1997).

With these evidence and citing various other works (Cloete and Steyn, 1987; Hirashi et al., 1989; Hirashi and Morishita, 1990; Auling et al., 1991; Wagner et al., 1994; Bond et al., 1995; Kampfer et al., 1996; I Made et al., 1998), Mino et al., (1998) concluded that *Acinetobacter spp* need no longer be considered as the principal organisms responsible for the EBPR process.

Many attempts have been made to isolate PAOs responsible for EBPR, but they all failed to show all the characteristics observed in typical EBPR processes (Mino et al, 1998; Oehmen et al., 2007). Despite difficulties to isolate microorganisms responsible, the use of molecular techniques have helped identifying some organisms which are predominant in sludges showing good EBPR performance (Bond et al, 1999).

Hesselmann et al., 1999 later named it as "Candidus *Accumlibacter phosphatis*". They are often abbreviated to *Accumlibacter* but are also referred to as *Rhodocyclus*-related bacteria. Later studies clearly showed abundance in many different full scale EBPR processes across the world (Oehmen et al., 2007), and the abundance of *Accumlibacter* clearly correlated to phosphorus content of the sludge, i.e. more the abundance of *Accumlibacter* more was the phosphorus content of the sludge. In some cases as high as 90% abundance of *Accumlibacter* was noted in a sludge with excellent EBPR performance.

Further studies identified that not all *Accumulibacter* species contained poly-P granules and there were other group of bacteria that contained poly-P granules. One of the group identified was *Actinobacter* species (Wong et al., 2005) which contained poly-P granules. In contrast to *Accumlibacter*, *Actinobacter* was shown not to uptake volatile fatty acids (VFAs).

VFA uptake is a major feature observed in many EBPR processes. These results point to the role of multiple organisms responsible for EBPR performance. Further studies are continuing to understand more about these organisms and other organisms that are present in full scale EBPR plants. The major breakthrough, however, is in identifying *Accumlibacter* as one of the PAOs. Despite identification of *Accumlibacter*, researchers are still struggling to isolate them.

## 4. Biochemical Aspects Of Enhanced Biological Phosphorus Removal Processes

The major feature of this process is that phosphorus stored in the sludge as high energy poly-P (polyphosphate) reserves are released in the form of ortho-P (orthophosphate) from the cell, as organic matter uptake and storage occurs during the anaerobic phase (Marias et al., 1983; Mino et al., 1988).

Under anaerobic conditions, organisms can take up carbon sources such as VFAs and store them intracellularly as carbon polymers, namely poly-β-hydroxyalkanoates (PHAs) (Satoh et al., 1992). Figure 3 shows the observed changes in a graphical form. In order to explain the biochemical pathways, researchers in the past bulked all organisms together and explained the phenomenon observed using known biochemical pathways.

This was because it was difficult to isolate PAO phenotypes, and hence the biochemical models discussed here should only be used with caution. With the availability of advanced molecular microbiological tools more information can be expected.

Acetate is the most studied substrate. This was because acetate fed reactors showed high phosphate accumulating capabilities and majority of the influent organic matter in most of the wastewater treatment plant contain acetate. The observed phenomena was later explained by a biochemical model. For formation of PHAs, reducing power is needed and this is supplied largely by the glycolysis of internally stored glycogen (Arun et al, 1988).

Experiments done by Sathasivan et al., (1993) showed that acetate addition in anaerobic condition induced additional glucose uptake in a glucose saturated sludge. In combination, the model that the reducing power necessary for formation of PHAs is supplied by glycolysis of glycogen was proposed. The model was later referred to as “the Mino model” although an alternative “the Comeau-Wentzel model” was proposed to explain the source of reducing power. Comeau-Wentzel model (Wentzel et al., 1991; Comeau et al., 1986; Wentzel et al., 1986) and Matsuo et al., (1982) proposed the source of reducing power to be supplied from tricarboxylic acid (TCA) cycle.

Figure 4 shows the biochemical pathway in anaerobic zone as shown in Mino et al., (1998). Although initial experimental evidences clearly pointed out to more functionality of Mino model, possibility of existence of Comeau-Wentzel model was also raised. Later experimental evidences, however, suggested that many other models are possible in anaerobic phase: Mino model (Glycolysis) has been proposed to be combined with full TCA cycle, the glyoxylate shunt or the split TCA cycle depending on the bacterial culture (or species of *Accumlibacter*) present in the sludge or on the prevailing environment to supply the necessary reducing power to form PHAs. If multiple organisms are responsible for EBPR, multiple pathways are likely to exist and hence gross biochemical models, as they are proposed now to explain the gross changes observed, may not completely work.

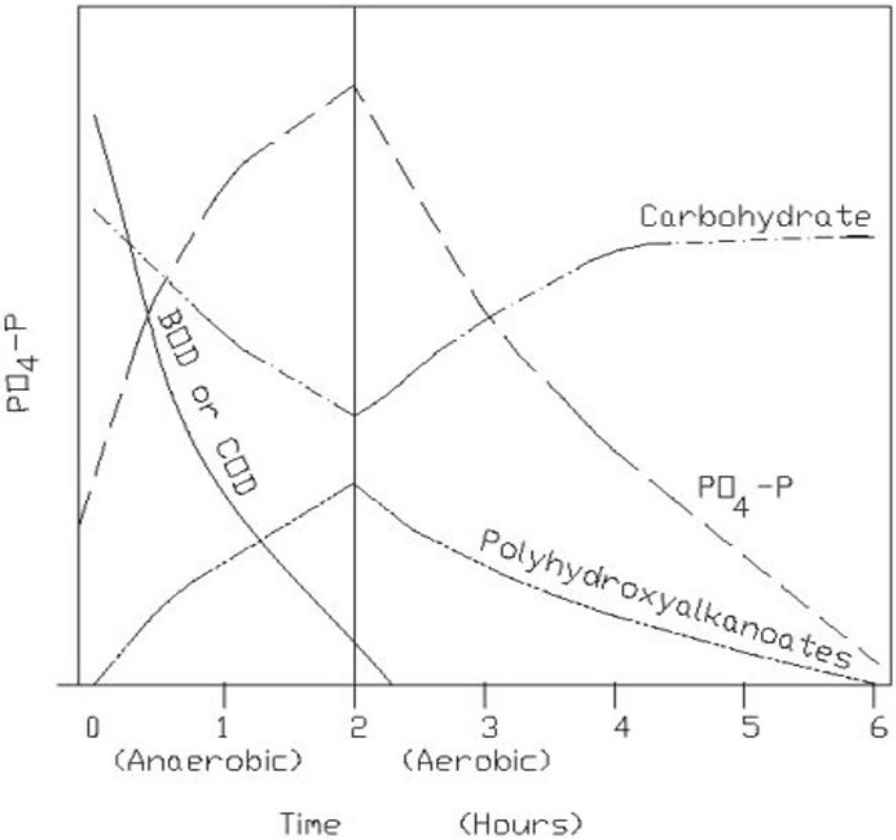


Figure 3: Typical intra- and extra-cellular observed in an anaerobic-aerobic batch experiment in enhanced biological phosphorus accumulating organisms (PAOs)

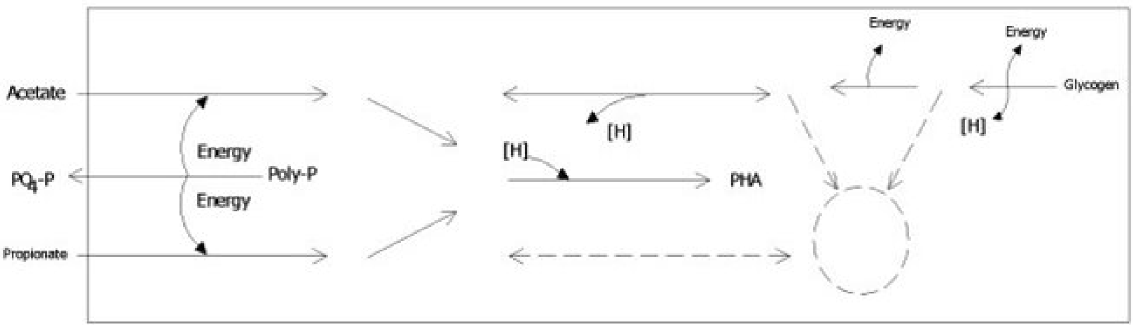


Figure 4: Anaerobic metabolism of PAOs- The Mino model. [H] represents the reducing power.

During the aerobic phase (Figure 5), the released phosphate is taken back into the cell and stored as poly-P reserves, as the terminal oxidation of the stored organic compound produces energy through oxidative level phosphorylation. During this process, glycogen replenishment is also reported (Arun et al, 1988). Net phosphorous removal is achieved through the removal of waste activated sludge containing a high polyphosphate content.

Presence of carbon and phosphate sources at the same time under aerobic or anoxic conditions has negative effects on phosphorous uptake (Smolders et al., 1994, Kuba et al 1994; Brdjanovic et al., 1998). Carbon sources available under these conditions will be primarily utilized for PHA formation. Only when the external carbon sources are exhausted, phosphorous uptake occurs (Mino et al., 1998). Therefore, simultaneous presence of electron acceptors (including carbon sources) and phosphate should be avoided.

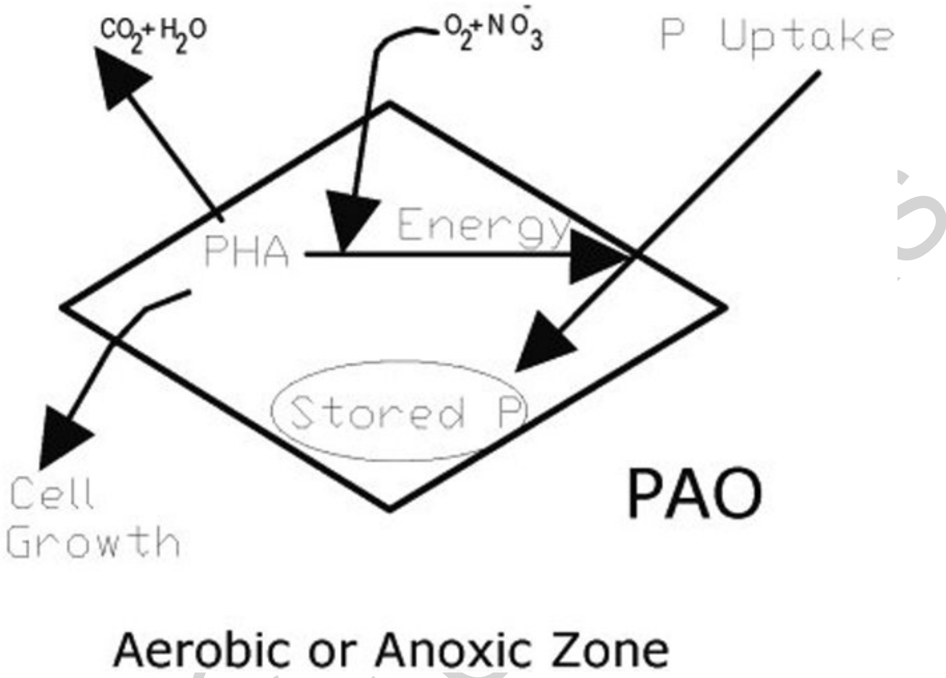


Figure 5: Biological Phosphorus Removal.

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## Biographical Sketch

**A. Sathasivan** has been working as a senior lecturer in the department of civil engineering and construction of Curtin University of Technology from 2006. He received his Bachelor of Science in Engineering (Hons,) in 1987 from the University of Peradeniya, Sri Lanka. He obtained his master degree (Master of Engineering in Water and Wastewater Engineering) from Asian Institute of Technology in 1991 and his doctoral degree (D. Eng. in Environmental Engineering) from The University of Tokyo, Japan in 1995. Prior to joining Curtin University, he worked for 5 years with Sydney Water on modelling and in investigating microbiological aspects of drinking water distribution preceded by another 2 yrs with Asian Institute of Technology as Assistant Professor. His major areas of research are nitrification in chloraminated systems, bacterial regrowth, biofouling in membranes and water reuse. His work in Sydney Water resulted in a new method development for microbiological decay measurement in chloraminated distribution system and a commercial *mEnCo* model to predict dissolved organic carbon (DOC) remaining after enhanced coagulation. The method to measure microbiological decay has shown that chloramine residual changes in distribution system is actually predictable, in contrast to the earlier believe that it is unpredictable. His interest in biological phosphorus removal started since 1990 when he did his masters research. He has many publications in modelling and fundamental aspects of drinking water quality in distribution system.