Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003)

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Abstract

Large quantities of phosphate present in wastewater is one of the main causes of eutrophication that negatively affects many natural water bodies, both fresh water and marine. It is desirable that water treatment facilities remove phosphorus from the wastewater before they are returned to the environment. Total removal or at least a significant reduction of phosphorus is obligatory, if not always fulfilled, in most countries. This comprehensive review summarizes the current status in phosphorus-removal technologies from the most common approaches, like metal precipitation, constructed wetland systems, adsorption by various microorganisms either in a free state or immobilized in polysaccharide gels, to enhanced biological phosphorus removal using activated sludge systems, and several innovative engineering solutions. As chemical precipitation renders the precipitates difficult, if not impossible, to recycle in an economical industrial manner, biological removal opens opportunities for recovering most of the phosphorus and beneficial applications of the product. This review includes the options of struvite (ammonium-magnesium-phosphate) and hydroxyapatite formation and other feasible options using, the now largely regarded contaminant, phosphorus in wastewater, as a raw material for the fertilizer industry. Besides updating our knowledge, this review critically evaluates the advantage and difficulties behind each treatment and indicates some of the most relevant open questions for future research.
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1. Introduction

Large-scale wastewater production is an inevitable consequence of contemporary societies. Wastewater is usually hazardous to human populations and the environment and must be treated prior to disposal into streams, lakes, seas, and land surfaces. Obligatory anaerobic treatment of domestic and agro-industrial wastewater releases large amounts of phosphorus and nitrogen into wastewater. These nutrients are directly responsible for eutrophication (extraordinary growth of algae as a result of excess nutrients in water bodies) of rivers, lakes, and seas worldwide (Lau et al., 1997; Trépanier et al., 2002). Consequently, disposal of wastewaters produces a constant threat to dwindling fresh water on a global scale (Montaigne and Essick, 2002).

Before discharging wastewater into water bodies, removing phosphate is usually obligatory, even though in many cases it is not performed, and leads to major contamination on a worldwide level. The wastewater treatment industry presently uses several methods to remove phosphorus (Duenas et al., 2003). Some are used in large-scale treatment facilities and some are only experimental projects, and therefore, still used on a small-scale basis (for a process-engineering point of view, see Stratful et al. (1999) and Van Loosdrecht et al. (1997). In all cases, phosphorus is removed by converting the phosphorus ions in wastewater into a solid fraction. This fraction can be an insoluble salt precipitate, a microbial mass in an activated sludge, or a plant biomass in constructed wetlands. These approaches do not recycle phosphorus as a truly sustainable product because it is removed with various other waste products, some of which are toxic. The nonsolubilized phosphates are either buried at landfills after incineration of the organic matter or used as sludge fertilizer, if the treatment facility eliminates human pathogens and toxic compounds.

This review analyzes the current status of phosphorus-removing approaches (inorganic, organic, and men-made organic phosphorus) and concentrates solely on developments reported since 1997. Several earlier fundamental studies are included only for clarification. It critically concentrates on each approach and on open questions that require more research. Special attention is given to biological phosphorus removal and its recycling as a raw material for the phosphate industry.

2. Precipitation of phosphorus by metal salts

Today, the main commercial processes for removing phosphorus from wastewater effluents are chemical precipitation with iron, alum, or lime (Donnert and Salecker, 1999a; Penetra et al., 1999), and to a lesser extent, biological removal (Stratful et al., 1999). Occasionally, auto-precipitation (like struvite, described later) occurs as an outcome of special conditions and composition of the wastewater (Van Der Houwen and Valsami-Jones, 2001). Phosphates (PO_4^{3-}) removal also can be achieved by air stripping CO_2 from anaerobic effluents (Kalyuzhnyi et al., 2000). Although phosphorus precipitation is a common commercial practice, further refining, fine-tuning, optimization, using leftovers and new materials from other industrial processes, and a few other innovations were proposed in recent years.

2.1. Iron–phosphorus precipitation

Creation of new recipes for better phosphorus precipitation with common materials from another industrial process was reported. For example, an industrial by-product, blast furnace slag, has a very high phosphorus adsorption capacity (Sakadevan and Bavor, 1998). Iron-based, layered double hydroxides were synthesized. The compounds released metal cations (Mg, Ca, and Fe). The released cations and their hydroxides effectively serve as coagulants for phosphorus removal (Seida and Nakano, 2002). A reaction-mixture composed of silica sand, crushed high-content calcium limestone, and several readily available metal oxides were compared. Iron/calcium oxides, produced during steel manufacturing, and fine-grained activated aluminum oxide outperformed other oxides. These materials removed greater than 99% of the phosphate from effluent within one hour. Continuous loading of a column composed of silica sand, limestone, and iron/calcium oxide, at representative groundwater flow rates over a four-year period, removed over 90% of phosphates from the water (Baker et al., 1998), showing the long-term potential of these combinations of substances to remove phosphorus.

Mechanisms of removing phosphorus by adding metal cations were studied. Adding $FeSO_4$ to three urban sewage sludges created vivianite, a non-soluble ferrous phosphate. In two anaerobically digested sludges, 60–67% of the sludge iron appeared as oxidized vivianite, whereas, in the activated sludge, 43% of the iron was accounted for by a non-oxidized vivianite. This process has an undesirable side effect, increasing the phosphorus content of the sludges (Frossard et al., 1997). Adding different concentration of $FeCl_2$ (20, 40 and 100 mg L^{-1}) to the aeration tank to control filamentous bulking also removed most of the phosphate. The iron salt also was effective in suppressing phosphate release and sulfide production from the wasted sludge by selectively enriching iron-reducing bacteria over sulfate-reducing bacteria. Consequently, iron-oxidizing bacteria outnumbered all the other bacterial populations (Yamamoto-Ikemoto et al., 1998).

Iron precipitation can work in concert with Ca, Cu, and Zn ions to eliminate common phosphorus pollutants, such as mono- and polyphosphonates (used in industrial and household applications such as water-cooling systems, oil production, textile production, and detergents). Normally, the main pathway of phosphonate removal is by adsorption onto surfaces. An evaluation of Ca, Cu, Zn, and Fe^{3+} on the adsorption of six phosphonates onto the iron (hydr)oxide, or goethite showed that, when Ca and Zn concentrations were in excess of the phosphonate concentrations ($10 \mu M$), considerable increases in adsorption occurred. This is presumably through a ternary surface complex formation and adsorption onto precipitated Zn (hydr)oxides (Nowack and Stone, 1999).

2.2. Alum-phosphorus precipitation

Aluminum hydroxide, $Al(OH)_3$, is a strong adsorption agent for orthophosphate and condensed phosphate. It precipitates them almost immediately. It precipitates organic phosphate only at the low pH of 3.6. A theoretical analysis of $Al(OH)_3$ and precipitation of $AlPO_4$ demonstrates that orthophosphate removal is not achieved via the precipitated $AlPO_4$ when applying conventional alum to the wastewater, but via precipitation of aluminum hydroxide phosphate. Laboratory tests of mixtures of alum sludge and wastewater indicated that phosphate is removed almost exclusively in the particulate fraction of the sludge. The removal of orthophosphate decreased with aging of the alum sludge (Galarneau and Gehr, 1997). The high adsorption capacity of aluminum oxide can be maintained over long periods, like the iron oxide mentioned earlier. A column packed with silica sand, limestone, and activated aluminum oxide removed over 99% of phosphorus, even after two years (Baker et al., 1998). Similarly, even air-dried, spent alum sludge adsorbed phosphorus rapidly, with phosphorus reduced 55% in the first 20 min after exposure (Huang and Chiswell, 2000). Low concentrations of phosphorus ($1 mg L^{-1}$) can be removed by activated alumina (Donnert and Salecker, 1999a; Donnert and Salecker, 1999b). Adsorption by aluminum sulfate can be enhanced by adding organic polyelectrolytes, like tannin, synthetic anionic polyelectrolyte, and clay (Ozacar and Sengil, 2003). Hydrotalcite, a double-layered mixed-metal hydroxide ($Mg–Al–CO_3$) adsorbed large quantities of phosphate from aqueous solutions (Lazaridis, 2003).

Aluminum compounds formed during precipitation depend on the type and quantity of organic matter present in the wastewater. Solid aluminum species with different surface properties are formed, depending on solution components. For example, addition of aluminum to wastewater in the presence of tannic acid forms aluminum hydroxyphosphate, hydroxy-alumino-tannate, and aluminum complexes containing both phosphorus and tannic acid. Tannic acid, as well as other organic matter, inhibits phosphorus removal. The extent of inhibition increases with increasing concentrations of organic matter. When aluminum is exposed to tannic acid, the organic matter coats the surface of the inorganic solid, preventing adsorption of phosphorus. It should be advantageous to add an alum treatment at the end of the cleaning process for harvesting more phosphorus when concentrations of organic matter are relatively low (Ozacar and Sengil, 2003; Omoike and Vanloon, 1999).

2.3. Calcium–phosphorus precipitation

Calcium–phosphorus precipitation is a common method of phosphorus removal, mainly because of low cost and ease of handling. Removal is achieved by direct precipitation of calcium phosphate (hydroxyapatite, $Ca_5(PO_4)_3OH$) (Yi and Lo, 2003), using calcite as seeding material (Donnert and Salecker, 1999a; Donnert and Salecker, 1999b). Additional artificial crystal seed material, consisting of calcium silicate hydrate (tobermorite crystals, manufactured by mixing siliceous and calcareous raw materials, pelletizing, and autoclaving), is also applicable for phosphorus removal by crystallization. "Card house"-shaped crystals develop on the seed material, where orthophosphate crystallizes as a calcium phosphate. The hydroxyapatite crystallizes at pH 8.0–8.5 without inducing the precipitation of calcium carbonates that usually negatively affect the process. Phosphorus removal efficiencies in this process ranged from 75% to 85% (Moriyama et al., 2001). Finally, lime was also suggested as a pre-treatment of municipal wastewater before the biological process (Marani et al., 1997).

Spontaneous calcium phosphate precipitation in EBPR aerobic systems (described later) may occur when systems are fed natural Ca-rich wastewater. However, before precipitation starts at neutral pH, the phosphate concentration must be at least $50 mg P L^{-1}$ and the calcium concentration at $100 mg L^{-1}$. This indicates that, in most cases, when dealing with municipal wastewater, calcium phosphate precipitation in EBPR processes is not a significant phosphorus removal mechanism (Carlsson et al., 1997). Supersaturation of the solution is necessary for spontaneous precipitation of hydroxylapatite. Addition of $1 mM$ citrate increases the supersaturation level required for precipitation, thereby inhibiting precipitation of hydroxylapatite (Van Der Houwen and Valsami-Jones, 2001).

Presence or formation of carbonates inhibits hydroxyapatite formation (Battistoni et al., 1997). At pH 8.0, the phosphate precipitation rate is significantly reduced by carbonate, but not at pH 9. This indicates that carbonates may decrease the precipitation rate of

calcium phosphate, and solution pH value is a key factor influencing the precipitation process. Carbonate and phosphate are competing for calcium. The effect of carbonate on the phosphate precipitation is attributed to the formation of ion pairs between carbonate and calcium and the decrease of free calcium ions. Additionally, carbonate may be co-precipitated from solution with phosphate, especially at pH 9–11, creating a precipitate with relative low phosphorus content (Montastruc et al., 2003; Song et al., 2002).

2.4. Magnesium–phosphorus precipitation

Magnesium salts are the least used of cations for phosphorus precipitation, apart from intentional formation of struvite (see later in this review). This precipitation is performed to assist sludge digestion leading to sludge stabilization. Removal of phosphate from the anaerobic supernatant is essential for reducing phosphate returned with the clarified supernatant to the head of the treatment plant. This may improve the overall efficiency of treatment. Applying $Mg(OH)_2$ to an anaerobic sludge digester resulted in a larger reduction in suspended solids and COD, a higher biogas production rate, and a lower level of phosphate and ammonia concentrations. Notwithstanding, high levels of phosphorus removal can be achieved. The required reaction time depends on the initial phosphorus concentration and the dose of $Mg(OH)_2$ (Wu et al., 2001). Similar to spontaneous or intentional struvite formation, adding magnesium salts removed ammonia and phosphorus from wastewater, where the magnesium source, bittern, the mother liquor obtained from seawater after removing NaCl and containing various magnesium salts, sulphate, chloride, bromide and iodine, was the most efficient. Removal of ammonia and phosphorus increased concomitantly with pH as high as 10.5, where 83% of the ammonia and 97% of the phosphorus were removed (Shin and Lee, 1998).

In sum, it seems that the relatively broad commercialization of chemical precipitation in wastewater treatment plants is attracting less research interest, as expressed in publicly available literature. Some chemical processes cannot be evaluated properly because commercial firms developed them, and they present less than credible data. The information is, however, available through the Internet. Currently, this section of the wastewater treatment field is a minor avenue and involves only small improvements of already known and studied processes from the past.

3. Cultivation of microorganisms in wastewater

Biological removal of nutrients in bioreactors is, by definition, performed by microorganisms. However, the species commonly used are non-specific and environmentally enriched from the sludge by the incubation conditions, as explained later for EBPR. Several attempts to intentionally use specific microorganisms as cleaning agents were reported, as has been done in earlier decades (for review of earlier cases: De la Noüe and De Pauw, 1988). These include bacteria and microalgae.

3.1. Bacteria

The tropical cyanobacterium *Phormidium bohneri* in domestic wastewater removed nitrogen and phosphate after growing 50 and 75 h, respectively. Adding monopotassium phosphate enhanced production of biomass by 56%, but did not significantly affect the time for completely removing these nutrients. The high biomass production by *P. bohneri* ($23–57 \, mg \, dw \, L^{-1} \, day^{-1}$), along with high rates of ammonium and phosphate removal (up to $20 \, mg \, L^{-1} \, day^{-1}$), indicates that *P. bohneri* has promise for wastewater treatment (Laliberte et al., 1997). The thermophilic cyanobacterium *P. laminosum*, eliminated nutrients during secondary sewage treatment when immobilized on hollow cellulose fibers at 43 °C. The concentration of PO_4^{-3} in the effluent decreased from 6.62 to $0.02 \, mg \, P \, L^{-1}$ after a residence time of 48 h (Sawayama et al., 1998a). Filamentous, mat-forming cyanobacteria (*P. tenue* and *Oscillatoria* O-210), isolated from Arctic and Antarctic environments, were tested for their growth and removal of nutrients, aimed at developing biological wastewater treatment systems for cold areas of Canada. Although all isolates grew well at 15 and 25 °C, growth was limited at 5 °C. At 15 °C, a satisfactory phosphorus removal rate of $0.6 \, mg \, P \, L^{-1} \, day^{-1}$ was obtained. This could not be repeated at the lower temperature. This failure indicates that tertiary biological wastewater treatment at low temperatures (5 °C) cannot be developed with the polar strains tested because they grow too slowly under extreme cold. However, they may have potential at moderately cool temperatures (about 15 °C and above), common from spring through fall in northern climates (Chevalier et al., 2000).

Bacteria cultivated for relatively short periods in wastewater present a feasible alternative to the longer EBPR process (described later). *Staphylococcus auricularis*, grown in sequencing batch reactors under repeated anaerobic and aerobic conditions, was able to remove between 5 and $50 \, mg \, P \, L^{-1}$, corresponding to more than 90% removal of phosphorus. These results, achieved after a short incubation period, demonstrated that a long adaptation time, one of the major problems in starting an EBPR process, could be addressed by a shorter approach (Choi and Yoo, 2000).

The purple photosynthetic non-sulfur bacterium *Rhodobacter capsulatus*, immobilized on cellulose beads,

removed organic carbon, ammonium ions, and phosphate ions from a diluted growth medium over a period of 19–22 days with a residence time of 10 h at 35 °C (Sawayama et al., 1998b). The photosynthetic bacteria, *Rh. sphaeroides* S, *Rb. sphaeroides* NR-3, and *Rhodopseudomonas palustris*, immobilized in porous ceramic under aerobic conditions, simultaneously removed COD, phosphates, nitrates, and H_2S from a synthetic sewage wastewater. In the batch treatment, 77% of the phosphates were removed effectively within 48 h. In semi-continuous treatments, this removal took about one month (Nagadomi et al., 2000).

3.2. Microalgae

Unicellular microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus* were capable of removing up to 55% of the phosphates from dairy industry and pig farming wastewaters (Gonzalez et al., 1997, for earlier studies see De la Noüe and De Pauw, 1988). Another strain of *Scenedesmus*, grown in artificial wastewater, also removed more than 50% of the phosphates (Voltolina et al., 1999). Production of starch yielded wastewater with a unique C:N:P ratio of 24:0.14:1. This effluent supported good growth of *Spirulina platensis.* Reductions in phosphate levels of the digested effluent reached over 99% (Phang et al., 2000). *S. platensis* could efficiently remove nitrates, ammonia, and phosphates from synthetic wastewater (Ogbonna et al., 2000).

These studies employed microalgae in a suspension. Their application is severely limited by the difficulties of harvesting the enormous microalgal population developed in the water after treatment. Therefore, the idea of entrapping microalgae for easy removal by sedimentation with spherical gels gained some momentum. For example, *C. vulgaris*, immobilized in two natural polysaccharide gels (carrageenan and alginate), was used to treat primary domestic wastewater. Although algal cells in the carrageenan and alginate beads grew far slower than the suspended cells, the immobilized cells were more metabolically active. Over 95% of ammonium and 99% of phosphates were removed from the wastewater in 3 days. This was much more efficient than the suspended cells that reduced only 50% of N and P in the same time period (Lau et al., 1997). Algal uptake of nutrients and adsorption on alginate gels were the major mechanisms involved in the removal of ammonium and phosphates (Tam and Wong, 2000).

Apart from the straightforward studies mentioned above, the notion that a combination of more than one microorganism is better than a single organism is gaining acceptance in agriculture and forestry, and is starting to appear in nutrient removal studies of wastewater. For example, when grown as a monoculture, neither *Rh. sphaeroides* nor *C. sorokiniana* could simultaneously remove acetate, propionate, ammonia, nitrate, and phosphate from synthetic wastewater, while a mixed culture could accomplish this (Ogbonna et al., 2000). A microalga (*C. vulgaris*) and a macrophyte (*Lemna minuscula*) could be applied in tandem for biological treatment of recalcitrant anaerobic industrial effluent, wastewater that otherwise prevented growth of any macrophyte. First, the *C. vulgaris* reduced ammonium ions (71.6%), phosphorus (28%), and COD (61%). Consequently, *L. minuscula* was able to grow in the treated wastewater, precipitate the microalgal cells by shading the culture, and reduced organic matter and color. However, *L. minuscula* did not significantly improve further nutrient removal (Valderrama et al., 2002).

The most radical combination of microalgae and bacteria suggested so far is the use of plant growth-promoting bacteria, used in agriculture, to enhance the growth and nutrient removal capacity of microalgae from wastewater. The bacteria of the genus *Azospirillum* are used as inoculants to promote the growth and yield of numerous crop plants, mainly by affecting the hormonal metabolism and mineral absorption of the plants (Bashan and Holguin, 1997). The underlying hypothesis assumed that the bacteria will enhance the performance of unicellular plants, like microalgae, and that the single-cell plant will respond similarly to bacterial inoculation like a higher plant. Co-immobilization of *C. vulgaris* and *C. sorokiniana* with *A. brasilense*, in small alginate beads, significantly enhanced all the growth parameters of the microalgae (Gonzalez and Bashan, 2000). Furthermore, these artificial combinations (thus far not found in nature) profoundly changed many cytological, physiological, and biochemical pathways and products within the microalgal cells (Gonzalez-Bashan et al., 2000; Lebsky et al., 2001; de-Bashan et al., 2002a). This co-immobilization, under semi-continuous synthetic wastewater culture conditions, significantly increased the removal of ammonium and soluble phosphate ions, compared to immobilization of the microalgae alone (de-Bashan et al., 2002b). Recently, these combinations were successful in significantly reducing ammonium and phosphate levels of municipal wastewater (de-Bashan et al., 2004).

In summary, no new technology has emerged from decades of research on intentionally using specific microorganisms for nutrient removal. Several proposals, especially the entrapment of microorganisms in polysaccharide gels and combinations of several organisms for simultaneous treatment of the wastewater, have the best potential for commercial use. However, as yet, it is a minor research avenue in the phosphate removal field in wastewater treatment.

4. Constructed wetlands

Constructed wetlands are a low-cost, low-tech process to control environmental pollution. Basically, it is a

container (as small as a bucket or as big as a very large pond) planted with mainly aquatic, but sometimes with terrestrial plants. Inflow wastewater current slowly flows either horizontally or vertically from one end to the other end and, in the process, the outflow is cleaner. Other major construction parameters are the type of substrate in which the plants grow or the container material. Both usually have some cleaning capacity by themselves. The roots of plants, especially aquatic macrophytes, both emergent and submerged, work as a giant biological filter that removes organic matter of all kinds. At the same time, microorganisms residing in the submerged roots in the wastewater are degrading other pollutants that are later absorbed by the plants. Afterwards, the treated wastewater is commonly discarded to natural water bodies or used for irrigation of inedible plants without any further treatment. Periodically, in some constructed wetlands, the plants need replacement. Usually wetlands are not designed to remove nutrients, such as phosphorus. They do so indirectly because the ions are nutrients for the plants.

The wetland concept is a popular way of treating wastewater for decades. Even though it is commonplace, reports about their ability to clear wastewater, refining of the process, and validation of the concept for rural areas were published in recent years. Several types of plants used in wetlands and their capacity to remove phosphorus are summarized in Table 1.

The removal capacity of phosphorus by a wetland can be substantial. Assessment of the contribution of duckweed *Lemna gibba*, a marcrophyte, and its associated microorganisms (algae and bacteria forming an attached biofilm) to remove nutrients showed that the biological floating mat complex (plants and microbes) is responsible for removing up to 75% of the nutrients in the wastewater. The macrophyte contributed up to 52% of phosphorus removal by its own growth; the associated organisms and microorganisms removed the rest (Korner and Vermaat, 1998).

Upgrading of the wetlands concept and increasing its economical value by directing the cleaning process and discarded effluent according to requirements and not by

Table 1
Recent reports on removal of phosphorus from wastewater in wetlands

Plant type	Type of wastewater	Phosphorus removal	Reference
Eichhornia crassipes (water hyacinth)	Untreated and partially treated domestic sewage	8 mg/g dw	Mohan and Hosetti (1998)
Gracilaria chilensis (macroalga)	Effluent from fish, oyster, and sea urchin cultivation	Small	Chow et al. (2001)
Lemna gibba (duckweed)	Domestic sewage	63–99%	Korner and Vermaat (1998)
Typha latifolia (emergent macrophyte)	Septic tank liquid	95%	Perdomo et al. (1999)
Zizaniopsis bonariensis *Typha subulata* (emergent macrophyte)	Anaerobically treated municipal wastewater	Small	Marques et al. (2001)
Cyperus sp.	Domestic sewage	39%	Ayaz and Akca (2000)
Cyperus papyrus *Phragmites mauritianus*	Municipal sewage	0.05 g/m^2/d 0.07 g/m^2/d	Okurut et al. (1999)
Mix of *Typha* sp., *Cyperus latifolius, Cyperus papyrus, Hydrocotyle* sp., *Hydrocleis* sp. and *Pontederia* sp.	Domestic sewage	88%	Nyakang'o and van Bruggen (1999)
Phragmites australis (reeds)	Irrigation drainage from nurseries or a synthetic sewage	65–90% (irrigation)	Headley et al. (2000)
		98–100% (sewage)	Drizo et al. (1997)
Phragmites karka	Domestic sewage	69%	Kurniadie and Kunze (2000)
Schoenoplectus validus	Synthetic sewage	>98%	Lantzke et al. (1998)
Juncus effusus, Scirpus validus, and *Typha latifolia*	Primary-treated domestic sewage	50–60%	Coleman et al. (2001)
C. papyrus, African marigold, peppermint,	Artificial sewage	>90%	Abe and Ozaki (1999)
Najas guadalupensis, Ceratophyllum demersum, and *Chara* sp. (submerged aquatic plants)	Agricultural runoff	>99%	Dierberg et al. (2002)

random, as is done in ordinary wetlands is a new notion. This might provide a better solution for operating wetlands in cold climates in winter and for treatment of recalcitrant water, which are difficult in common artificial wetland systems. Such an idea has been called an "engineered wetland." The terms "engineered wetland" and "constructed wetland" are used interchangeably, although they significantly differ. By definition, all engineered wetlands are constructed wetlands, but not all constructed wetlands are engineered wetlands. A constructed wetland normally refers to passive flow wetlands. An engineered wetland is a wetland that can be changed at will, according to the ever-changing conditions of both climate and the type of wastewater; the process conditions and operations can be modified, manipulated, and controlled by the facility management. Examples for possible "engineering" of a wetland can be by controlling the flow rate of the influent, effluents from various sites in the wetland system may be recycled to other sites more appropriate to the type of pollution needed to be treated, and addition of substrates capable of adsorbing certain pollutants. If necessary, heat, chemicals (in restricted areas), and air can be applied, and the species of vegetation may be selected for its phyto-remediating capacity (Higgins et al., 2000). The engineered wetland can also be combined with other cleaning systems to achieve clean water. This process was suggested for integration with downstream trickling filtration or activated sludge processes in EBPR (Shipin et al., 2000).

The substrate of the constructed wetland and the plants used are essential components of the wetland concept and significantly affect performance. Performance of such wetlands in removing pollutants, including phosphorus, can be enhanced by using a reactive sorbent. The sorbent must have a high phosphorus-sorption capacity and an adequate hydraulic conductivity. Gravel alone provided significant improvement of effluent quality in wastewater treatment, but adding vegetation, especially the macrophyte *Typha* spp., further improved treatment efficiencies (Coleman et al., 2001). Effective phosphate removal was observed in a topsoil in Brazil used in an upflowing wetland system, which has a high surface adsorption area (Farahbakh-shazad et al., 2000). A constructed wetland with horizontal subsurface flow of synthetic sewage, using readily available shale as a substrate and with and without reeds (*Phragmites australis*), was evaluated for removal of phosphate and ammonium. Shale was selected for its physico-chemical properties and its potential for phosphate removal. Both the planted and unplanted wetlands showed extremely high phosphate removal (98–100%) during about a year of evaluation (Drizo et al., 1997). In a laboratory experiment comparing several substrates for phosphorus adsorption from sewage effluent (two gravels and a Hawksbury

sandstone used in constructed wetlands and six by-products from steelworks), all by-products from steelworks had adsorption capacities superior to the constructed wetland gravels and Hawkesbury sandstone (Mann, 1997). Similarly, in another test, crystalline blast furnace slag had the highest phosphorus-sorption capacities (Johansson, 1999). Incorporation of these materials in construction of wetlands is environmentally questionable for the time being. Comparison of phosphorus adsorption capacities of industrial iron by-products, soils, and a clinoptilolite material (zeolite, used to remove ammonium ions from wastewater) was studied to evaluate their potential as substrates for phosphorus removal from constructed wetlands. Apart from iron by products that obviously adsorbed phosphorus as well as in the previous example, soil samples collected from an operating constructed wetland system in northern New South Wales, Australia was the best, followed by zeolite. The other surface soils collected from two other regional constructed wetland systems were found to have the lowest phosphorus removal capacity. This variability in substrate performance clearly demonstrates that careful selection of substrate material for construction of wetland should be done (Sakadevan and Bavor, 1998).

An out-of-the-ordinary solution for construction of common wetlands capable of removing nutrients, including phosphorus, was suggested in Africa to treat municipal wastewater; a dual function of beauty and usefulness as a wastewater treatment facility. The 0.5 ha wetland was designed to treat wastewater as well as to provide an aesthetically pleasing and environmentally sensitive landscape with ponds and ornamental plants for recreation (Nyakang'o and van Bruggen, 1999). Similarly, plant bed filter ditches containing papyrus and flowers (used by rural communities for the production of handicrafts, cut flowers, and ornamental purposes) were used for efficient removal of N and P from eutrophic pond water. The system supplied clean water and additional income, together with vistas of ornamental streams (Abe and Ozaki, 1999).

In summary, wetlands systems are a feasible approach for all wastewater treatment, and can be constructed with limited resources in practically every location, and without problems relating to the technological capacity of the local society. The operational and maintenance costs are minimal, and are affordable, even in very small operations in developing countries (Nyakang'o and van Bruggen, 1999; Kurniadie and Kunze, 2000). Improvements in plant selection, size and type of substrate, and especially combined with better design for the control of the wetland as an "engineered wetland", might make the wetland system the method of choice for wastewater treatment where land is available and resources are scarce.

5. Enhanced biological phosphorus removal (EBPR)

Removing phosphate by biological means can be accomplished by two independent mechanisms: (i) direct absorption of phosphorus by suspended growing cells and plants described earlier, and (ii) enhancing the storage capacity of phosphorus as polyphosphate by the microbial biomass in activated sludge at the treatment facility. Because of relatively low efficiency of phosphorus removal by techniques described previously, engineers developed the EBPR approach.

5.1. Procedure

EBPR is a wastewater treatment based on the selective enrichment of bacteria accumulating inorganic polyphosphate as an ingredient of their cells. It involves microbial metabolic cycling via several microbial-accumulated biopolymers (polyphosphate, PHA, and glycogen). This metabolic cycling is induced in microorganisms by alternating the incubation conditions of the wastewater between initially carbon-rich, strictly anaerobic incubation (no oxygen or nitrate are present), followed by carbon-poor, aerobic incubation. In essence, during the anaerobic phase, microbes residing in the sludge (applied as an inoculum to the wastewater) deplete the organic matter and carbon sources from the wastewater, accumulate storage biopolymers (mainly PHAs and glycogen), and release soluble orthophosphate from the sludge.

The energy source for these processes is derived mainly from polyphosphate. Polyphosphate is a high-energy storage molecule that, upon hydrolysis, can supply ample energy for biochemical reactions within the cell. This molecule is particularly useful during the anaerobic stage of EBPR, where it supplies the needed energy for uptake of the organic substrates. Polyphosphate is stored in the cells of the same bacteria residing in the applied sludge of previous wastewater treatment cycles. The other biopolymer, glycogen, which usually serves as a regulator of redox balance in cells, also provides additional energy, helping polyphosphate-accumulating organisms (PAOs) to absorb organic substances under anaerobic conditions.

When conditions in the bioreactor change to aerobic, PHA molecules serve as energy and carbon sources for uptake of even larger amount of orthophosphate than the amount originally released during the anaerobic process, and this enhanced uptake includes the phosphorus arriving with the new wastewater. The aerobic process reincorporates the orthophosphate into new intracellular microbial polyphosphate. This leaves the wastewater phosphate-poor, and in case of complete EBPR success, phosphate-free.

The activated sludge produced after the process (containing organic matter and a large microbial population) is even richer in phosphates, usually discarded, and may be used for biogas production (see later discussion). A small part of the sludge is recycled and used as an inoculum for new wastewater. Theoretically, the process may continue indefinitely. By withdrawing the phosphate-rich sludge from the system as excess sludge, the EBPR system achieves, with time, high phosphate removal rates. Technically, anaerobic–aerobic cycles can be easily and commercially obtained by assigning spatial zonation (anaerobic–aerobic) in a continuous flow system (with sludge recycling serving as inoculum) or by arranging temporarily anaerobic and aerobic incubation periods in sequenced batch bioreactors (Fig. 1).

EBPR is attributed to PAOs, mainly bacteria. An obligatory requirement to achieve high and stable EBPR is maintaining PAOs in the system. While the appearance and disappearance of microbial biopolymers is known, the metabolic pathways involved in biomass synthesis and energy production in these bacteria are far from being understood and were reviewed recently (Mino, 2000; Mino et al., 1998). A characteristic of the EBPR process is the interaction between different microbial intracellular biopolymers. Therefore, an understanding of the effects of these biopolymers on EBPR is helpful for the practical application of the process (Mino et al., 1998; Pramanik et al., 1999).

Recent papers report that, in long-term operations of conventional anaerobic-anoxic-aerobic EBPRs, the process exhibited high performance in removing organic matter, nitrogen, and phosphorus. According to Chuang and Ouyang (2000), efficiency in this system was 96.5% (organic matter), 70% (total nitrogen), and 100% (total phosphorus). EBPR achieved complete phosphate removal in two different systems: an anaerobic-anoxic sequencing batch reactor and an anaerobic–aerobic sequencing batch reactor (Merzouki et al., 1999). Phosphorus removal was high as long as the carbohydrate content in the sludge was low. Efficiency of phosphorus removal declined concomitantly as the sludge carbohydrate content increased. They concluded that the carbohydrate content of the sludge is a reliable parameter for representing the capacity of sludge for phosphorus removal. Therefore, reducing the carbohydrate content of the sludge to low levels would be a prerequisite for achieving EBPR process (Liu, 1997, 1998).

5.2. Addition of nutrients

An essential component in a successful EBPR procedure is the addition of nutrients, mainly acetate and, to a lesser extent, glucose, during anaerobic incubation. Both are added as an additional carbon source to the wastewater. Propionate, butyrate, and lactate were once tested. Consequently, the composition
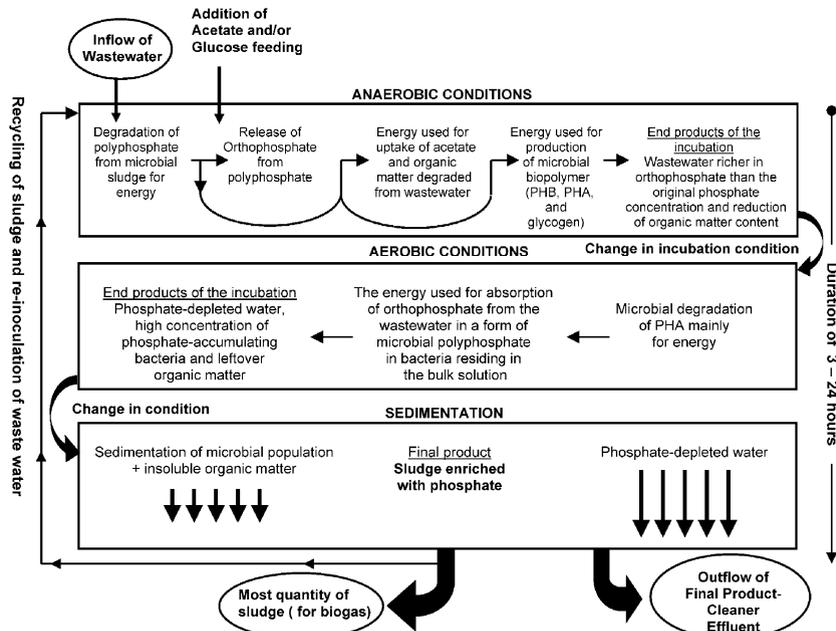
Fig. 1. Conceptual model for enhanced biological phosphorus removal.

of the polymers formed was dependent on the substrate used (Lemos et al., 1998; Satoh et al., 1992). Polyphosphate hydrolysis is used as an energy source to take up acetate very rapidly, releasing orthophosphate in the process, and store PHAs in the cells. All of this results in more efficient EBPR. Injecting acetate into the sludge layer during settling and decanting in the anaerobic phase enhanced phosphorus liberation inside the sludge layer and nearly all phosphorus was absorbed during the subsequent aerobic phase (Ahn et al., 2001a). Fractionation of anaerobic and aerobic sludge from acetate-induced EBPR demonstrated that most of the phosphorus is absorbed in the form of polyphosphate. Only 4% of the total phosphorus in the aerobic sludge and 2% in the anaerobic sludge were metal-bound phosphates (Christensson et al., 1998). When glucose was used instead of acetate as the dominant substrate to induce and maintain successful EBPR, less orthophosphate was released. Additionally, the biopolymer 3-HV-enriched -PHA, rather than 3-hydroxybutyrate-enriched PHA, was accumulated during the anaerobic phase. The predominance of 3-HV-enriched PHA is probably used to balance the internal redox during the anaerobic phase. It is likely that, for anaerobic glucose metabolism, the Entner-Doudoroff pathway was used when the bacteria showed good EBPR because this pathway requires polyphosphate as an energy source (Wang et al., 2002).

Alternatively or simultaneously, glucose can be converted under anaerobic conditions to glycogen as the storage compound, in addition to PHAs (Sudiana et al., 1999). However, glycogen accumulation sometimes does not yield EBPR. For example, glycogen can increase absorption of different substrates from the wastewater, storing PHAs as well, but without performing the complete EBPR process of releasing and absorbing phosphorus (Carucci et al., 1999). Sometimes, significant amounts (up to 12%) of glycogen-accumulating bacteria were detected in EBPR, but without negative effects on the overall EBPR performance (Lee et al., 2003a). Another reason why EBPR could not be accomplished using glucose as an additional carbon source was that it selects for the domination of bacteria not capable of accumulating polyphosphate (but accumulating glycogen instead). These bacteria absorb glucose without the release of orthophosphate under anaerobic conditions, a fundamental step necessary for successful EBPR (Mino et al., 1998). Despite this, Jeon and Park (2000) demonstrated that release of orthophosphate and synthesis of PHAs during the anaerobic phase were related to the total concentration of organic carbon in the wastewater, rather than to the concentration of glucose. EBPR with glucose was accomplished by at least two kinds of microbial populations—lactic acid-producing organisms (LPO) described by (Jeon et al., 2000) and PAOs. As glucose was converted into other storage compounds (inferred to be lactic acid polymers) by the LPO, the PHAs synthesized less than the amount expected after glucose was added. Regardless of this, PHAs were synthesized by PAO. Later,

during the aerobic phase, PHAs and other stored compounds were metabolized and phosphate absorption occurred, and EBPR was accomplished (Jeon and Park, 2000). Apparently, a correlation between soluble phosphorus uptake and volatile fatty acid, a potential substrate for enrichment of PAO, was found (Lie et al., 1997; Thomas et al., 2003). These volatile fatty acids, originating from prior fermentation of supplemental glucose, could cultivate a bacterial population capable of EBPR (Randall et al., 1997).

The interactions among the biopolymers participating in EBPR and adsorption of orthophosphate from the wastewater are of essential importance for the success of the process. For example, the uptake rate of orthophosphate from the wastewater, under aerobic conditions, was highly dependent on the concentration of PHB in the microbial biomass. The presence of orthophosphate increased the degradation rate of PHB significantly and consequently, the uptake rate of orthophosphate was a function of the PHB concentration (Petersen et al., 1998). Not all phosphorus removed during EBPR can be accounted for by PAO. Cell clusters with associated EPS, on average, contained 57–59% phosphorus, while EPS alone, on average, contained 23–30% phosphorus. Therefore, EPS is acting as a phosphorus reservoir (Oosthuizen and Cloete, 2001).

5.3. Effect of environmental parameters

Environmental parameters governing the incubation of wastewater, like pH, COD, phosphorus, magnesium, calcium, and potassium, incubation time, sludge retention time, temperature, excessive aeration, and the time when aeration starts, all play a major role in removal of phosphorus from wastewater.

Recent findings showed that cultures obtained from activated sludge of five different wastewater treatment plants performing EBPR showed increases between 50% and 143% in levels of orthophosphate uptake when pH during growth was reduced to 5.5, rather than the common 7.5. Of 100 individual sludge microbial isolates evaluated, 34 demonstrated capacities for EBPR when the optimum pH for the process ranged between 5.0 and 6.5 (McGrath et al., 2001). Contrary to this, in a bioreactor showing poor EBPR, one of the changes in operation that led to improved performance of the bioreactor was allowing the pH to rise during the anaerobic period. This promoted anaerobic orthophosphate release and possibly caused selection against bacteria unable to remove phosphate (Bond et al., 1999; Bond et al., 1995).

Biomass density during EBPR increased at a rate of $1.2\,\mathrm{mg\,L^{-1}}$ for every 1% increase in biomass phosphorus content. Samples taken at the end-aerobic phase had an average 25% higher buoyant density than samples from the end-anaerobic phase (Schuler et al., 2001). Influent COD, influent phosphorus, and the timing of aeration were critical factors that governed PHA dynamics and its efficient regulation during orthophosphate uptake in the aerobic phase (Arnz et al., 2001; Dassanayake and Irvine, 2001). However, adequate retention time is required for the enrichment of PAOs. Therefore, the initial anaerobic period should not be shortened during the start-up phase of EBPR. Otherwise, facultative bacteria, unrelated to EBPR, will efficiently compete with the PAOs. Furthermore, PAOs facing long aerobic periods may not need the polyphosphate accumulation mechanism to survive short anaerobic periods. In this case, even if COD removal is observed during the short initial anaerobic phase, further phosphate accumulation during the aerobic phase will be insignificant (Castillo et al., 2000). For complete phosphorus removal, no less than 15 days were needed as sludge retention time (Merzouki et al., 2001). Excessive aeration (aeration during starvation) has a strong negative effect on EBPR processes. The reason is that, in an over-aerated process, phosphorus uptake stops because of a gradual depletion of PHB. If another organic substrate is introduced to the EBPR, phosphorus release is immediately resume, but the released phosphorus cannot be fully re-absorbed because the low PHB content limits the uptake rate. As a result, incomplete phosphorus uptake leads to temporary reduction of EBPR efficiency. This effect may explain the deterioration of EBPR processes observed after heavy rainfall or during weekends when oxygen control is deficient (Brdjanovic et al., 1998a). Efficiency of EBPR improved as temperature decreases and EBPR performed better at colder temperature (5 °C). Better performance of the system was ascribed to reduced competition for substrate in the non-oxic zones, which resulted in an increased PAO population (Erdal et al., 2003). The impact of long-term (weeks) changes in temperature on the kinetics of the anaerobic and aerobic phases of EBPR showed that temperature had only a moderate impact on the aerobic orthophosphate uptake. However, a strong temperature effect was observed for PHA consumption, oxygen uptake, and bacterial growth (Brdjanovic et al., 1998b).

Synthesis and degradation of polyphosphate by PAOs are also controlled by the energy state of the cell concomitantly with extracellular phosphate levels in the wastewater. Limited phosphate loading in the wastewater may suppress the development of PAOs. Incorporation of excess phosphate into polyphosphate under phosphate- and energy-rich growth conditions permits PAOs to survive when phosphate or energy are depleted or in short supply. Under phosphate-starvation conditions, phosphate can be hydrolyzed from polyphosphate. Under energy starvation, energy can be obtained either from ATP or by hydrolysis of polyphosphate. Subsequently, orthophosphate must be secreted for recharging the transmembrane proton gradient

(Keasling et al., 2000). Thus, it is required that PAO communities are established under phosphorus-rich loading conditions. If not, phosphorus-poor effluent may establish microbial communities lacking the capacity to accumulate polyphosphate. These microbial communities were dominated by glycogen-accumulating organisms (Sudiana et al., 1999), and they were responsible for the deterioration of the EBPR process (Falkentoft et al., 2001; Rodrigo et al., 1999; for a review of similar earlier cases, see Mino et al., 1998). Doubling the concentration of magnesium in the influent (from 15 to 31 mg L^{-1}) enhanced phosphorus removal from 85% to 97%. Ca, Mg, and K were the principal metal components of polyphosphate granules within the cells. Varying the metal composition created different types of polyphosphate granules. Ratios of Ca, Mg, and K in the polyphosphate granules were dependent on the concentration of these metals in the influent and these metal ratios presumably control EBPR (Schoenborn et al., 2001).

Although EBPR is usually stable for phosphorus, external disturbance episodes may interrupt the activity. Such cases are: excessive loading, high rainfall, excessive aeration, shortage of potassium, and excessive nitrate loading in the anaerobic zone (Mino et al., 1998; Brdjanovic et al., 1998a). However, EBPR might collapse as well from the development of glycogen-accumulating non-polyphosphate organisms (Thomas et al., 2003). These organisms have a metabolism very similar to PAOs, except that they do not hydrolyze polyphosphate for energy and use glycogen as the major source of energy. Although morphologically these bacteria and PAO are different, there is no definite proof that these are not the same bacteria, which operate differently for, as yet, undefined reasons (For review, see Mino et al., 1998).

5.4. Relation between phosphorus removal and nitrogen removal

Phosphorus removal from wastewater is apparently connected with nitrogen removal. The presence of a small amount of nitrate at the start of the anaerobic phase stimulated the growth of denitrifying, phosphate-accumulating organisms in an anaerobic–aerobic system. This selection caused a switched oxygen to nitrate as an electron acceptor and enhanced anoxic orthophosphate uptake activity (Merzouki et al., 1999; Ahn et al., 2001b, 2002; Falkentoft et al., 2002; Ostgaard et al., 1997. Increasing ammonium concentration in EBPR during sufficiently long anoxic period was mandatory to stimulate and eventually implement simultaneous nitrification and phosphorus removal in the wastewater (Gieseke et al., 2002). The other metabolic pathways (recovery of glycogen and polyphosphate through consumption of stored PHAs) are also operating when

nitrates are present. Since low concentrations of nitrite are not detrimental to anoxic phosphate uptake, it can also serve as electron acceptors in this set of pathways (Lee et al., 2001). Exposure to high nitrite concentrations completely inhibit phosphate uptake under anoxic conditions and severely inhabit uptake under aerobic conditions. The inhibiting effect of nitrite may last at least several hours after exposure (Meinhold et al., 1999a). Limitation of acetate in influent and high degree of nitrification during the anaerobic phase yielded released phosphorus inside the sludge layer. If the sludge layer functions as a blocking layer, phosphorus concentrations in the supernatant may not be influenced by the released phosphorus inside the sludge (Ahn et al., 2001b).

Evaluation of possible interactions between nitrogen removal potential of PAOs with their phosphorus removal capacity under anoxic conditions (in batch reactor operated in a sequence of anaerobic, anoxic, and aerobic phases) showed, as expected, that the phosphate uptake rate under anoxic conditions was lower than under aerobic conditions. However, in the presence of external carbon sources, like glucose and acetate, phosphate removal was directly dependent on the type of added carbon. In the presence of nitrate, release of phosphate occurred only in the presence of acetate, but not glucose. Similarly, the rate of nitrate uptake was also much lower with glucose than with acetate (Artan et al., 1998). Exposing sludge microorganisms performing EBPR, in sequencing batch reactors, to three different electron acceptor conditions (oxygen only, nitrate only, and oxygen and nitrate) changed the microbial community structure according to the electron acceptor used. Furthermore, microorganisms cultivated in the presence of both nitrate and oxygen significantly increased the amount of phosphorus uptake under anoxic conditions compared to either of the electron acceptors alone (Ahn et al., 2002). PAOs capable of both denitrification and phosphorus uptake may reduce oxygen consumption. As lower initial COD is required in the wastewater for nitrogen removal, the excess COD can be removed from the wastewater by sedimentation, saved, and later be used for methane production. Consequently, this process leads to lower sludge production and ultimately to lower CO_2 emissions (reduced net energy consumption). These PAOs accumulated more phosphate than conventional EBPR organisms (Shoji et al., 2003). Hao et al. (2001) proposed that excess energy production from methane production is greater than the energy needed for aeration, dewatering, and incineration in common wastewater treatments. These results support the hypothesis that PAOs in EBPR systems consist of at least two major groups: (i) denitrifying PAO that are capable of using oxygen and nitrates (Ahn et al., 2002), and (ii) non-denitrifying PAO that are capable of using oxygen only (Meinhold et al., 1999b).

5.5. Phosphate accumulating organisms participating in enhanced biological phosphorus removal

PAOs, mainly bacteria, are the cornerstone of EBPR. EBPR alternates incubation periods (anaerobic followed by aerobic) selected for the development of populations of PAOs. PAOs containing high phosphorus content are specifically selected and become dominant in the wastewater during the EBPR process, although they are considered slow growers. Originally, it was assumed that EBPR would be dominated with a single or a few groups of microorganisms, as is common in other enrichment cultures. Despite this, it appears that there are no specific bacterial genera directly responsible for accumulating polyphosphates. EBPR appears to consist of a consortium of phylogenetically and taxonomically diverse groups of bacteria, some more numerous than others. Many are unculturable and could be detected only by molecular methods (Mino, 2000; Mino et al., 1998; Erasmus et al., 2000; Kampfer et al., 1996; Morohoshi et al., 2003). There is a possibility that PAOs cannot grow as a single culture and interactions among different species are necessary, as is known from other microbiological systems. At present, no clear experimental proof has shown that the presence of a few or many bacterial populations is indispensable for the growth of PAOs. A few culturable species that may participate in EBPR are: *Acinetobacter* spp., *Microlunatus phosphovorus*, *Lampropedia* spp., and members of the *Rhodocyclus* group (Table 2). As yet, the general microbial community structure has not been described, and therefore, the ecological mechanisms selecting for PAOs over other bacterial communities in EBPR are vaguely understood.

PAOs are environmentally selected. When sludge is applied to wastewater containing organic material under anaerobic conditions, sludge-borne microorganisms capable of using the organic matter quickly and efficiently is advantageously selected. This is the *fundamental* requirement to achieve a functional EBPR system. Although the sludge added to the wastewater is bacterially rich, none of the bacterial species isolated from the sludge possess all the key characteristics of a whole EPBR sludge. Furthermore, none of the isolated species has been definitely shown to be primarily responsible for EBPR. The metabolic cycling of biopolymers in EBPR is energy-expensive for the bacteria, therefore, not favorable to microorganisms. However, this cycling allowed PAOs to win the microbial selection process in EBPR. Sometimes, the amount of PAOs developed is small. In municipal wastewater treated to remove nitrogen and phosphorus, heterotrophs were the major microorganisms responsible for biomass production, while a minute amount of PAOs were present (Chuang and Ouyang, 2000).

PAOs have been evaluated using diverse direct (culturing and observation under light and electron microscopes) and indirect methods (molecular and biochemical analyses). A strain of polyphosphate-synthesizing, phosphate-releasing *Acinetobacter johnsonii* was isolated from a wastewater treatment facility operating with EBPR (Boswell et al., 2001). Its study provided novel insight into the transport of inorganic phosphorus and divalent cations in prokaryote cells (van Veen, 1997). Another phosphorus-starved strain of *Acinetobacter* was capable of initial, rapid phosphate uptake, in addition to its participation in EBPR Pauli and Kaitala, 1997). Mino (2000) and Table 2 however, list the evidence against this species formerly considered *the* main bacterial species in EBPR (Fuhs and Chen, 1975). With our current knowledge, it is unlikely that this species is the primary one responsible for EBPR, yet, there is no doubt that *Acinetobacter* spp. participate in EBPR.

Recent molecular identification techniques, such as fluorescence in situ hybridization (FISH) analysis, use oligonucleotide probes that complement the 16S and 23S regions of rRNAs, showing that the main microbial populations in EBPR operations is composed of bacteria of the beta-2 subclass of *Proteobacteria* and *Acinetobacteria*. These microorganisms have extremely good phosphorus removal capacity (Bond et al., 1995). Another FISH analysis combined with confocal laser scanning microscopy of EBPR bioreactors, where the oxygen was replaced with nitrate as the electron acceptor, revealed major shifts in bacteria populations within two weeks of the change. Most bacteria belonging to the alpha subclass were replaced by the filamentous, beta subclass *Proteobacteria* (Falkentoft et al., 2002). The combination of FISH and microautoradiography performed on two EBPR systems showed that *Rhodocyclus*-related bacteria (beta-proteobacteria) were present in both systems in significant numbers (up to 28%) throughout the 2.5-year period of evaluation (Lee et al., 2003a). Similarly, an analysis of the sludge bacterial community, using a clone library based on the 16S rRNA gene, revealed that *Acinetobacter* strains constitute only 9.8% of the clones (Christensson et al., 1998). In another EBPR sludge, *Acinetobacter* spp. represented 4% of cultured bacteria (Melasniemi et al., 1998). PCR-denaturing gradient gel electrophoresis of 16S ribosomal DNA fragments showed that *Rhodocyclus* spp. and *Dechlorimonas* spp. where the dominant denitrifying-PAO in EBPR. However FISH analysis confirm only the identity of *Rhodocyclus* spp. (Ahn et al., 2002), where in another FISH analysis using a specific probe for these bacteria failed to detect them (Falkentoft et al., 2002). Comparative analyses by FISH, terminal restriction-fragment length polymorphism, or comparative 16S rDNA of two EBPR plants (with and without nitrification and denitrification)

Table 2
Bacterial species and genera that might be involved in induction of or participating in Enhanced Biological Phosphorus Removal (EBPR)

Bacterial genera or species	Evidence for involvement in EBPR	Evidence against involvement in EBPR	Reference
Acinetobacter spp.	1. Predominance in EBPR, based on culture media growth. 2. Strains accumulate polyphosphate and PHAs under aerobic conditions.	1. Culture media detection method is selective for *Acinetobacter* spp. 2. Fluorescent antibody staining, quinone profile or fluorescent in situ hybridization with oligonucleotide probe specific for *Acinetobacter* showed that the species is not primarily responsible for EBPR and is present as a small percentage in the sludge ($<10\%$ of total bacteria). 3. No strain possesses the typical metabolic pathways of EBPR-like acetate uptake and its conversion to PHA and hydrolysis of polyphosphate and release of orthophosphate under anaerobic conditions. 4. As dominant respiratory mechanism, bacteria uses quinone Q-9 when PAO processes are Q-8 or MK-8(H$_4$). 5. Polyamine pattern show that *Acinetobacter* has the polyamine diaminopropane, where EBPR processes have almost no presence of the compound.	Mino et al. (1998), Christensson et al. (1998), Fuhs and Chen (1975)
Microlunatus phosphovorus	1. Bacteria accumulate large amounts of polyphosphate under aerobic conditions. 2. Capable of anaerobic uptake of glucose.	1. Bacteria do not take up acetate and do not accumulate PHA under anaerobic conditions. 2. A 16s rRNA-targeted oligonucleotide probe, specific to the species, showed that its population is $<3\%$ of total bacteria in the sludge, when PAO are about 9% of the total population. 3. Contains Q-9 as a major quinone and not the common Q-8 and MK-8(H$_4$) of PAO-enriched-sludge.	Eschenhagen et al. (2003), Nakamura et al. (1995), Kawaharasaki et al. (1998), Santos et al. (1999)
Lampropedia sp.	1. Bacteria possess key metabolic characteristics of PAO. Bacteria accumulate polyphosphate and poly β-hydroxybutyrate. 2. Bacteria take up acetate and store it as PHA with polyphosphate degradation and release of orthophosphate.	1. Has a unique sheet-like organization, which is uncommon in EBPR processes.	Stante et al. (1997)

Rodocyclus sp.	1. Bacteria possess key metabolism of PAO.	No available data.	Lee et al. (2003), Bond et al. (1999), Ahn et al. (2002), Eschenhagen et al. (2003)
	2. Fluorescent in situ hybridization show that the group dominates the EBPR process (>81% of the population).		
Tetrasphaera japonica, *Tetrasphaera australiensis*	Phosphate-accumulating coci.	Microscopically similar to glycogen-accumulating bacteria from activated sludge.	Eschenhagen et al. (2003), Maszenan et al. (2000)
Tessaracoccus bendigoensis	1. Contains intracellular polyphosphate granules. 2. Morphologically similar to dominant microorganisms in activated sludge	Resembles glycogen-accumulating bacteria.	Maszenan et al. (1999)
Paracoccus denitrificans	Polyphosphate-accumulating bacterium.	Does not need the alternating anaerobic/aerobic cycle for phosphate accumulation. Can accumulate PHB, but does not accumulate polyphosphate when cells are rich in PHBs.	Barak and van Rijn (2000)
Burkholderia cepacia	Contains intracellular polyphosphate granules.	No available data.	Mullan et al. (2002)
Agrobacterium sp. *Aquaspirillum* sp. *Micrococcus* sp. *Staphylococcus* sp. *Acidovorax* sp. *Microsphaera multipartite*, *Dechlorimonas* spp. Unidentified yeast, Cytophaga-Flavobacteria group	Found in large and dominate numbers in EBPR processes.	No available data.	Merzouki et al. (1999), Ahn et al. (2002), Melasniemi et al. (1998), Melasniemi and Hernesmaa (2000), Van Ommen Kloeke and Geesey (1999)

PHA = polyhydroxyalkanoate; PAO = Phosphate accumulating organisms.

revealed similar highly diverse bacterial populations, where *Tetraphaera* spp. dominated the PAO community. However, other PAOs, such as *Microlunatus* spp. and members of the *Rhodocyclus* group, were also present (Eschenhagen et al., 2003). Identification of strain isolates from EBPR systems by the Biolog system and API 20 NE identification kit revealed that the most active PAOs were also the most efficient denitrifying bacteria and were identified as *Agrobacterium tumefaciens* B, *Aquaspirillum dispar*, and *Agrobacterium radiobacter* (Merzouki et al., 1999). Analysis of whole cell fatty acids of bacterial communities exhibiting EBPR capacity in municipal wastewaters belonged to more than twenty genera, with *Micrococcus*, *Staphylococcus*, and *Acidovorax* the most common (Melasniemi et al., 1998). Large vegetative yeast-like cells and spores of yeast, assessed microscopically after polyphosphate staining, was the principal PAO in an EBPR system. The cells of the PAO were the biggest cells in the sludge, clearly larger than average sludge bacteria (Melasniemi and Hernesmaa, 2000). In the aerobic, activated sludge phase of wastewater treatment, Cytophaga-Flavobacteria, as a group, is important in mediating the liberation of inorganic orthophosphate from detrital organic phosphate (Van Ommen Kloeke and Geesey, 1999).

A critical difficulty in the above studies is that none of the bacteria isolated from EBPR systems present all the typical characteristics of EBPR, and none were suggested, without any doubts, as the primary cause of EBPR. In the meantime, all the above bacterial species and genera should be taken as candidates for participating in EBPR.

In summary, EBPR is a major avenue for contemporary and future development of phosphorus removal with microorganisms. Perhaps it will become as important as metal precipitation methods that are now widely employed. However, with phosphorus recycling taking root (See later in this review), it is possible that the process will gain rapid importance and popularity in the wastewater industry.

6. Use of recovered phosphate as fertilizer

Mined rock phosphate is an abundant and relatively cheap source of phosphate for fertilizer production. At the current rate of exploitation, the high quality portion of the resource will be largely depleted in less than 100 years, if another source of high-quality phosphate is not identified (Isherwood, 2000). This will place a burden on agriculture production, because lower-grade phosphates will have to be used, significantly increasing production costs. Phosphates recovered from wastewater plants might be a viable source of industrial raw material for manufacture of phosphate fertilizers. The activated sludge (bio-solids) commonly discarded in fields from

wastewater treatment plants contains considerable phosphorus. Recovery of this phosphorus would help to alleviate the stringent legal restrictions on sludge disposal that require low levels of phosphorus in discarded sludge. Currently, this phosphorus is regarded more as a contaminant than a resource. This perspective has started to change in recent years.

The most common approach for removing phosphate from wastewater is metal salt precipitation, which makes the precipitate unrecoverable for possible industrial processing into fertilizer (Donnert and Salecker, 1999a; Donnert and Salecker, 1999b). Phosphate recovery from municipal wastewater is possible without metal salt precipitation, using existing technologies (Stratful et al., 1999; Driver et al., 1999; Durrant et al., 1999; Strickland, 1999; Woods et al., 1999). It is economically feasible to recover 10–80% of the phosphorus flowing into wastewater treatment facilities. Recovered phosphorus product can be superior in quality to currently available phosphate rock. Today, the recovery approach is attractive only for wastewater treatment plants that use biological nutrient removal (Gaterell et al., 2000; Jeanmaire and Evans, 2001) (Fig. 2).

6.1. Struvite

The most promising compound for recovery from wastewater plants is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$), commonly known as struvite, which precipitates spontaneously in some wastewater processes (Booker et al., 1999; Stratful et al., 2001; Williams, 1999). If formation and collection are controlled and cost-effective, struvite might have potential in the fertilizer market. Small quantities of recovered struvite are currently being tested as fertilizer, mainly in Japan. The theoretical potential is much larger, and approaches 67,000 tons of P_2O_5 fertilizer per year from the UK alone, as well as 270,000 tons from Western Europe (Gaterell et al., 2000). Because the compound is receiving increasing attention, this review will elaborate more on its formation, potential, and limitations.

6.1.1. Formation

Struvite precipitates spontaneously in wastewater treatment environments where high concentrations of soluble phosphorus and ammonium are present. Additional essential conditions are low concentration of suspended solids and pH above 7.5. Precipitation of struvite requires that its components are available simultaneously in the wastewater in the molecular ratio $1(Mg^{2+}):1(NH_4^+):1(PO_4^{3-})$. Normally, municipal wastewater and several other wastewaters tend to be rich in ammonium, but deficient in magnesium, so supplementation of magnesium is required, and this helps to increase solution pH (Lee et al., 2003a; Chimenos et al.,

Fig. 2. Future avenues of recovering phosphorus from wastewater and the potential use of the product.

2003; Munch and Barr, 2001; Nelson et al., 2003; van Rensburg et al., 2003). Similarly, addition of magnesium chloride or bittern, a low-cost magnesium, forced precipitation of phosphorus and reduced the concentration of soluble phosphorus in swine waste within a 10-min reaction time (Burns et al., 2003; Lee et al., 2003b). The pH can also be elevated by adding NaOH, an expensive process, (Stratful et al., 2001), by air stripping (Kalyuzhnyi et al., 2003), where aeration of wastewater removes CO_2 and pH increases in the process (Battistoni et al., 1997) or by ion exchange using phosphate-selective sorbents (Petruzzelli et al., 2003). Struvite crystals are orthorhombic, ranging from X-shaped to flat trapezoidal, depending on the degree of solution saturation. Crystals can grow significantly in 3 h. A natural aging with phosphate precipitation can be obtained in a few days with a different percentage of struvite and hydroxyapatite formed, depending on the sludge liquor composition; supersaturation of the solution with different ions play a major role in dictating the outcomes (Battistoni et al., 2000). The struvite production rate, a very important parameter in reactor design and efficiency estimation, increased with the concentration of struvite and mixing intensity in the reactor (Yoshino et al., 2003). Adding the chelating agent EDTA at high concentration can dissolve most of the precipitated struvite into solution even at pH values greater than 7.5 (Doyle et al., 2003).

6.1.2. Potential

The implication of theoretical knowledge on struvite formation is that, if these conditions can be duplicated and exploited in a practical engineering process, there is potential to economically extract struvite from wastewater in commercial quantities. This might be done by precipitating struvite in a dedicated reactor, instead of allowing spontaneous formation (Stratful et al., 2001; Munch and Barr, 2001; Hao and van Loosdrecht, 2003). This spontaneous formation fouls pipes and other inner surfaces of the treatment process, making operation of the plant inefficient and costly because the struvite must be dissolved with sulfuric acid or broken down manually with hammer and chisel (Durrant et al., 1999; Stratful et al., 2001; Williams, 1999). Another advantage of a dedicated reactor is that excess ammonium, which is a normal product of anaerobic wastewater digestion, might yield relatively pure struvite. Today, many wastewater processes do not fulfill the basic requirements for struvite formation, and the best medium for formation and recovery of struvite is the supernatant of sludge obtained from EBPR. Phosphorus can be obtained from sludge only with biological processes because the commonly used chemical precipitation of phosphorus described earlier in this review, which involves addition of iron and aluminum salts, produces a product that cannot be recycled for industrial recovery

of phosphorus (Donnert and Salecker, 1999a; Donnert and Salecker, 1999b).

There is no common method to recover struvite from biological processes, and equipment ranges from complex patented reactors employing ion exchangers, to simple stirring tanks (Gaterell et al., 2000; Williams, 1999). Recently, a struvite crystallization reactor was developed in Japan. Struvite was produced from the filtrate of anaerobic sludge digestion by adding magnesium hydroxide, and adjusting the pH to 8.2–8.8 with sodium hydroxide. A retention time of 10 days allowed the growth of pellets 0.5–1.0 mm in diameter. The recovered struvite was sold to fertilizer companies (Ueno and Fujii, 2001). Another experimental industrial process provided simultaneous removal of phosphate and ammonium ions by selective ion exchange and recovery of the product by chemical precipitation in the form of struvite (Liberti et al., 2001).

Since struvite contains phosphorus and nitrogen, removal will affect the content of both elements in the leftover sludge, which is used by farmers as a soil improvement agent and fertilizer. Struvite recovery from wastewater might have marginal effect on the net content of nitrogen, but greater impact on the concentration of phosphorus in the sludge. Gaterell et al. (2000) calculated that, because sewage has a typical N:P ratio of 8:1 and struvite 1:1, a theoretical maximum of 12.5% of the nitrogen load could be removed as struvite. The practical limit is lower because not all phosphorus can be recovered as struvite with today's technologies. Struvite removal leaves less phosphorus in the sludge. This is beneficial from two perspectives: (i) Sludge applied to fields usually has phosphorus in excess of the needs of plants. Subsequent eutrophication of water bodies from leached phosphorus compounds would be reduced, and, (ii) Struvite recovery would help to meet legal requirements imposed on sludge disposal and reduce the area needed for disposal.

6.1.3. Use

Struvite has many uses. The most obvious is as a raw material for the fertilizer industry (Gaterell et al., 2000). It can be used as a material in fire-resistant panels and in cement (Sarkar, 1990; Schuiling and Andrade, 1999). If cheap production methods are developed, it could be used in detergents, cosmetics, and animal feed, all of which use phosphates (Gaterell et al., 2000).

While struvite has many potential uses as fertilizer, as yet, none have been proven commercially profitable. The most promising application is as a slow-release fertilizer that can be applied in a single high dose without damage to growing plants. The plants suggested are ornamentals, vegetables, forest out-plantings, turf, orchard trees, and potted plants. This fertilizer might have low leach rates and slowly release nutrients during the growing

season (Munch and Barr, 2001; Li and Zhao, 2003; Johnston and Richards, 2003).

Highly soluble orthophosphate, serving as the initial phosphorus supply for establishing container plants, could be used together with struvite in a mixed fertilizer product. Struvite could also replace the major fertilizer diammonium phosphate, which is produced by neutralizing phosphoric acid with ammonia. Mixing struvite with phosphoric acid might even yield a superior fertilizer; part slow-release $MgHPO_4$, and part fast-release, highly soluble ammonium phosphate $(NH_4)_2HPO_4$. This might be considerably more cost-effective than commonly used diammonium phosphate fertilizers. Another possible product is untreated granular struvite that can be mixed with peat to serve as a lightweight potting mix (Gaterell et al., 2000). High solubility is not an asset in many fertilizer applications, as in grasslands and forests, where fertilizer is applied once in several years. Slow-release fertilizers are preferred for these cases, and struvite is even more solubilized than the currently used mostly insoluble rock phosphate. The presence of magnesium in struvite makes it attractive as an alternative to contemporary fertilizers for a few crops, like sugar beets, that require magnesium (J. Driver in: Gaterell et al., 2000).

The phosphate industry is using rock phosphate for detergents, food, and cosmetics by applying a high-energy, high-temperature industrial process to purify the phosphate. It is not likely that struvite will be used in the short term because struvite purification technology is unknown, small amounts of phosphate is needed by these industries, and well-known rock phosphate purification technology. However, as high-quality rock phosphate increases in price, and the industry is forced to use low-quality rock phosphate, pressure will increase to develop purification methods for struvite, which is sometimes purer than rock phosphate.

6.2. Alternative methods

Other technologies for recovering phosphorus from wastewater, with sometimes P-removal principle not well defined, were also proposed in recent years. Alternative for leftover sludge after phosphorus removal is as a source of ash, which can be recovered, if iron is not used for phosphate precipitation in the treatment plant. Applying Ca, Al, or EBPR increases the recycling potential for ash. Currently, Cu and Zn are still highly concentrated in the ash, prohibiting its use as fertilizer (Schipper et al., 2001). An alternative technology for phosphate removal by conventional precipitation produced phosphate pellets having high-purity and extremely low water content. The process was patented in The Netherlands. The main advantage, apart from removing phosphorus from the water, is that the pellets can be reused in industry (Giesen, 1999). Additionally, a new

material, the macroporous (100 nm diameter) fibrous structure of TiO_2 removed large quantities of phosphoric anions by crystallization as hydroxyapatite (Nagamine et al., 2003). In energy terms, production of struvite from urine is less demanding than the conventional ways used by the wastewater industry (Maurer et al., 2003).

A simpler option for phosphate recovery as a beneficial raw material is to heat the sludge. Heating activated sludge at 70 °C for one hour released most of the phosphorus from polyphosphate. Precipitation of the phosphorus by $CaCl_2$ yielded 75% of the phosphorus in the sludge without the need of pH adjustment, as is the case for struvite. The precipitate contained more P and less Ca than typical natural rock phosphate. Hence, this has potential as a simple recovery process for usable phosphorus from wastewater material (Kuroda et al., 2002).

Another option is to use a strongly adsorbing filter material that retains phosphorus efficiently, and after phosphorus saturation, using the discarded filter as a fertilizer. Blast furnace slag showed high phosphorus sorption capacity and was used for abiotic sorption of wastewater phosphorus. During this process, the calcium concentration also decreased, forming the compound hydroxyapatite. This implies Ca–P precipitation as the predominant phosphorus removal mechanism in the slag. The low solubility of hydroxyapatite might have important implications for its possible production for fertilizer by direct formation (Johansson and Gustafsson, 2000). Seed crystal material made of calcium silicate hydrate (tobermorite crystals), applied for phosphorus removal by crystallization, yielded precipitated hydroxyapatite that can be incorporated into soil and had characteristics of a good plant fertilizer (Moriyama et al., 2001). The direct use of non-soluble phosphate, such as hydroxyapatite, as a fertilizer, will require an effective and cheap means of solubilization. However, this problem might be solved with the use of phosphate-solubilizing bacteria (PSB) and phosphate-solubilizing fungi (PSF) (Richardson, 2001; Whitelaw, 2000).

6.3. Phosphate-solubilizing microorganisms in relation to exploitation of recovered phosphorus

PSB and PSF are common types of soil microorganisms. They inhabit almost all agricultural and forest soil, belong to numerous genera, and are employed worldwide, mainly in developing countries, as inoculants together with rock phosphate as substrate (Leggett et al., 2001; Rodriguez and Fraga, 1999). Although several methods for mobilization of non-soluble phosphorus (Fe– and Al–P in acid soils, Ca–P in alkaline soils) are documented, the most common is by production of organic acids and release of protons by the microbes (Jones, 1998).

We propose that precipitated phosphate recovered from wastewater, together with common PSB and PSF,

be used as a fertilizer. The organic acids produced by these microorganisms are strong enough to dissolve the struvite and hydroxyapatite obtained from wastewater. There are significant economic and environmental advantages in developing these materials in a wide range of fertilizers. These include very slow-release fertilizers for pasture and forest (phosphates as recovered), a mix of slow- and fast-release fertilizers for most agricultural practices (recovered phosphate mixed with phosphate that is partly dissolved by PSB and/or PSF), and fast-release fertilizers combining recovered phosphate with strong phosphate-solubilizing microorganisms for crops needing rapid start up.

In summary, present consumption of phosphorus raw material is over one million tons per year. The most likely use of struvite is as a substitute for rock phosphate in the fertilizer industry, since no additional industrial treatment is required, and the material can be used "as is" or with very simple further processing, like granulation and mixing. Furthermore, routine simple treatments used by the fertilizer industry, like application of phosphoric acid, could be used to alter the solubility of struvite, making it more desirable as a fertilizer. Future use of struvite depends mainly on industrial operations, that are simple and cost effective, and positive field trials that have not been undertaken. Without knowing how much struvite can be recovered efficiently, the cost effectiveness of the industrial operation, and the desire of consumers to use struvite as a fertilizer, it is nearly impossible to assess how soon struvite-based products will enter the market. Perhaps legislative pressure to keep the environment free of phosphorus contamination originating from sludge disposal and exhaustion of the high-quality rock phosphate supplies, with attendant increase in price, will increase the economic attractiveness of struvite for industrial development.

7. Concluding remarks and directions for future research

'Eutrophication of water bodies is a major, global environmental problem. Its main cause is disposal of nutrients (N and P) directly from wastewater plants or indirectly from agriculture runoff and leaching from sludge deposited in landfill and fields. As stringent laws now require that the level of these nutrients be significantly reduced (Stratful et al., 1999; Gaterell et al., 2000) boost to the existing wastewater industry was given.

The contemporary focal issue is not eliminating phosphorus per se (that has been done efficiently using metal and polymer precipitation), but recycling it. Recycling of phosphorus is converting an acute disposable problem into a raw material that will benefit industry and society, as phosphorus has a significant contribution, as fertilizer, to the well being of human societies. All tertiary wastewater facilities eliminate phosphorus (either by chemical or biological removal) as a non-recyclable material (metal-phosphate precipitates or precipitates together with numerous other waste materials as sludge). What is needed is a process of phosphorus removal that separates it from other waste components, so that it can be recycled as a fertilizer or an ingredient in other valuable phosphorus products.

The emerging technology of struvite crystallization (magnesium-ammonium phosphate) and hydroxyapatite (calcium phosphate), as an alternative phosphorus removal technology may serve as a catalyst for removing phosphorus as a recyclable product. The best way is to combine EBPR with struvite and hydroxyapatite crystallization. It will save chemicals for precipitation, reduce the size of the treatment facility, reduce the volume of effluent to be treated (more concentrated phosphorus in present effluent), and consequently reduce costs (Stratful et al., 1999; Jeanmaire and Evans, 2001).

If biological phosphorus removal is the cornerstone of the phosphorus recycling trend, EBPR systems are the major players, and therefore, a better understanding of processes and microbial communities involved is indispensable. To that end, there is a need to employ better microbial detection techniques than the conventional cultivation techniques that have been used and failed to detect the major organisms in EBPR, partially because some are probably not culturable. Ribosomal RNA-targeted oligonucleotide probes have become valuable tools for the detection of microorganisms involved in important biotechnological processes. Hybridization techniques have become fast and reliable alternatives to traditional detection techniques (Grady and Filipe, 2000; Lipski et al., 2001), and some have started to emerge in recent years for analyzing bacterial populations in EBPR. To define the general microbial community structure in EBPR processes, it is essential to look more closely into the occurrence and behavior of each species of PAO in various EBPR processes, mainly by molecular methods. Also as a common EBPR metabolism appears in phylogenetically diverse microbial population of PAO, this suggests the possibility that key genes of the EBPR metabolism are common among different bacterial species (Mino, 2000). At this stage, available data on microbial communities and species participating in EBPR is so limited that no definite conclusions can be drawn about the microbial community structure of the EBPR process.

There are several lines of research that are needed to assist our understanding of the process of phosphorus removal and recycling of phosphorus as a valuable product.

1. Attempts to isolate culturable bacteria and molecularly characterize communities in EBPR that cannot be cultured at this time should continue. Simultaneously, measurements of essential parameters like PHA, glycogen, acetate, and orthophosphate, to-

gether with phosphorus content of the sludge should be done. This will show the possible involvement and relationship of the characterized bacteria to a particular aspect of the EBPR process (Mino et al., 1998).

2. Manipulation of the genes responsible for polyphosphate metabolism can also be used to improve gene expression (Keasling et al., 2000).

3. An economic, separate, and efficient industrial process for recovering struvite and hydroxyapatite from wastewater should be developed (Stratful et al., 1999; Gaterell et al., 2000).

4. Fertilization tests of crops under field conditions with these two potential fertilizers and their possible industrial derivatives should be performed.

5. The combination of phosphate solubilization bacteria with struvite and hydroxyapatite should demonstrate if this combination is an alternative to chemical treatment of these less soluble phosphate products.

6. The idea of "engineered wetlands" should be examined more closely.

Acknowledgements

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