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New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste

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1. Introduction

Phosphate rock is a life essential and non-renewable resource for the production of phosphorus (P) based chemicals used in numerous agricultural and non-agricultural applications. The largest part of all mined phosphate is used for the production of mineral fertilisers (Cordell et al., 2009). However, the quality of global phosphate reserves is decreasing and the quantity is limited. One of the solutions to overcome potential P scarcity is implementing relatively basic phosphorus recovery techniques on various phosphorus containing waste streams, such as domestic wastewater (Acevedo et al., 2015). Based on an average excretion of 2 g P per capita per day and P from detergents, food waste, food additives and other products (Verstraete et al., 2009), the wastewater treatment sector is considered as one of the major hotspots for P depletion mitigation (Acevedo et al., 2015). Even though the biggest concern is the removal of P from wastewater in order to meet the discharge limits and to protect surface waters from eutrophication (Verstraete et al., 2009), the removed P has the potential to be reused as a substitute for mined P fertiliser (Cornel and Schaum, 2009). The most popular P removal techniques applied on wastewater are biological P removal and the more widely used chemical P removal (Verstraete et al., 2009). According to Cornel and Schaum (2009), recent investigations showed that P recovery is particularly successful in combination with biological P removal. In this context, the research of innovative biological techniques for phosphorus recovery from wastewater shows interesting opportunities.

2. Phosphorus in wastewater treatment

Phosphates are the most common P specie found in domestic wastewater and their concentrations are generally low (<10 mg L⁻¹), which implies that it is necessary to concentrate them to make P recovery and reuse feasible (Geerts et al., 2015). Throughout the wastewater treatment cycle, most of the incoming P, approximately 90%, is concentrated in sewage sludge. First of all, during primary settlement, approximately 11% of the incoming P load is removed within the primary sludge. In biological wastewater treatment systems another 28% of the total P-load is incorporated in the biomass and removed within the surplus activated sludge even without specific P removal processes. Thus, based on the European maximum allowable discharge concentration of 1mg L⁻¹ (>100,000 Inhabitant Equivalents) or 2 mg L⁻¹ (10,000–100,000 Inhabitant Equivalents) (European Directive 91/271), another 50% of the incoming P load has to be removed additionally by chemical or biological processes or a combination of both (Cornel and Schaum, 2009). In most wastewater treatment plants (WWTPs), the remaining P is removed through chemical precipitation with metal (aluminium, iron or magnesium) salts or lime (Verstraete et al., 2009). Alternatively, P can also be removed by incorporation in the biomass through biological phosphorus removal processes.

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Since almost all (90%) incoming P from the wastewater is concentrated in the sludge, direct use of the P rich sewage sludge in agriculture is the most straightforward way for the valorisation of nutrients recovered from wastewater. However, the application is limited, since increasing concerns about contamination with pathogens, heavy metals and toxic organic compounds led to the fact that some European countries have forbidden the direct use of sewage sludge in agriculture (Schoumans et al., 2015). Furthermore, the phosphate in sewage sludge under the form of iron or aluminum phosphate after chemical precipitation is strongly bound, which makes the phosphorus hardly available for plant uptake under typical pH conditions found in soils (Sartorius et al., 2012). For those reasons, techniques for P recovery from sewage sludge needed to be developed.

The variety of existing techniques for P recovery from municipal wastewater can be applied at different points throughout the wastewater flow at the treatment works (Schoumans et al., 2015): phosphate can be recovered from the liquid phase, the sludge phase or from sewage sludge ashes. Since in WWTPs without P recovery, about 90–95% of the total P load is retained in the sewage sludge, the theoretical P recovery potential from sludge is higher in comparison to the liquor phase (<50–60%) (Cornel and Schaum, 2009). However, most of the current techniques aim at recovering phosphate from dewatering rejection streams, since the low concentration of suspended solids makes it relatively easy to separate the phosphate precipitates from the wastewater (Desmidt et al., 2015). The existing recovery techniques applied on chemically or biologically accumulated phosphorus in sewage sludge are described underneath.

3. Physicochemical processes for phosphorus recovery

The physicochemical techniques are summarized in Fig. 1. P removal by chemical precipitation is a relatively easy and effective method. In the past, chemical precipitation was often used in WWTPs to improve the removal of total suspended solids (TSS) and biological oxygen demand (BOD). Since the 1970s, the need to remove organic compounds and nutrients (N and P) more completely, has drawn new attention to chemical precipitation (Tchobanoglous et al., 2013). This technique is efficient but has some negative effects. The presence of iron in sewage sludge results in a lower biogas production during anaerobic digestion. In addition, chemical P removal rules out some of the P recovery possibilities, since phosphate ions are too strongly bound in the sludge matrix (Parsons and Smith, 2008).

To mobilize a higher percentage of chemically precipitated P in the sludge, a wet chemical process, such as acidic leaching, has to be applied. That process allows to recover large amounts of P by transferring P fixed in the solid (sludge) to a liquid (water) phase and it is required to obtain P recovery from chemically treated sludge, but also can be applied on WWTPs with either biological or chemical P elimination (Ewert et al., 2014). Those processes, however, require large amounts of chemicals and special acid resistant equipment, and generate many different residual byproducts which must be disposed of at high costs (Sartorius et al., 2012).

Similar to sewage sludge, the direct use in agriculture of ashes obtained after sewage sludge incineration is limited due to the elevated heavy metal content. The technologies for P recovery from sewage sludge ashes are based on two different approaches, namely the wet-chemical or thermochemical treatment (Adam et al., 2015). Thermochemical treatment of sewage sludge ash removes heavy metals and improves P bioavailability for crops. Incinerated ash can also be used as a secondary resource in the fertiliser industry as substitute for phosphate rock. This possibility has only been tested and is not fully established yet (Adam et al., 2015). Recovering P from sludge or sludge ash by wet-chemical or thermal processes results into larger recovery rates than those from

the liquid phase. Up to 90% of the phosphorus can be recovered from sludge or sludge ash, but on the other hand large amounts of chemicals and/or energy and a more downstream process are required, which leads to higher capital and operating costs in comparison to P recovery from the liquid phase (Desmidt et al., 2015).

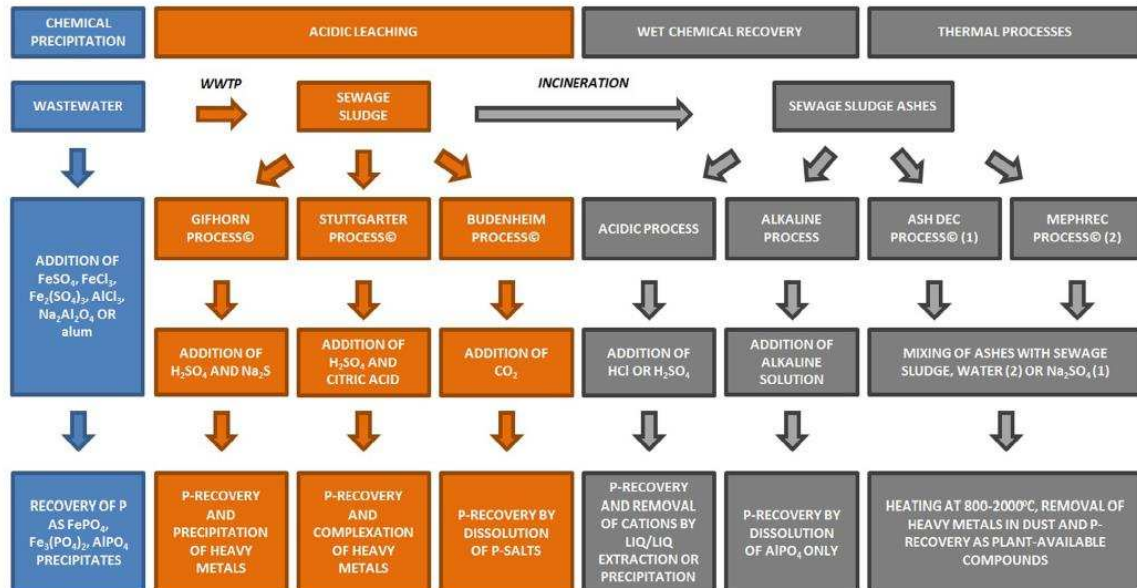


Fig. 1. Physicochemical P-recovery techniques applied to wastewater, sewage sludge and sewage sludge ashes to date. Derived from Cornel and Schaum (2009), Ewert et al. (2014), Petzet et al. (2012), Sartorius et al. (2012), Wilfert et al. (2015).

4. Towards biological processes for P recovery?

Besides using physicochemical precipitation, P can also be removed through incorporation in the biomass by means of biological P uptake. This type of P removal is based on enhancing the ability of microorganisms, known as polyphosphate (Poly-P) accumulating organisms (PAOs), to take up more P than required for growth under alternating anaerobic and aerobic/anoxic conditions, often referred to as Enhanced Biological P Removal (EBPR). Under these conditions, PAOs are able to remove diluted phosphate and other ions (such as K^+ and Mg^{2+}) from the liquid phase and convert and concentrate them into the sludge biomass under the form of intracellular Poly-P (Yuan et al., 2012). P removal is then achieved by discharge of the P rich sludge.

Biological P removal has several benefits by comparison with to chemical P removal. The sludge originating from biological P removal processes has a better agricultural value due to the higher content of plant available phosphates and the lack of other undesirable chemicals (Nieminen, 2010). The main advantages of EBPR are the reduced sludge production and the elimination of the use of chemicals associated with physico-chemical recovery processes. The Poly-P stored in the microorganisms after EBPR are partly released under anaerobic conditions (i.e., during anaerobic digestion (AD)) leading to an increase of the free phosphate content in the sludge systems (Desmidt et al., 2015), while if the P would have been chemically removed with iron or aluminium salts, the phosphates would remain bounded to these metals after anaerobic treatment (Parsons and Smith, 2008). P enriched EBPR sludge contains 5–7% P, whereas normal activated sludge has only a P content of 1–2%. P can be recovered from the P enriched sludge or its rejection liquor after dewatering in a precipitation/crystallization tank where magnesium or calcium salts and if necessary seed crystals are added to remove P as calcium phosphate or magnesium ammonium phosphate (Cornel and Schaum, 2009).

Even though biological P removal has many advantages in comparison to chemical P removal, it is not yet a widely applied technique. Wilfert et al. (2015) postulate that future energy producing WWTPs will rely on iron addition for P and COD removal, but highlights the problem associated with P recovery from the iron, even though in environmental systems, P is efficiently mobilized from various iron–P compounds through (microbial) oxidation and reduction, under alkaline or acidic conditions, etc. Therefore, the authors believe that the current poor understanding of iron and P chemistry in wastewater treatment systems is inhibiting the development of processes to recover P from iron-P rich sewage sludge. They propose to develop biomimetic processes to recover P from iron–phosphates, as an important step to make WWTPs act as energy and nutrient factories.

Recently, there is increasing interest in the integration of forward osmosis (FO) with a biological wastewater treatment process, known as osmotic membrane bioreactor (OMBR) (Qiu and Ting, 2014). In the reactor organic matter and ammonia are removed through biological activities. Since they are rejected by the FO membrane, phosphate, magnesium, calcium and unconverted ammonium ions are concentrated in the reactor (Qiu and Ting, 2014). This phosphorus rich supernatant can be used for phosphorus recovery through precipitation, even without any addition of Ca^{2+} , Mg^{2+} , K^{+} and NH_4^{+} . The recovery of these ions from the bioreactor supernatant results in additional benefits such as lower salt accumulation within the bioreactor and reduced potential scaling of the of the FO membrane (Qiu and Ting, 2014).

Most of the phosphate recovery processes are developed to be applied on industrial and municipal wastewater and only a few techniques are developed for P recovery from manure and digestate (Desmidt et al., 2015). Similar to sewage sludge, the solid fraction of manure can be incinerated to produce bio-energy and P can be recovered from the ash (Schoumans et al., 2015). One of the major challenges is the high content of organic compounds present in manure and AD effluents, negatively influencing the precipitation or crystallization process (Cerrillo et al., 2014). From currently published literature, it can be inferred that P can be recovered from manure at high rates of up to 95% through struvite precipitation (Siciliano and De Rosa, 2014) or up to 90% as calcium containing P precipitate (Szögi et al., 2015).

5. Biological systems used for phosphorus removal from wastewater

5.1. Phosphorus removal by bacteria

The design of microbial processes that use PAOs to remove P from wastewater requires understanding the metabolism of phosphate in microorganisms. P is often a limiting nutrient required for cell growth compared with carbon and nitrogen, which are more easily accessible. Consequently, the microorganisms have developed pathways to accumulate P to cope with the starvation conditions (Blank, 2012). P is essential to bacteria and is found in the different components of the cell. It has many functions and represents about 9% of the mass of nucleic acids (DNA and RNA). It is a component of phospholipids (about 4% of the total biomass) and provides the cells with energy through Adenosine Tri-Phosphate (ATP). It has also an important function in the posttranslational processing of proteins (Elser, 2012).

Poly-P metabolism depends on many enzymes. Among the most important ones, the polyphosphate-adenosine diphosphate phosphotransferase (EC 2.7.4.1), also called polyphosphate kinase (PPK), catalyzes the elongation of the Poly-P chain by transferring a phosphate group from ATP to the Poly-P chain. Three types of PPK have been described, but only PPK1 catalyzes this reaction. PPK2 removes phosphate groups from Poly-P and links it to a guanosine-diphosphate (GDP) molecule to form guanosinetriphosphate (GTP). It can also catalyze the same reaction with the other nucleosides

di-phosphates. With the ability to both produce and consume Poly-P, PPK can regulate the ribonucleoside concentration in the cytoplasm and thus participate in the regulation of the cell division. PPK3 uses Poly-P to phosphorylate cytidine-diphosphate (CDP) into cytidine-triphosphate (CTP) (Achbergerová and Nahálka, 2011). The exopolyphosphatase (PPX) removes a phosphate group at the end of the Poly-P chains. It is composed of two subunits with a molecular mass close to 58 kDa. A wide variety of PPX have been reported, but they all require high concentrations in K⁺. Their activity decreases with Poly-P chains showing a low DP. The endopolyphosphatase (PPN) hydrolyzes the bonds in the middle of the Poly-P chain and provides PPX with action sites (Günther et al., 2011).

5.1.1. Anaerobic metabolism of PAOs

PAOs need a carbon source for their growth. During the anaerobic phase, PAOs take up carbon substrate and store it in case if the time spent without oxygen lasts. Generally, they take up acetate from their environment since it is the largest volatile fatty acid (VFA) found in wastewaters. Inside the cell, the acetate is converted into acetyl-CoA. This activation requires energy and therefore the consumption of ATP. This ATP comes from the transfer of an energy-rich phosphoric group from polyphosphate to ADP. This hydrolysis releases H₂PO₄⁻ and the associated cations (e.g., K⁺, Mg²⁺) outside the cell. Next, acetoacetyl-CoA is formed by the condensation of two molecules of acetyl-CoA. Acetoacetyl-CoA is then reduced by the coenzyme nicotinic amide dinucleotide (NADH) into 3-hydroxybutyryl-CoA. This last compound is polymerized to form poly-β-hydroxybutyrate (PHB). PHB belongs to the family of poly-β-hydroxyalkanoates (PHAs) that are intracellular carbon polymers. In some cases, PAOs also produce a smaller extend poly-β-hydroxyvalerate. Those steps occur in anaerobic conditions (Oehmen et al., 2007).

5.1.2. Aerobic metabolism of PAOs

The aerobic metabolism of PAOs is similar in all the models that have been proposed. In aerobic conditions, bacteria use oxygen as an electron acceptor. The stocks made during the anaerobic phase are used for the cell growth and the reconstitution of the polyphosphate reserves. PHB and PHV are degraded, leading to acetyl-CoA, processed through the TCA cycle. It produces energy from oxidation and carbon for new cell growth. A part of the energy is used to take up soluble phosphate from the environment and to incorporate it into polyphosphate. A part of carbon and energy is also used to regenerate glycogen (Tchobanoglous et al., 2013).

5.1.3. Polyphosphate accumulation in bacterial cells and functions

Poly-P consists of phosphates linked together, forming a linear polymer. Metallic cations neutralize the negative charge (Ca²⁺, Mg²⁺, K⁺) of the macromolecule with a chemical formula M_(n+2)P_nO_(3n+1). Its degree of polymerization (DP) can reach a value of 10⁶, while the maximum polymerization degree of PHA is 30,000. Poly-P can form different complexes with various molecules like polyhydroxybutyrate (PHB), nucleic acids and proteins. With PHB and Ca²⁺, Poly-P forms a complex that consists of a double helix in which PHB is the outer chain and Poly-P is the inner chain, both stabilized by Ca²⁺. This complex is located in bacterial membranes. In prokaryotic cells, Poly-P is not located in specific organelles because bacteria do not have vacuoles or nucleus membranes, which are known in eukaryotic cells to contain a large amount of Poly-P. Its location depends on the specie considered. Poly-P can be found in the cytoplasm, in the periplasm, at the flagellar pole, in the cell membrane and on the cell surface. All the Poly-P molecules are organized in small granules which can have a diameter comprised between 48 nm and 1 μm, and one single microbial cell can contain many Poly-P granules (Günther et al., 2011).

5.2. Phosphorus removal by microalgae

Microalgae have been extensively used for the treatment of domestic wastewater worldwide; in particular, the use of the symbiotic relationship between microalgae and bacteria in Waste Stabilization Ponds (WSPs) has been the main important feature of this low-cost, natural wastewater treatment system. In simple terms, heterotrophic bacteria metabolise organic carbon and other nutrients under the presence of oxygen for growth and energy, and produce oxidized forms of carbon (CO₂), nitrogen (N under the form of NH₄⁺, NO₂⁻ and NO₃⁻) and P (PO₄³⁻); in return, photosynthetic microalgae utilize CO₂ and nutrients (NH₄⁺, NO₃⁻ and PO₄³⁻) for growth and the production of oxygen to support bacterial activity (Craggs, 2005). Wastewater can be considered as a culture medium to grow microalgae due to the presence of nutrients and various pollutants. Microalgae can also be used in many industrial applications, such as food processing, pharmaceuticals, fertilisers, animal feeding, biofuels and biogas production. Therefore, they can lead to high-value products by valorizing nutrients which cause eutrophication (Abdulsada, 2014).

Microalgae are the most abundant algae in the environment. These unicellular microorganisms are photosynthetic and mainly eukaryotic cells (only cyanobacteria are prokaryotes). Their growth rates are much higher than other terrestrial plants. The most common microalgae found in wastewater are *Chlorella* and *Scenedesmus* (Abdulsada, 2014). Other genera were also reported: *Nitzschia*, *Navicula*, *Euglena*, *Phacus*, *Chlamydomonas* and *Micractinium* (Eland, 2012). Their growth is stimulated by light through photosynthesis. However, they can also work in the absence of light as heterotrophic organisms, using oxygen to assimilate organic carbon. They have a positive role on pathogen destruction by the increase of pH in their environment and produce antibacterial molecules (Abdulsada, 2014).

Nutrient removal by microalgae depends on different types of mechanisms. At first, the simple diffusion of dissolved nutrients depends on the thickness of the boundary layer of water outside the cells, and turbulence is necessary for increasing diffusion into the microalgae. The second mechanism is precipitation and is directly applicable to P. Microalgae consume CO₂ and HCO₃⁻, leading to an increase of pH. This alkaline pH induces a precipitation of P with the available cations. Finally, the increase of pH leads to the stripping of ammonia, released into the atmosphere (Abdulsada, 2014). Sedimentation, biological uptake of N and P, denitrification, nitrification and mineralization are supposed to play a key role in nutrient removal in WSPs (Eland, 2012). It has been estimated that WSPs remove 50–70% N and 20–50% P from wastewater (Eland, 2012).

Phosphorus is the second essential nutrient for microalgae after nitrogen. It comprises about 1% of the cell's dry weight, and they can accumulate P under the form of Poly-P like bacteria (Abdulsada, 2014). This mechanism of "luxury P-uptake" is observed in specific conditions and is defined as the uptake of P beyond that required for growth and storage of phosphate within the biomass as Poly-P (>1% P dry weight). The growth of microalgae, and consequently P-uptake, depends on many parameters which must be controlled during the process: light exposure, temperature, aeration, mixing, carbon and nutrients' concentrations, pH, presence of grazer organisms and viruses (Abdulsada, 2014).

Microalgae accumulate P reserves as Poly-P in acidocalcisomes when exposed to stressful environmental conditions, which at the same time stimulate lipid production and in-cell accumulation. However, some specific alga strains continue accumulating high amounts of P even in nutrient-rich environments (i.e., luxury P uptake) by using biochemical mechanisms not well understood. In the long term, there is a real opportunity to develop a novel, relatively simple and

sustainable technology to harvest nutrients via organic N and Poly-P accumulation by microalgae in wastewater treatment works. The accumulation of Poly-P granules inside the cells creates the need for separating microalgae from wastewater. This harvesting can be accomplished through different technologies: centrifugation, flocculation, sedimentation, filtration and dissolved air flotation. Immobilization may also be used but the matrices are weak and costly (Abdulsada, 2014).

Modern WWTPs with energy recovery via anaerobic digestion of sewage sludge have great potential for allowing the implementation of algae cultivation, as they provide: (a) continuous source of nutrients for algae growth – i.e., CO₂ contained in biogas from AD reactors and flue gas from combined heat and power (CHP) units; and N and P compounds in both wastewater and digestate; (b) downstream processes in place for harvesting algal biomass –i.e., polymer dosing for improving sedimentation of algal biomass, sludge thickening and dewatering; and (c) advanced digestion systems that will render the algal biomass suitable for mesophilic AD with subsequent generation of heat and power through CHP units (Horan and Camargo-Valero, 2013).

Microalgae have proven their ability to effectively remove both ammonium and phosphate from wastewater even under temperate climate conditions (up to 10% N and 3% P in dry algal biomass) (Camargo-Valero et al., 2010), therefore they may provide a new route for biological nutrient recovery and potentially removing all together the need for nitrification/de-nitrification processes and phosphate removal by P-accumulating bacteria in large WWTPs.

5.3. Phosphorus recycling by other organisms

Mycetes were also reported as P-accumulating organisms, but they are much less studied than bacteria. Filamentous fungi are much easier to harvest than bacteria because of their growth in mycelial structure, which simplifies a potential industrial extrapolation. The accumulation of P in Poly-P granules has also been reported in these organisms. For instance, *Mucor circinelloides* reached a cellular P-content of 7.08% (Ye et al., 2015). Several yeasts were also reported as P-accumulating organisms, and the mechanisms of P-accumulation have already been studied at a genetic level in *Saccharomyces cerevisiae* (Watanabe et al., 2008). Other yeasts were reported as P-accumulators, such as *Hansenula fabianii* and *Hansenula anomala* (Watanabe et al., 2008). However, yeasts do not form biological structures like filamentous fungi, and the recovery of P is therefore more difficult. However, the use of mycetes in the topic of P-recovery is a promising area. Fig. 2 shows the possible uses of microorganisms in the field of P-recovery.

Animals are also concerned in P-recycling. More specifically, vermicomposting consists in composting of organic wastes by earthworms. *Eisenia fetida* and *Eisenia andrei*, common earthworm species, are usually used in this process. The technique is quite new, and allows transforming organic wastes in worm castings. The worms coat the organic material with mucous excretions which contain microorganisms. This leads to a preliminary digestion and produces a pre-digested compost, consumed by the earthworms to release an organic worm-humus and mineral components (Perera and Nanthakumaran, 2015). These organisms are known to improve soil fertility by excreting humus, N, P, K, micronutrients, beneficial soil microbes and growth hormones. “Vermicompost”, or compost obtained by the action of earthworms, acts as a slow-release fertiliser and has been found to be cheaper and more efficient than expensive chemical fertilisers. The concentration effect of nutrients in vermicompost is sometimes more efficient than conventional composting, which means that vermicomposting offers many opportunities (Sinha et al., 2010).

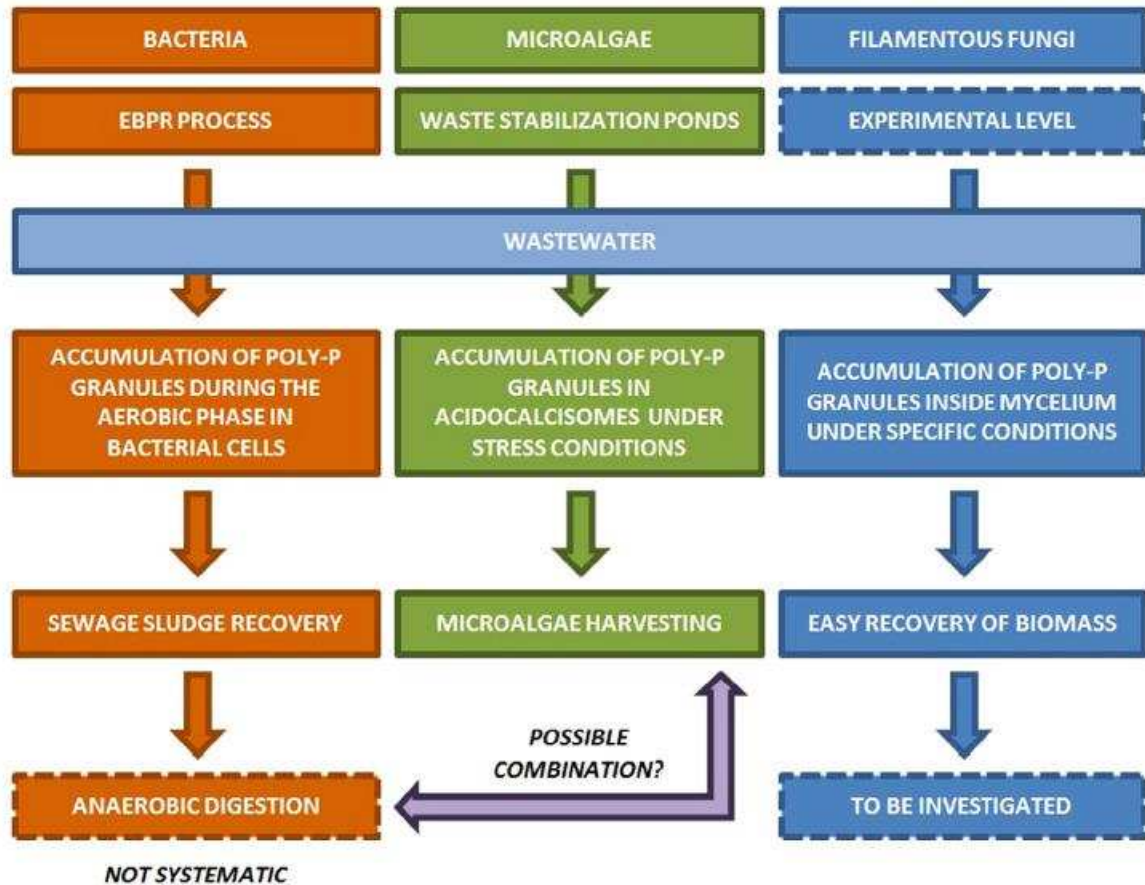


Fig. 2. Possible uses of microorganisms as P-recovery systems.

5.4. Current bottlenecks of biological processes

PAOs have been cited in many papers, but there is clearly a lack of a model PAO. Indeed, many strains have been cited in research studies, but the uncultivable family of *Candidatus Accumulibacter phosphatis* is the only one recognized as a true PAO. It represents 5–20% of EBPR sludge community (Yuan et al., 2012). The *Accumulibacter* cluster is composed of two types (I and II), and each type is divided into several clades (IA-E, IIA-G). Among these clades, Clade IIA has been found to be able to perform the metabolic characteristics found only in PAOs (Yuan et al., 2012). However, no isolation of pure strains has been accomplished so far. Gram-positive *Tetrasphaera*-related organisms also have shown characteristics of PAOs and are common in EBPR plants. However, their metabolism is poorly understood (Yuan et al., 2012). Other bacterial strains have been isolated and were able to accumulate significant quantities of Poly-P, such as *Acinetobacter* sp., *Burkholderia* sp., *Rhodocyclus* sp. and *Microlunatus phosphovorus* but they cannot be considered as true PAOs because they lack the characteristics of *Accumulibacter* (Aravind et al., 2015).

Studies of PAOs did not lead to the culture of pure isolated strains. Many media have been tested but there is no rapid method for isolating and screening them (Chaudhry and Nautiyal, 2011). On the other hand, many techniques have been found to be able to detect Poly-P granules, such as staining techniques (Neisser, DAPI, methylene blue), electron microscopy, Nuclear Magnetic Resonance, Fluorescence In Situ Hybridization techniques, antibodies, etc. (Günther et al., 2011). However, it has been possible to cultivate PAOs under the form of enriched consortia. In this context, the control of non-PAOs must be undertaken. More specifically, Glycogen Accumulating Organisms (GAOs) are also able to grow under alternating anaerobic and aerobic/anoxic conditions. These bacteria perform the

same carbon transformation than PAOs, but do not contribute to P-removal. The two groups compete for the carbon source and the environmental conditions have a direct effect on this competition. Temperature, pH and the type of volatile fatty acids in solution have a strong impact on the PAO/GAO balance (Yuan et al., 2012).

Whether this concerns PAOs' isolation or EBPR control in fullscale plants, many key parameters must be considered. Moreover, it must be kept in mind that the EBPR process commonly used in WWTPs works but is far from using the PAOs with a maximal efficiency. The key parameters to take into account are aeration, time of anaerobic and aerobic/anoxic phases, pH, temperature, volatile fatty acid composition, PO_4^{3-} , SO_4^{2-} , NH_4^+ , K^+ , Na^+ , Mg^{2+} , Ca^{2+} and other metal ion concentrations (Aravind et al., 2015; Chaudhry and Nautiyal, 2011; Yuan et al., 2012).

6. Innovative bioprocess design for phosphorus recovery

6.1. From improved EBPR design to new microbial processes

As outlined in previous sections, biological phosphorus recovery can be achieved by using microbial communities through EBPR. However, EBPR cannot be applied in each case because its efficiency depends on specific conditions which do not go along with a good carbon and N treatment. The removal of P is based on the metabolic activities of PAOs, subjected to alternating anaerobic and aerobic/anoxic conditions. To reach a good efficiency, the carbon to phosphorus ratio (C:P) has to be close to 30. Three EBPR variants have been described. The first EBPR process was simply composed of an anaerobic/aerobic sequence and was called "Phoredox" or A/O (anaerobic/aerobic) process. A recirculation of the sludge was made from the clarifier to the head of the process. However, no denitrification occurred in this process. As the treatment of N is mandatory, this process was quickly abandoned. The A2O (anaerobic/anoxic/aerobic) process carries out denitrification because of the presence of an anoxic tank in the central section. A mixed liquor recirculation is required to supply the anoxic tank with nitrite and nitrate that will be further reduced in molecular N. The MUCT (University of Cape Town) process achieves phosphorus removal and denitrification on wastewater the BOD/P ratio of which is low. The recycled sludge is sent to the beginning of the anoxic tank to avoid that the nitrite and nitrate generated in the aerobic phase promote the use of VFAs for denitrification instead of the formation of PHA by PAOs. The mixed liquor (MLSS) recirculation from the end of the anoxic tank to the beginning of the anaerobic tank is set up to increase carbon utilization and biomass recycling. The three processes are described in Fig. 3. Besides these classical EBPR configurations, specific designs (called EBPRr, as process incorporating nutrient recovery) can be found, such as the SIPER process where sludges are recirculated for providing optimal COD for P recovery (Yan et al., 2015). Some countries using seawater toilet flushing have adopted Sulphate reduction Denitrification and Nitrification Integrated (SANI). The SANI systems can be now extended to P removal leading to a reduction of sludge volumes significantly enriched in P (Wu et al., 2014). Another important innovation is the discovery of denitrifying phosphate accumulating organisms (DPAOs), allowing the simultaneous assimilation of N and P (Wang et al., 2015). The discovery of DPAOs have been exploited through the design of new bioprocess for P removal, such as the anaerobic, anoxic/denitrifying two sludges process coupled with chemically induced crystallization (Shi et al., 2012), the improvement of the MUCT process by integrating denitrifying P removal (Zeng et al., 2013), the extension of anammox process to P removal through bio-augmentation (Ma et al., 2013).

Membrane technologies have also been considered for the improvement of biological P removal processes, such as osmotic membrane bioreactor (OMBR) (Qiu and Ting, 2014). In the reactor organic matter and ammonia are removed through biological activities. Since they are rejected by

the FO membrane, phosphate, magnesium, calcium and unconverted ammonium ions are concentrated in the reactor. This phosphorus rich supernatant can be used for phosphorus recovery through precipitation, even without any addition of Ca²⁺, Mg²⁺, K⁺ and NH₄⁺. The recovery of these ions from the bioreactor supernatant results in additional benefits such as lower salt accumulation within the bioreactor and reduced potential scaling of the FO membrane (Qiu and Ting, 2014). On the same basis, OMBR coupled with reverse osmosis (OMBR-RO) can be used for the simultaneous recovery of P and production of clean water (Luo et al., 2016).

Granule-based EBPR processes have also been developed for increasing long term performances of P removal systems (Zheng et al., 2013). Formation of granules allows an optimal sludge recovery after clarification, but these granules are sensitive to free ammonia (Zou et al., 2015). Besides classical EBPR, very innovative P recovery devices can be considered on the basis of bio-electricity generating reactors (Hirooka and Ichihashi, 2013). Indeed, microbial fuel cells (MFC) allows P removal through chemical precipitation (around 80%) and microbial uptake (between 4% and 17%) (Tao et al., 2014). By using MFC, generation of electrical power and the simultaneous recovery of P from waste are now possible (Ichihashi and Hirooka, 2012).

Another very innovative way for recovering P is the use of seawater-based urine phosphorus recovery (SUPR) system (Tang et al., 2015). In this system, urine is biologically hydrolyzed by microbial ureases, the released ammonium and pH changes leading to P precipitation (Dai et al., 2014). However, SUPR system needs to be more thoroughly investigated before any industrial implementation (only two publications are available to date). All these systems are summarized at Table 1.

Table 1

Overview of the different microbial systems that can be used and/or extended to the recovery of phosphorus (EBPR: enhanced biological phosphorus process with recovery, SIPER: sludge reduction, inorganic solids separation, phosphorus recovery and enhanced nutrient removal, SANI: sulphate reduction autotrophic denitrification and nitrification integrated, OMBR-FO: osmotic membrane bioreactor – forward osmosis, OMBR-RO: osmotic membrane bioreactor – reverse osmosis).

Strategy		Lab-scale	Pilot-scale	Industrial-scale	Specificities	P removal	References
Denitrifying phosphate accumulating communities (DPAOs)	Anaerobic, anoxic/nitrifying to sludges process	✓	✓			99.2%	Shi et al. (2012)
	Modified MUCT process	✓	✓	✓		90%	Zeng et al. (2013)
	Extended anammox process	✓	✓	✓		96%	Ma et al. (2013)
EBPR	SIPER process	✓	✓	✓	Treatment of seawater toilet flushing systems	74.5%	Yan et al. (2015)
	Extended SANI process	✓	✓	✓		NA	Wu et al. (2014)
Membrane technology	OMBR-FO	✓	✓		Production of clean water	>95%	Qiu and Ting (2014)
	OMBR-RO	✓	✓			>95%	Luo et al. (2016)
Granule-based EBPR		✓	✓		Long term stability of granules impaired	93%	Zheng et al. (2013), Zou et al. (2015)
Microbial fuel cells		✓			Allows generation of electrical power	97%	Hirooka and Ichihashi (2013), Tao et al. (2014), Ichihashi and Hirooka (2012)
Microbial ureolysis	Seawater-based urine phosphorus recovery (SUPR)	✓			Only 2 publications available to date	NA	Tang et al. (2015), Dai et al. (2014)

6.2. System and synthetic biology for improving biological P recovery

Most of the biotechnological processes have been significantly improved during the past decade by the advancement of knowledge in system biology, these processes including also environmental applications (Nikel et al., 2014a). This scientific discipline allows for a deeper understanding of the microbial cell and its behavior at a system level, i.e. including metabolome, proteome and transcriptional network. Since it has been shown in the previous sections that the characterization of PAOs still needs further improvements, an alternative for the design of more efficient P recovery biological process could be the use of well characterized microorganisms, such as *Escherichia coli* or

Pseudomonas putida. In this context, the well-characterized microbial chassis *P. putida* KT2440 containing expression vector for polyphosphate kinase has been used in a dedicated process for phosphorus recovery, overcoming the limitations associated with the use of complex microbial communities (Du et al., 2012). Since phosphorus is a central element in microbial metabolism, many other metabolic engineering strategies are also available for enhancing biological P accumulation, e.g. by playing on elements involved in ATP homeostasis (Blank, 2012). The robustness of this approach will be strengthened in the future by the design of artificial metabolic pathways by synthetic biology. Indeed, synthetic biology found many applications, including the engineering of artificial parts for the detection and accumulation of elements by microorganisms in the environment (Nikel et al., 2014b). The use of synthetic part for environmental application will also be made possible, since the design can also incorporate systems for insulating these artificial DNA parts from the surrounding microbial cells (Wright et al., 2013).

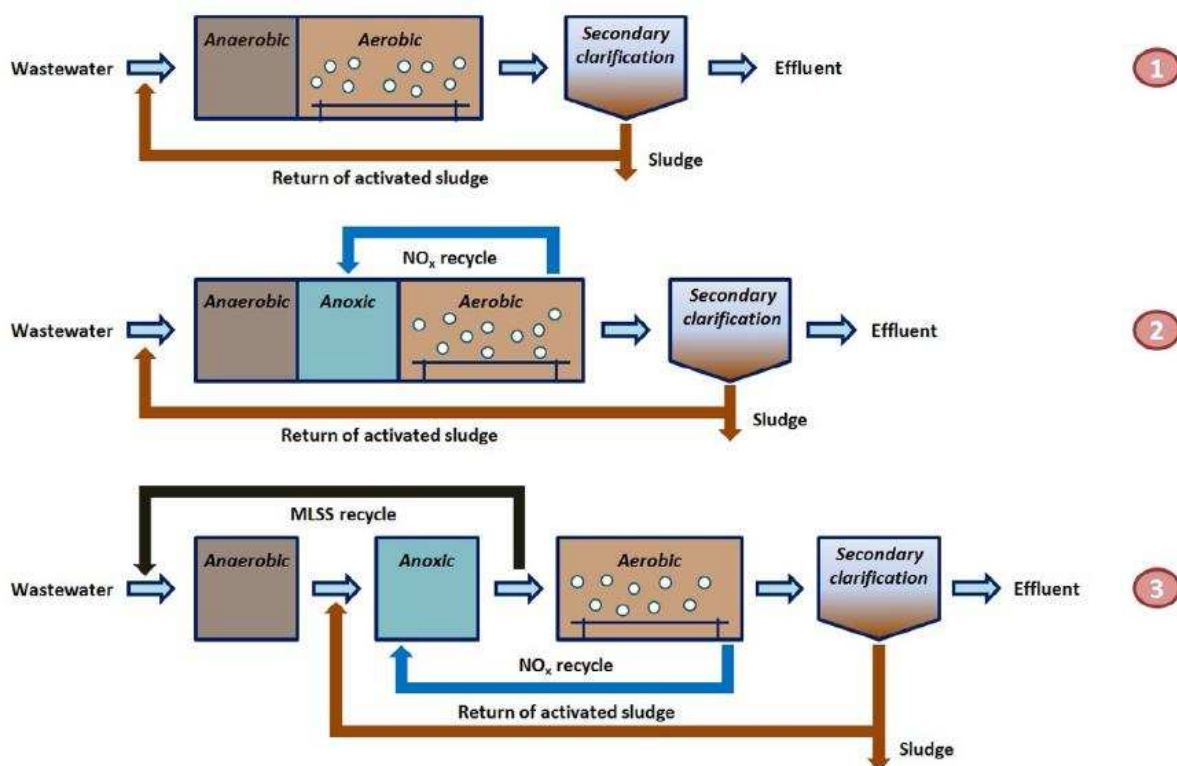


Fig. 3. EBPR processes (adapted from Tchobanoglous et al. (2013)). 1 – basic EBPR process (A/O process); 2 – A²O process; 3 – UCT process.

7. Economic context of recovered phosphorus

7.1. Nutrient use efficiency and potential side-effects of recovered phosphorus

7.1.1. Nutrient use efficiency assessment methods

To assess the fertilizing potential and efficiency of new fertilisers, obtaining insights in their P release over time is of crucial importance (Vaneckhaute et al., 2015). Since there are several methods to measure the efficiency of plant nutrient use, the interpretation of the recovery and efficient use of P applied to the soil is dependent on the definitions adopted and whether crop yield, P uptake or soil data are used in the calculation, (Syers et al., 2008). In general, the performance of a fertiliser can be evaluated by (i) product fractionation, (ii) biological plant response assessment and/or (iii) chemical extractions of soil-fertiliser mixtures (Vaneckhaute et al., 2015). The fractionation of fertiliser products (i) based on the solubility of P uses different solvents with a variety of strength and

electivity (Vaneckhaute et al., 2015) such as mineral acid, citric acid, neutral ammonium citrate and water. Besides the P solubility, the fertiliser efficiency can be expressed as bio-availability indices which can be determined using biological methods (ii) by measuring the plant response (Vaneckhaute et al., 2015).

The most commonly used methods, based on Syers et al. (2008) and Cassman et al. (1998), are: direct method, difference method, balance method, partial factor productivity index and physiological efficiency index. The direct method can only be used in case the fertiliser can be isotopically labeled, e.g. by ^{32}P - or ^{33}P -labeled fertiliser, to measure the nutrient uptake from the fertiliser directly (Achat et al., 2014). By comparing the specific activity (SA, ratio of the radioisotope and the stable isotope) in the plant to the SA of the labeled fertiliser, the amount of P derived from the fertiliser taken up by the plant can be assessed (Nanzer et al., 2014). For complex fertilisers containing multiple P species, an indirect labelling approach, where plant available soil P is labeled, can be used. The indirect labeling method is based on the plant available soil P can be labeled homogeneously and that any dilution of the SA comes from the (unlabeled) P fertiliser (Nanzer et al., 2014). In this case, P derived from the fertiliser is assessed by comparing the SA of a fertilized plant to the SA of an unfertilized plant grown on the same soil (Fardeau et al., 1996). A difficulty using the indirect labeling is the contribution of (unlabeled) seed P to the P uptake by the plant (Nanzer et al., 2014), which can be accounted for using the method described by Pypers et al. (2006). The difference method can be used using yield, $(\text{Y}_N - \text{Y}_O)/\text{FN}$, or nutrient uptake, $(\text{UN} - \text{UO})/\text{FN}$, where Y_N and Y_O are the crop yields with and without addition of nutrients, UN and UO are the nutrient uptake with and without nutrients added and FN the amount of nutrients applied, all expressed in kg ha^{-1} . The method using yield is often referred to the 'agronomic efficiency' of the applied nutrient, while the method using nutrient uptake is considered to be the 'apparent recovery' or 'apparent efficiency' of the applied nutrient (Syers et al., 2008). However, this method is less appropriate for P, since the largest portion of P taken up by the plant comes from soil P reserves (Johnston and Syers, 2009).

Therefore, Syers et al. (2008) and Johnston and Syers (2009) state that the balance method which uses the total amount of the nutrient in the crop and the amount of the nutrient added, UN/FN seems more accurate. This method has the advantage that the recovery of P from soil reserves is accounted for and that there is no need for a blank. Furthermore, the partial factor productivity of the applied nutrient is the ratio of the amount (kg) of product produced and the amount (kg) of nutrient applied, Y_N/FN . Finally, the physiological efficiency on the other hand is calculated as the amount (kg) of product increase over the amount (kg) of nutrient taken up by the plant, $(\text{Y}_N - \text{Y}_O)/(\text{UN} - \text{UO})$ (Syers et al., 2008). Furthermore, the fertiliser efficiency of secondary phosphates can be expressed relative to the efficiency of its mineral counterpart. Cabeza et al. (2011) define the relative fertiliser efficiency (RFE) as the increase in phosphorus uptake ($\text{UN} - \text{UO}$) coming from a specific fertiliser compared to the increase in phosphorus uptake from the standard water soluble P fertiliser triple super phosphate ($\text{UTSP} - \text{UO}$).

Besides time consuming extensive bio-assays, chemical extractions of fertilized soil (iii) give insights in the P bioavailability of the fertiliser. However, the search for suitable extractants has resulted in a large variety of soil tests which differ per country and per region (van Rotterdam-Los et al., 2013). The amount of extracted P of the most common soil P tests decreases in the order $\text{P-total} > \text{P-oxalate} > \text{P-AL}$ (ammonium lactate) $> \text{P-Mehlich3}$ (acetic acid + ammonium nitrate + ammonium fluoride + nitric acid + EDTA) $> \text{P-Bray}$ (ammonium fluoride + HCl) $> \text{P-AAEDTA}$ (ammonium acetate + EDTA), P-CL (calcium lactate), P-CAL (calcium lactate/acetate) $> \text{P-Olsen}$ (sodium bicarbonate) $> \text{P-}$

AAAc (acid ammonium acetate), P-Morgan (sodium acetate) > PH₂O > P-CaCl₂ (Neyroud and Lischer, 2003).

From these results it can be concluded that a single measurement cannot provide information on both P in the soil solution and in the solid phase, which makes these tests of limited use. However, by combining extraction methods, more information on the different P fractions can be obtained (Sanchez-Alcala et al., 2015). Sequential chemical extractions can be used to investigate the transformations of fertiliser P and understand its availability to plants (Nanzer et al., 2014). A frequently used method to identify the different chemical forms of P in the soil is the sequential extraction method developed by Hedley et al. (1982) using 6–7 extractants. Even though this method is widely used over the last decades, it has its limitations since it is quite complex and time consuming (Nanzer et al., 2014). A few years ago, Tiessen and Moir (2007) made a modification to this method, reducing the complexity of the method. Alternatively, van Rotterdam-Los et al. (2013) showed that by using 2 types of solvents, a quite mild extractant such as P-CaCl₂ represents the quantity of P that is directly available for the plant (P intensity), while with harsher extractants such as P-AL, it is possible to find out the total capacity for P replenishment (P quantity).

7.1.2. Nutrient use efficiency of recovered phosphorus

Experiments on the fertiliser potential of recovered P from wastewater showed that struvite products are as efficient as commercially available phosphate fertilisers such as triple super phosphate. From a 2-year pot experiment with maize (*Zea mays* L., cv. Atletico) Cabeza et al. (2011) concluded, based on P uptake measurements, that magnesium ammonium phosphate products from sewage treatment plants and triple superphosphate (TSP) were equally effective in a wide range of soil pH (neutral and acidic soils). The results were based on the relative fertiliser efficiency (RFE), which compares the increase in P uptake caused by a specific fertiliser to the increase in P uptake caused by the standard water soluble P fertiliser TSP, $(UN - UO)/(UTSP - UO)$. Results of the same study showed that calcium phosphate was only effective in acidic soil. The evaluation of pot experiments performed by Johnston and Richards (2004) using perennial ryegrass (*Lolium perenne*) on a sandy loam soil and a sandy clay loam soil with different pH and Olsen P content indicated that the dry matter yield and P uptake using struvite recovered from sewage didn't show significant differences in comparison to synthetic monocalcium phosphate (MCP) and to each other. Recovered calcium phosphate was also tested, but resulted in the lowest P uptake compared to the different struvite products and MCP.

7.1.3. Potential side-effects of recovered phosphorus

The application of sewage sludge to agricultural soil, as is the case in some European countries, can lead to contamination with toxic heavy metals, such as Cd, Cu, Zn, Ni and Pb, and toxic organic compounds and pathogens, imposing health and environmental risks (Contin et al., 2015). Therefore, the application of organic waste is regulated by the European Directive 86/278/EEC to prevent adverse effects on the environment and living organisms. However, Huguier et al. (2015) state that harmonized ecotoxicological strategies to assess these effects still lack within the existing regulatory framework and that the adverse effects are still underrepresented due to the fact that the eco-toxicological assessment of organic waste is not obliged before application on agricultural soils and the existing assessments are mainly based on physicochemical characteristics which is not sufficient to determine potential eco-toxicological effects. For this reason, the authors propose a test strategy using terrestrial bioassays (mainly plant and earthworm bioassays) using a direct approach which seemed to be more sensitive, relevant and discriminant in comparison to aquatic bioassays to estimate the hazard of using organic waste in agriculture.

Clarke and Smith (2011) also highlight that most risk assessments on organic pollutants demonstrate that recycling bio-solids from sewage sludge on agricultural land doesn't pose human health at risk. Though they emphasize that continued attention is required to assess the implication of emerging organic contaminants such as polychlorinated alkanes (PCAs) or perfluorochemicals (PFCs) in sludge to ensure sustainability and security of bio-solids' management.

7.2. Economic feasibility of phosphorus recovery

At this moment, there is no economic incentive to recover P from wastewater since it is still cheaper to use rock phosphate for the production of mineral fertilisers in comparison to recycled P (Molinós-Senante et al., 2011). However, various predictions show that even without total depletion of the current P reserves, it is very likely that the cost will increase significantly due to increasing oil prices which raises the cost for transportation and chemicals, such as ammonia and sulphuric acid, necessary for the production of phosphate fertilisers (von Horn and Sartorius, 2009). Furthermore, an investment in additional processing techniques might be necessary to handle the increasing heavy metals (uranium and cadmium) content caused by a more intense exploitation of the mines (Sartorius et al., 2012). Therefore, it is necessary to start to close the P cycle by phosphate recycling and reuse. However, struvite is not considered yet as a common commercial product, even though its market value is expected to be high (684 EUR ton⁻¹) (Desmidt et al., 2015). It also has to be highlighted that next to the environmental benefits obtained from lower P discharge and the production of a mineral fertiliser substitute respectively, P recovery from domestic wastewater has other additional economic advantages. Results from a full scale pilot installation where struvite is recovered from digested sludge, showed that the dewaterability of the treated sludge was enhanced after P removal and the scaling speed was reduced leading to less operational problems such as pipe clogging and valve freezing (Marchi et al., 2015).

8. Conclusions

Adopting a more sustainable way of life is becoming a top priority. In this context, the building of WWTPs is a considerable advantage in respect of the valorization of P from wastewater. However, the quality of sewage sludge products is altered by various pollutants. Another problem is the conflict between P removal, P recovery and energy generation. A final point is that the microbiology of phosphorus removal is still not well characterized, and additional efforts are needed at this level, notably for detecting and genotyping PAOs. Then, the control of innovative P-removal processes will be much more accessible.

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