Seqestration Options for Phosphorus in Wastewater

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Abstract

Inefficient wastewater treatment introduces huge amount of nutrients mainly phosphorus and nitrogen to the natural waterbodies. Excessive phosphate in the water leads to the growth of algae or eutrophication. One-third of the aquatic ecology has been destroyed by eutrophication worldwide including China, Japan, Europe, South Asia and South Africa. Artificial eutrophication affects the water ecology around the world by decreasing the quality standards of water and alters the ecosystem structure and function. Phosphorus is known to be a limiting factor, and it is crucial to remove the phosphate from the effluent prior to exoneration into waterbodies.

Intracellular phosphate content of certain important species of bacteria influences phosphate removal in wastewater treatment. A variety of polyphosphate-accumulating organisms (PAOs) are involved. Under alternating anaerobic and aerobic conditions, these PAOs store phosphate in the form of polyphosphate. Among PAOs, *Accumulibacter* sp., *Pseudomonas* sp., *Aeromonas hydrophila*, *Tetrasphaera* sp. and gram-positives are the major role players as phosphate removers. As compared to chemical method, biological way of nutrient removal proved to be cost-effective, and it reduces the sludge production. An integrative approach towards phosphoregulation is a key aspect of dealing with the problem.

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6.1 Introduction

Wastewater management, eutrophication and phosphoregulation have an indispensable connection. Water scarcity is the immense problem faced worldwide due to increased growth of population, climate change and inefficient wastewater management. It was reported in the fourth World Water Development Report that only 20% of globally produced wastewater is currently receiving proper treatment (UN report 2012). There is an urgent need to conserve water and reuse the properly treated water for agricultural or other non-portable use.

To meet the needs other than drinking purposes, 1 billion gallons of treated wastewater have been used in the United States. An EPA estimate suggests that almost 91 billion is spent to assert and improve treatment systems all over the nation.

The discharge of untreated wastewater/improperly treated wastewater leads to the major calamity for water sources called "eutrophication" as this wastewater contains a huge amount of nutrients in it. According to UNEP newsletter and technical publication, the untreated wastewater or wastewater treated by conventional mechanical-biological techniques still contains 25–40 mg/l and 6–10 mg/l of nitrogen and phosphorus, respectively [1].

Eutrophication is the enrichment of waterbodies when a huge amount of nutrient-containing wastewater is dumped into it (Yewalkar-Kulkarni et al. 2016). It causes excessive growth of algae leading to the condition called algal bloom, causing the decrease in dissolved oxygen content and death of normal aquatic flora and fauna. Decomposition of these dead matters releases nutrients which amplify the process of eutrophication.

Dodds et al. (2008) showed that combined cost of $2.2 billion (approx.) was spent annually for recreational water usage, waterfront real estate, recovery of lost biodiversity and drinking water as a result of eutrophication in US freshwaters.

To check this deleterious effect, it is essential to control nutrient amount, and prime focus has been given to removal of phosphorus. Wastewater treatment plants or nutrient removal plants take advantage of polyphosphate-accumulating organisms (PAOs) which have the capability of accumulation of polyphosphate by removing phosphate from wastewater. Alternating anaerobic and aerobic condition is required for phosphate removal. Under anaerobic conditions PAOs uptake volatile fatty acid (VFA), e.g. acetate; polyhydroxybutyrate (PHB) is formed from this acetate and further used for cell growth and polyphosphate synthesis under aerobic conditions (Strom 2006). Based on the statistics for wastewater treatment, biological removal seems to be a simple and ecologically balanced way for phosphate removal and to curb eutrophication. Through this chapter we have tried to compile the issues regarding nutrients in wastewater; their ill effects, i.e. eutrophication; how this eutrophication and changing climate is related to each other; and the measures taken to get rid of eutrophication and reduce the nutrient loading into natural waterbodies by WWTPs. In this chapter, we have also illustrated the role of (meta)genomics approach to reveal the bacterial community structure so that the better modelling and designing of the WWTPs could be possible and best quality effluent would be generated to circumvent the harmful effects of effluent and sludge disposal to natural waterbodies. Not only the harmful effects of algal bloom but their use in WWTPs in nutrient sequestration is also discussed.

6.2 Nutrient Issues in Wastewater Management

The combined effluents that come from domestic use, industrial use, urban runoff and agricultural runoff are considered as wastewater. Wastewater management is a process of treatment of wastewater/sewage prior to introducing it to the waterbodies so that the ecology of the water should be maintained. Wastewater contains various pollutants such as:

- Plant nutrients (phosphorus and nitrogen mainly)
- Heavy metals (cadmium, mercury, nickel, lead and zinc)
- Pathogens (bacteria, viruses and other microorganisms)
- Other organic pollutants (UN report 2015)

All these pollutants have detrimental effects on both environment and human health; therefore, it is indispensable to treat the wastewater before disposing it to the natural water sources. If the wastewater management is neglected, then it will lead to two major impacts: one is chemical and nutrient contamination and the other one is microbial pollution.

6.2.1 Phosphate/Nutrient Induced in the Sewage

Wastewater comes from the industries, urban runoff and household; these are the major sources which introduce the nutrients into the sewage (Romero et al. 2013; UN report 2015). Household wastewater mainly consists grey water (kitchen and bathing wastewater) and black water (excreta, urine and faecal sludge) (UN report 2015). SeaWeb in their newsletter reported sewage and septic tanks as a major source of nutrient pollution as many soap and detergents contain phosphorus, whereas human excreta are known to be nitrogen rich [2]. In relation to microbiota, diversity and species richness are the factors to be considered for balancing the nutrient flow in any ecosystem. It has been found in many ecosystems that functional diversity dictates equilibrium in the ecosystem rather than species number (Mulder et al. 2012). This is an important factor as diversity is dictated by environment and nutrient cycling. Environmental sensing of phosphate has important roles in wastewater treatment (phosphate removal). High phosphate causes eutrophication in an aqueous environment. The intracellular phosphate content of certain important species influences phosphate removal in wastewater treatment (Ruiz-Martinez et al. 2015). Thus phosphate homeostasis could in part influence the rate of removal of phosphate in wastewater and reduce eutrophication.

6.2.2 Nutrient Management in Sewage

Various physical and chemical methods like filtration, membrane technologies and precipitation, respectively, are used to remove phosphate, but the biological method (EBPR) is known to remove phosphate in a cost-effective manner. It also reduces the phosphate level to acceptable standards (Strom 2006) and is also more environmentally friendly (Gunther et al. 2009). According to FAO Corporate Document Repository, after treatment of the sewage water by conventional methods, it still has the nutrients in concentration, phosphorus (P), 10 mg/l; potassium (K), 30 mg/l; and nitrogen (N), 50 mg/l [3]. EBPR is known to achieve around <0.1 mg/L effluent P levels (Barnard 2006). Phosphate removal by struvite precipitation was also studied recently (Lu et al. 2016).

6.2.3 Consequences of Untreated/Partially Treated Sewage Disposal to the Different Waterbodies

The biggest drawback of sewage disposal is that it demolishes the aquatic biodiversity by harmful effect of eutrophication. It also causes dissolved oxygen depletion to satisfy the BOD of organic matter present in sewage (Diaz and Rosenberg 2008). The introduction of sewage also leads to other harmful effects like the introduction of pathogenic organisms and production of unpleasant smelling gases, e.g. H_2S (Klein and Perera 2002; Topare et al. 2011). The sewage disposal intensifies the presence of faecal coliforms (Rim-Rukesh and Agbozu 2013); these pathogenic organisms are known to cause waterborne diseases. According to an article in GE step ahead 2015, annually around 1 lakh causalities occur in India due to these waterborne diseases like cholera, jaundice, diarrhoea and typhoid [4]. The partially treated sewage when introduced into fresh waterbodies greatly reduces biological and physicochemical qualities of receiving waterbodies. Rim-Rukesh and Agbozu (2013) studied the impacts of partially treated wastewater on Epie Creek (Nigeria). In their study, they use Malaysian Water Quality Index (WQI) to assay the water quality. They report that Epie Creek is equitably polluted on the basis of their findings: dissolved oxygen (DO) 3.73–5.20 mg/l, chemical oxygen demand (COD) 17.3–53.2 mg/l, biochemical oxygen demand (BOD) 12.4–36.7 mg/l, total faecal coliforms 2,120–20,800 cfu/ml, total phosphorus (TP) 0.73–1.73 mg/l and ammoniacal nitrogen 4.10–5.0 mg/l. With the obvious effect of destroying the waterbody composition, untreated wastewater has long-time consequences including the destruction of waterbody ecosystem by altering the species distribution.

6.3 Eutrophication

The natural ageing process of waterbodies is called eutrophication. In this process of natural ageing, a large, profound, nutrient-poor lake successively turns in to be nutrient rich, and with the course of time, it becomes a pond and then converts to a marsh. The anthropogenic activities have increased the rate of this process, and it became so common that the term eutrophication itself sounds like a terrible condition of waterbodies. In the United States alone, eutrophication accounts for almost one-half of the impaired lake area and 60% of damaged river (Smith 2003). The United Nations Environment Programme (UNEP) in their newsletter and technical publication, volume 3, states that the eutrophic waterbodies are classified into four classes based on their nutrient concentrations (mainly nitrogen and phosphorus): these are oligotrophic, mesotrophic, eutrophic and hypereutrophic containing phosphorus and nitrogen (Table 6.1) [1].

6.3.1 Source of Nutrient to the Waterbodies

Nutrients can be deposited to the aquatic systems by two means either naturally or by human activities (anthropogenic). It will take centuries for a lake to become eutrophic by natural means (Gao 2015), but the anthropogenic activities speed up the process by heavy deposition of nutrients (Chislock et al. 2013; Erisman et al. 2013). Natural rock weathering (Carpenter 2008) and atmospheric depositions (Anderson 2002) are the examples of natural ways, while erosion and leaching from fertilized agricultural areas, development of aquaculture and sewage from cities, urban runoff and industrial wastewater are the main source of anthropogenic activities. Expansion of aquaculture plays a role in eutrophication by discharging the unused animal food and excreta of fish into the water (Klein and Perera 2002). It is quite easy to control the point source of nutrient pollution, whereas the non-point sources are difficult to control.

6.3.2 Consequences of Eutrophication

Ultimately whatever may be the source of nutrient to the waterbodies, the effect will almost always be the same. Availability of the high concentration of nutrients enhances the primary productivity of the waterbodies by increasing the metabolic

Table 6.1 Classification of eutrophic waterbodies based on nitrogen and phosphorus concentration

Sr. No.	Status of waterbody	Average total phosphorus (μg/l)	Average total nitrogen (μg/l)
1.	Oligotrophic	8.0	661
2.	Mesotrophic	26.7	753
3.	Eutrophic	84.4	1875
4.	Hypereutrophic	>200	High

rates which result in the increased production of phytoplankton and *Cyanobacteria* out of which some are poisonous and some are non-poisonous. Apart from nutrient availability, physical factors like temperature, renewal of water and light also play an essential role (Klein and Perera 2002). It was observed that in the Chesapeake Bay during the spring season owning to nutrient-rich environment, phytoplankton biomass increases (Anderson 2002). Blooms of *Cyanobacteria* result into foul-smelling scum and cause problems in drinking water by reducing its taste (Carpenter 2008); it also prevents the penetration of light to the bottom (Lehtiniemi et al. 2005). The major effect of eutrophication is depletion of oxygen and formation of dead zone near the bottom. After the eventual death of algal blooms during the process of decomposition, bacteria consume oxygen, and even some bacteria use sulphates (SO_4^{2-}), and as a result, free S^{2-} take up available dissolved O_2 and make it unavailable for organisms (Klein and Perera 2002); this creates hypoxic condition and results into loss of actual biodiversity by killing normal aquatic flora and fauna. In 2005, 146 coastal marine dead zones had been documented around the world, out of which 43 were in the United States (Dybas 2005). The extensive dead zone was created during summer due to nutrient discharge from the Mississippi and Atchafalaya rivers in the northern Gulf of Mexico (Rabalais et al. 2002). Diaz in 2008 observed that dead zones have been reported to affect more than 245,000 km^2, which accounts for more than 400 marine ecosystems. Smith (2003) mentioned there were 3,164 reported events of poisoning and 148 deaths of humans in the Asia-Pacific region alone. Economic losses may exceed US $1 million per event, and monitoring efforts may cost up to US $50,000 for each affected area. According to NOAA forecast, the prediction of dead zone ranges from 5204 to 6823 mi^2 in the Gulf of Mexico in the year 2016. This forecast is based on the nutrient runoff, river and stream data from the United States Geological Survey (USGS). According to USGS around 20,800 metric tons of phosphorus and 146,000 metric tons of nitrate are received by the Gulf of Mexico from Mississippi and Atchafalaya rivers in May 2016. This makes 25% and 12% above the long term (1980–2015) of nitrate and phosphorus, respectively [5].

6.3.3 Correlation of Eutrophication and Climate Change

Not only by the excessive loading of nutrient by the anthropogenic activities but the climate change also affected the eutrophication. Climate change is mainly the consequence of pollution (especially introduction of greenhouse gases through the burning of fuels) caused by anthropogenic activities (Vijayavenkataraman et al. 2012). Basically, we look at eutrophication as the excessive growth of cyanobacteria (harmful algal blooms). The climate change (altered rainfall patterns, increased storms, melting glaciers and warming soil) increases the signs of eutrophication (Jeppesen et al. 2010; Jeppesen et al. 2011). Rising temperature correlates with eutrophication by various ways like increasing the nutrient loading due to increased mineralizing rate and reducing the water level which causes the concentration of existing nutrients; this is accompanied by low intense storm which causes nutrient loading due to soil erosion (Rustad et al. 2001; Brookshire et al. 2011). Paerl and Paul (2012) studied the anthropogenic and climatic control on a harmful algal bloom. They state that growth of cyanobacteria and the bloom-forming ability are highly influenced by the nutrient enrichment as well as climatic changes like hydrologic changes, global warming and increased frequencies and intensities of tropical storms and droughts. Temperature rise has a positive impact on the cyanobacterial growth (Watkinson et al. 2005). The cyanobacterial photoprotective and photosynthetic pigments absorb light and add on to the increased water temperature (Paerl and Paul 2012).

So far we have discussed the direct effect of climate change on eutrophication and growth of (cyano) harmful algal blooms, but climate change is also influenced indirectly by the consequence of eutrophication, i.e. dead zones. Recently, Altieri and Gedan (2015) reviewed the impact of climate change on dead zone. They also examined various climatic parameters like temperature, precipitation, wind, storm, ocean acidification and sea-level rise and concluded that it can affect the O_2 availability and response to hypoxia. The main reason of dead zone is the appearance of a hypoxic condition in the waterbodies and death of aquatic fauna due to low oxygen availability. The temperature rise plays a key role in promoting the hypoxic condition. Warm water has low capacity to hold oxygen; thus the rise in temperature causes low availability to aquatic animals (Altieri and Gedan 2015). Another effect of the rise in temperature is that it causes stratification of the surface water and prevents mixing of oxygenated water (Cloern 2001). The climate change is also known to affect the time and rate of production of phytoplanktons (Winder and Sommer 2012). Similar kind of observation was made by Alheit et al. (2005); they observed early bloom formation in the Baltic Sea for the warmer period. As compared to phytoplanktons, their grazers are more sensitive towards temperature change; in many examples, it is seen that with the increase in temperature, grazing ability also increases. Climate warming also results in widening of existing dead zones and change in duration by the following ways: sea-level rise, season stretching and hypoxic thermal kill zones (Altieri and Gedan 2015).

Thus, climate change has an impact on eutrophication. It seems subtle if we take into consideration a short time period. But, over the course of time, this pools together to create more impact on enhancing rates of eutrophication.

6.3.4 Members of Algal Bloom

Among the bloom-forming organism, blue-green algae (cyanobacteria) usually override other algal species (Smith 2001; Wang and Lei 2016). Paerl and Paul (2012) reported about the algal bloom-forming toxigenic cyanobacteria, and these are *Anabaena*, *Cylindrospermopsis*, *Microcystis* and *Oscillatoria* (*Planktothrix*), while others are *Phaeocystis* and several dinoflagellates (*Prorocentrum*, *Gymnodinium*, *Dinophysis*) (Klein and Perera 2002). The composition slightly differs for different waterbodies, but the effects remain the same.

6.3.5 Remedy of Eutrophication

In order to mitigate the harmful effects of eutrophication, various approaches suggested by many people are compiled by Chislock et al. (2013). These approaches are:

- Diversion of excess nutrients
- Altering nutrient ratios
- Application of potent algaecides and herbicides

But it seems to be more important to check the primary causative agent of eutrophication, i.e. nutrient loading. To circumvent eutrophication, only the control of reactive nitrogen is not enough. Measures to control phosphorus are essential and must be included in management programmes (Carpenter 2008). In the early 1990s, the deposition of about 50% of total P to Tar-Pamlico watershed of the Albemarle-Pamlico estuarine systems in North California by the largest phosphorus mine reduced to more than 90%, placing the example of point source control (Anderson 2002). This is the first line as the deposition of excessive phosphorus in any form is controlled beforehand. To eradicate phosphate from the point sources, various strategies are applied by the wastewater treatment plants; these are discussed in details in the other section of this chapter.

6.4 Phosphoregulation

So far we have discussed the phosphate content in wastewater, phosphate pollution in the natural waterbodies and its harmful effects. But phosphate itself does not harm directly; it's the excessive growth of algal bloom which is responsible for the detrimental effects on water ecosystem. In this section, we discuss the importance of phosphate in life, phosphate regulation in wastewater and other things.

6.4.1 Phosphate Flow in Ecosystems

Phosphorus is considered as the fifth most essential element for growth. Not only bacteria but plants as well as humans all require phosphorus in their nutrition. It plays a role in various biological processes like synthesis and maintenance of membrane and is involved in cell signalling as a second messenger and component of genetic material and energy metabolism (Santos-Beneit 2015). Bergwitz and Juppnner (2011) have reported that, if not properly regulated, phosphate can cause many diseases in humans like muscle myopathy, tumour-induced osteomalacia, cardiomyopathy, neuropathy and haemolysis. Misregulation of phosphate in the environment causes an environmental problem (Santos-Beneit 2015).

In general, bacteria utilizes the inorganic form of phosphorus (phosphate ion, i.e. Pi) present in its environment. In the case of unavailability of inorganic phosphate,

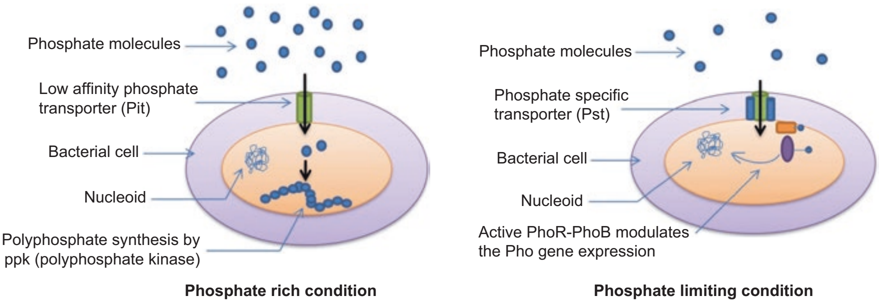

Fig. 6.1 The phosphorus utilization by bacteria and cellular components involved in the utilization

the bacteria use organic compounds containing phosphate like phosphonates and glycerol-3-phosphate. They have two types of transporters for phosphate ion depending upon the availability of Pi; these are Pi-specific transporter (Pst) and low-affinity phosphate transporter (Pit) expressed under low and high availability of Pi, respectively. In the case of organic compounds containing phosphate, related transporters and enzymes are involved in their metabolism. Under the Pi-rich condition, the bacteria take up an excess amount of inorganic phosphate and with the help of enzyme polyphosphate kinase (ppk) forms a long polymer called polyphosphate (poly P) which severs as a source of energy under Pi-starving conditions (Fig. 6.1) (Santos-Beneit 2015).

6.4.2 Phosphorus Sequestration in Natural Waterbodies

We know that natural waterbodies receive phosphorus from both natural and anthropogenic activities (which may include the point source or non-point source) such as agricultural runoff and municipal and industrial sewage effluents (Howarth et al. 2000). As per United Nations Environment Programme (UNEP), the excessive nutrient loading can be prevented by wetlands; this ascertains a natural way to eradicate the problem of eutrophication. The basic concept is that wetland soil adsorbed the phosphorus present in the effluent/polluted water, whereas the nitrate is released as nitrogen in the atmosphere, after its conversion. Díaz et al. (2012) report the effectiveness of constructed wetland to reduce the agricultural runoff pollutants before discharging it in Sacramento-San Joaquin river system (California).

Many studies reported the phosphorus removal by adsorption methods, e.g. on activated aluminium oxide (Genz et al. 2004), oxide tailings (Zeng et al. 2004), zeolite (Karapinar 2009), ferrihydrite (Carabante et al. 2010) and titanium dioxide (Delaney et al. 2011). Zamparas et al. (2012) studied the effectiveness of modified bentonite (Zenith/Fe) in phosphorus sequestration in natural waterbodies and report 80% removal of phosphorus. Earlier used sand, gravels and soil do not remove nutrient with great efficiencies (Park 2009). Recent researches suggest that the industrial wastes and by-products would aid to improve the nitrogen and

Table 6.2 List of agricultural by-products (ABPs) used for phosphorus removal

Sr. No.	Form	Agricultural by-products (biosorbents)	References
1.	Natural	Sawdust of Aleppo pine	Benyoucef and Amrani (2011)
		Palm surface fibre	Ismail (2012)
2.	Modified	Diethylenetriamine – cross-linked cotton stalk and wheat stalk	Xu et al. (2011)
		2-hidroxypropyltrimethyl ammonium chloride modified coconut shell fibre	De Lima et al. (2012)

phosphorus removal if used as the substrate (Ahmad et al. 2016). Ahmad et al. (2016) reviewed the use of water treatment sludge as a substrate in constructed wetlands with improved efficiencies in nutrient removal.

Some materials can form hazardous species; e.g. it has been viewed in many cases that aluminium causes toxicity to living organisms (Haghseresht 2004). The agricultural by-products (ABPs) can be successfully used as biosorbent as these can be a prevalent source because of its low cost, eco-friendliness, and utilization of agricultural wastes (Nguyen (TAH) et al. 2012). The agricultural by-products are either used in its natural form or can be modified in order to increase their effectiveness (Table 6.2). The efficiency of ABPs is also governed by parameters like pH, temperature, adsorbent dosage, interfering ions and contact time (Nguyen (TAH) et al. 2012).

6.4.3 Phosphorus Sequestration Through Designed Bioreactors

Strom (2006) reported that phosphorous can be removed by various methods like filtration of particulate phosphate, membrane technologies, precipitation, crystallization, adsorption, constructed wetlands and enhanced biological phosphorous removal – EBPR. Various reactors and treatment techniques have been designed and practised with the aim of improving the effluent quality (to reduce the nutrient loading into natural water system). In this section, we discuss the bioreactors used in nutrient removal except for EBPR system; because of its huge serving in the nutrient removal, it is discussed separately. Seow et al. (2016) reviewed wastewater treatment technologies and discussed aerobic granulation, biofilm technology and microbial fuel cell techniques and their merits and demerits. All three techniques are used to treat various kinds of wastewater, but aerobic granulation shows effective results in phosphate/nutrient removal. The aerobic granulation technique is known to remove nutrients (nitrogen and phosphorus) from the slaughterhouse wastewater, livestock wastewater and domestic sewage with the values 99.3% and 83.5%, 73% and 70%, and maximum volumetric conversion rates for nitrogen and phosphorus were 0.17 and 0.24 kg/m^3 in respective wastewater (Othman et al. 2013; Liu et al. 2015; Pronk et al. 2015). In the Netherlands under the trade name of Nereda®, a full-scale domestic sewage treatment plant is operated using aerobic granulation technique (Pronk et al. 2015).

Electrocoagulation is a technique used with increasing rate to treat not only the industrial wastewater but also raw domestic sewage as well as tertiary treated to reduce the microbes, nutrients and other personal care pollutants. Energy efficiency and less expensive nature make it popular to use in the wastewater treatment process. Percent mean reduction of phosphorus in both raw and tertiary-treated wastewater sample was found to be 95.79% and 96.33%, respectively (Symonds et al. 2015). Ozyonar and Karagozoglu (2011) perceived 98% phosphorus removal from domestic sewage by using electrocoagulation technique and concluded that aluminium electrode effectively removes phosphorus.

The microalgal culture is used to remove phosphorus because of its ability to assimilate this nutrient for its growth in photobioreactor which employed light source for the cultivation of microalgae. Abdel-Raouf et al. (2012) reviewed the use of microalgae in wastewater treatment. He states different treatment systems, like immobilized cell system, dialysis culture, tubular photobioreactor, stabilization pond and algal mat, and also the use of harvested biomass for energy production. The microalgal membrane technique is highly popular because of its ease in harvesting the biomass. Singh and Thomas (2012) worked on a unique microalgae membrane photoreactor (mMR) with the aim of furnishing the effluent obtained from domestic wastewater treating aerobic membrane bioreactor. They reported good rate of removal of NO_3, NO_2 and PO_4 under both batch and continuous modes of operation. The algal-bacterial biofilm reactor reported to remove phosphorus, nitrogen and carbon from domestic wastewater at 10 days HRT (hydraulic retention time), 85 ± 9%, 70 ± 8% and 91 ± 3%, respectively (Posadas et al. 2013). Sukacova et al. (2015) studied the phosphorus removal by microalgal biofilm under different light conditions and reported 97 ± 1% of phosphorus under nonstop synthetic illumination.

6.4.4 Mechanism of Phosphate Sequestration

Wastewater treatment plants use enhanced biological phosphorus removal (EBPR) process to remove phosphate from sewage. The EBPR process is basically an activated sludge process functioning under anaerobic and aerobic conditions and introduces the influent wastewater in anaerobic phase (Kristiansen et al. 2013); this enriches the polyphosphate-accumulating organisms (PAOs). Under the anaerobic condition where the influent wastewater is mixed, the bacteria are able to uptake the carbon source/volatile fatty acid (e.g. acetate) and convert it into polyhydroxybutyrate (PHB), deriving the energy from stored poly P, and release orthophosphate in medium (Martin et al. 2006; Wong and Beiko 2015). The reducing equivalent required is obtained from either conversion of stored glycogen to PHB and CO_2 or by partial oxidation of acetyl CoA through the TCA cycle (Skennerton et al. 2015). Under aerobic conditions poly P is synthesized by accumulating the phosphate present outside the cell; it also synthesizes glycogen by using the energy from anaerobically stored PHB. Thus the rate of P accumulation is much higher than the rate it is released during the anaerobic phase; this is how the PAOs are able to remove the

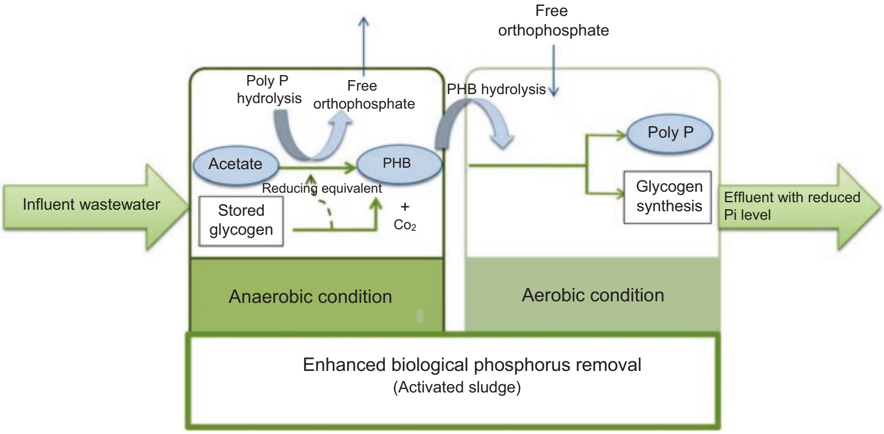

Fig. 6.2 The mechanism involved in EBPR process in wastewater treatment plant

higher amount of phosphate from the wastewater (Lu et al. 2016). This mechanism is diagrammatically represented (Fig. 6.2).

6.4.5 Microbial Community Structure: Its Relation to Phosphorus

Enhanced biological phosphorus removal (EBPR) process is not performed by a single dominant bacterial species, but a mixed population of bacteria is able to utilize carbon source/VFA, store PHB, accumulate poly P, store and maintain glycogen, etc. Some heterotrophic bacteria are known to accumulate polyphosphate and use it as the source of phosphate and energy under phosphate-starving condition (Santos-Beneit 2015). Many researchers have found pure cultures, e.g. members of genera *Acinetobacter*, *Tetrasphaera*, *Microlunatus* and *Lampropedia*, with the traits of PAOs, but they were not found to be significant for wastewater treatment plants (Seviour et al. 2003). In 2009, Gunther et al. developed a dual polyphosphate/DNA fluorescent staining approach which explores the knowledge of noncultivable PAOs. Fluorescent staining determines the number of poly P granules, whereas DNA contents and cell size explain the active PAOs. For isolation and identification of the representative PAOs, cell sorting and terminal restriction fragment length polymorphism (T-RFLP) profiling of 16S rRNA gene were used. The dual staining divides the complex community into subcommunities on the basis of their growth rate and poly P content. 16s rRNA gene sequencing verified the staining technique specificity and generated clone library. This library showed a low diversity composed of phylotypes of *Candidatus Accumulibacter* and members of *Pseudomonas* and *Tetrasphaera* genera. The tetracycline (TC) and 4′-6′-diamidino-2-phenylindole (DAPI) are considered as a reliable method for the detection of PAO activity. In other studies, it was reported that the *Accumulibacter* sp. and *Tetrasphaera* sp. are the abundant poly P accumulators (Oehmen et al. 2007; Nielsen et al. 2010; Nguyen et al. 2011). The *Tetrasphaera* sp. comprises 30–35% of the microbial community, whereas *Accumulibacter* sp. forms only 3–10% (Gu et al. 2008; He et al. 2008; Nielsen et al. 2010; Nguyen et al. 2011). Sidat et al. (1999) carried out a study using a culture-dependent method and isolated 39 monocultures from the sludge procured from Johannesburg full-scale BNR system, out of which 24 isolates showed the phosphorus-accumulating ability. Gram negative forms 58% and gram positive forms 42% of total phosphorus-accumulating population isolated from sludge. Their study showed that gram positive and *Pseudomonas* sp. form the 50% of the population.

For successful operation of EBPR and its further optimization, it is essential to have the knowledge of PAO biodiversity (Blackall et al. 2002). To explore the microbial community dynamics, Ju and Zhang (2015) revealed a statistical method based on the correlation between bacterial community networks and the associated taxonomic affiliations through this predict co-occurrence and co-exclusion patterns of the community. Through this work they studied the 16s rRNA gene sequencing data, and species-species association (SSA) network was constructed which comprised of 3899 pairwise significant SSA correlations which connect 170 species-level OTUs. During their work, they found that apart from the closely related bacteria (taxonomically) which share the similar niche taxonomically, less related species were also observed and posed competition to community assembly.

6.5 GAOs as Competitor of PAOs

Glycogen-accumulating organisms (GAOs) are the potential competitor of PAOs for volatile fatty acids, and they do not accumulate phosphorus (Oehmen et al. 2006; Gu et al. 2008). *Candidatus Competibacter phosphatis* (B12%) and *Defluviicoccus vanus* (9%) were found to be the abundant GAOs in EBPR system (Saunders et al. 2003; Burow et al. 2007). If *Competibacter* sp. is present along with *Accumulibacter* sp. in WWTPs, then it will hamper the EBPR process by not achieving the target level of phosphorus removal (Zhang et al. 2011). Metabolically the GAOs are the same as that of PAOs except they do not accumulate poly P and use it as a source of energy under anaerobic phase (Cydzik-Kwiatkowska and Zielińska 2016).

6.6 Carbon and Phosphorus Metabolism in EBPR

EBPR system places a good example where one can study the correlated carbon and phosphorus metabolism. All PAOs have the same or related genes and their function for phosphate and central carbon metabolism. Here we take *Accumulibacter* as an example organism to discuss the metabolic process involved in EBPR.

The phosphate metabolism of PAO involves the gene for low-affinity phosphate transporters (*pitA*) and phosphate-specific transporters (*pstABC*) and genes responsible for the formation (*ppk1*) and hydrolysis (*ppx*) of polyphosphate. The metabolic pathways are almost similar for PHA production from acetate, glycolysis and the

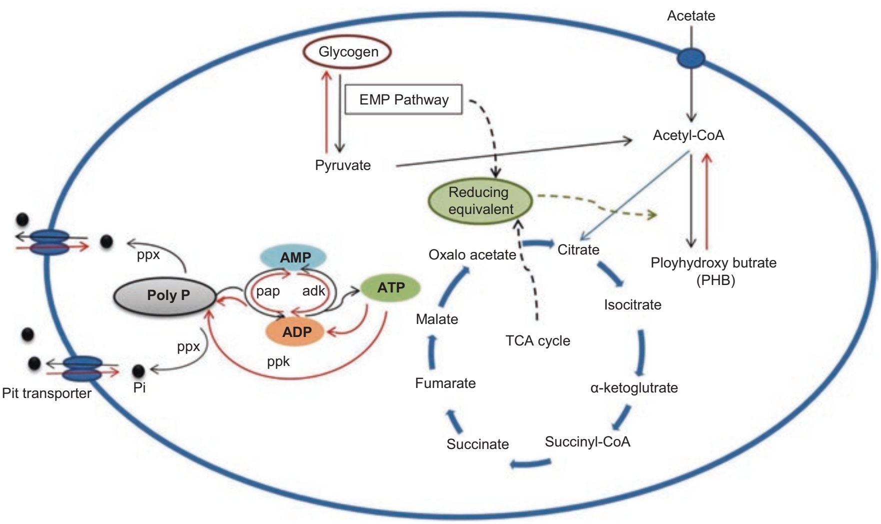

Fig. 6.3 Metabolic event that occur in the *Accumulibacter* EBPR system. Events that occur in anaerobic conditions are depicted with black arrows and aerobic events with red arrows; black dot (●) represents the inorganic phosphate, (⬭) represents the reducing equivalent, (⬬) represents AMP, (⬬) represents ADP and (⬬) represents ATP

TCA cycle (Skennerton et al. 2015). There is a mystery regarding *Accumulibacter* carbon metabolism, either the EMP or ED pathway is followed during anaerobic glycolysis (Oehmen et al. 2007). Recently sequencing of all the *Accumulibacter* genomes showed the presence of the EMP pathway (Hesselmann et al. 2000). The source of reducing the power required during the formation of PHB presented another topic of conflict. Reducing power was either provided by degradation of store glycogen or by anaerobic operation of the citric acid cycle, i.e. TCA cycle (Skennerton et al. 2015). For anaerobic TCA to occur, it is mandatory to reoxidize the reduced quinones; quinol reductase helps in this process which is found in the genome of *Accumulibacter* UW-1 (Martin et al. 2006). A pictorial representation of this metabolic event that occur in *Accumulibacter* is given (Fig. 6.3). The biochemical network of EBPR is thus much more complex than just utilization and removal of phosphate as it has inputs from central energy metabolism. But, studying this in detail will help engineer bacteria through potentially suggesting a carbon source which ultimately assists phosphate removal. Such conditioned consortia would most likely have increased EBPR.

6.7 Metagenomic Approach

Due to the immense contribution of (meta)genomic approaches in exploring the phylogenetic composition and functional prospective of a complex community in the EBPR process, this section is purely devoted to metagenomics and its application in WWT plants. Initially, the metagenomic approaches were used to identify the uncultivable microbes; now these approaches are used to study the dynamics of bacterial communities (Meena et al. 2015; Ambardar et al. 2016; Sharma and Lal 2017). The first metagenomic study was applied to obtain a draught genome of *Accumulibacter phosphatis* UW-1 from lab-scale EBPR reactors (Martin et al. 2006). Phylogenetic studies are not only limited to 16s rRNA gene but also carried out by using *ppk1* gene. Study of these genes categorized the *Accumulibacter* into two clades, i.e. type I and type II, which is again subdivided into IA-IE and IIA-IIF, respectively (Flowers et al. 2013; Skennerton et al. 2015). Deep knowledge was gathered from the independent work of many researchers who use next-generation sequencing methods and expand our knowledge regarding the genetic variety of *Accumulibacter*; their work produces additional draught genomes, and these are as follows: one of *A. phosphatis* UW-2 from clade IA (Flowers et al. 2013), one of *Accumulibacter* sp. strain HKU-1 from Class IB (Mao et al. 2014) and one from clade IA, one from clade IC, three from clade IIC and three from clade IIF (Skennerton et al. 2015). Thus as a number of draught genomes for these bacteria increase, our knowledge and ability to build an effective as well as safe consortia for EPBR increase. It is quite difficult to compile the metagenomic studies; we have tried to gather the information and compile in a tabular form for easy understanding (Table 6.3).

6.8 Algae: Problem as Well as Boon

So far we have seen the algal community as the culprit of the worst situation of the aquatic systems called eutrophication, and we have also discussed its harmful effects. The ability of the algae to show robust growth in the presence of nutrient-rich conditions is only the dark shade of algae. This nature of algae can be exploited by using algae in the nutrient removal systems in WWT plants. This idea has been used in the early 1950s, and now this has been used in many lab scales and pilot-scale plants with more innovative techniques and improved efficiencies. Many researchers have reported the use of microalgal culture in the wastewater treatment with the ability of microalgae in nutrient stripping as well as removal of heavy metals and toxic compounds, and the biomass can be successfully used for other purposes (Kumar and Goyal 2010; Pittman et al. 2011; Abdel-Raouf et al. 2012; Ruiz-Martinez et al. 2012; Yewalkar-Kulkarni et al. 2016). The obtained biomass can be further used for the production of biofuels like biodiesel (Schenk et al. 2008), fertilizers and animal feed and pharmaceutical industry (Singh and Thomas 2012). The use of microalgae in wastewater treatment is popular because of its skill to use solar energy for biomass production which to some extent shorts down the cost of the process and can be grown in the outdoor solar bioreactors (photobioreactor) (Abdel-Raouf et al. 2012). For the enhanced removal of nutrient, either the monoculture or the polyculture is being used. Ruiz-Martinez et al. (2012) in their work used a polyculture with the idea that the strain will get selected and evolve according to the need and condition.

Table 6.3 The metagenomic approaches used for defining the microbial community structure and bacterial abundance

Sr. No.	Sample collection site	Wastewater type	Metagenomic approach	Bacterial abundance	References
1.	13 Danish full-scale wastewater treatment plants		Illumina sequencing of 16s rRNA amplicon (V_4 region)	*Nitrotoga*	Saunders et al. (2016)
2.	Aalborg east wastewater treatment plant, Denmark, (57.044565°N) (10.047598°E)	Domestic wastewater	Illumina sequencing and q-FISH	*Accumulibacter* clade IIA strain UW-1	Albertsen et al. (2012)
3.	30 Danish full-scale wastewater treatment plants		MAR-FISH	*Candidatus halomonas phosphatis*	Nguyen (HTT) et al. (2012)
4.	Municipal wastewater treatment plants, China		PCR-DGGE and FISH	*Pseudomonas* and *Acinetobacter* (in suspended sludge) *Nitrosomonas*, *Nitrospira* and *Nitrobacter* sp.(in biofilm)	Bai et al. (2016)
5.	Municipal wastewater treatment plants, China		PCR-DGGE	*β-Proteobacteria* (*Candidatus Accumulibacter*, *Rhodocyclus* or *Dechloromonas*), *Bacteroidetes* and *γ-Proteobacteria* (*Pseudomonas*, *Alcaligenes* and *Acinetobacter*)	Lv et al. (2014)
6.	9 lab- and full-scale EBPR and 4 non-EBPR systems		16s rRNA sequence analysis and FISH	Novel cohesive clusters with 7 subgroup in *γ-Proteobacteria*	Kong et al. (2002)
7.	8 activated sludge samples Municipal wastewater treatment plants		Illumina HTS-based metagenomics	Proteobacteria followed by *Actinobacteria*, *Chloroflexi*, *Bacteroidetes*	Ju et al. (2014)

(continued)

Table 6.3 (continued)

Sr. No.	Sample collection site	Wastewater type	Metagenomic approach	Bacterial abundance	References
8.	Skagen wastewater treatment plants, Skagen, Denmark	Industrial wastewater (fish)	MAR-FISH	PAOs related to *Rhodocyclus*, 2 morphotypes related to *Tetrasphera*	Kong et al. (2005)
9.	25 Danish full-scale wastewater treatment plants		FISH	*Accumulibacter* clades IA, II A, II C, II D *Tetrasphera* clades I, II, III	Nielsen et al. (2012)

q-FISH quantitative fluorescent in situ hybridization, *MAR-FISH* micro-autoradiography fluorescent in situ hybridization, *PCR-DGGE* polymerase chain reaction denaturing gradient gel electrophoresis, *HTS* high-throughput sequencing

Like bacteria microalgae are also known to store phosphorus in the form of polyphosphate (poly P) and will use it later on under the phosphate starvation conditions (Eixler et al. 2006; Larsdotter 2006; Powell et al. 2008, 2009, 2011; Rao et al. 2011). Apart from this biological way of phosphorus removal, chemical reactions are also known to remove the phosphate in the culture. As the photosynthesis occurs, it will reduce the CO_2 which results in the increase in the pH. This increased pH causes the precipitation of phosphate after complex formation with metal ions (Pires et al. 2013). There are multiple safe ways to remove phosphorus, and it depends upon environmental conditions prevalent in waterbodies and level of eutrophication whether to use a single or a combination of ecologically safe methods.

6.8.1 Microalga Used in WWT

Cai et al. (2013) discussed the efficiency of microalgal species in nutrient removal from various wastewater sources. They account the efficacy of *Chlorella* sp., *C. pyrenoidosa*, *C. sorokiniana*, *C. vulgaris*, *Scenedesmus* sp., *S. obliquus*, *Oscillatoria* sp. and *Arthrospira* sp. in the removal of nitrogen and phosphorus. Likewise various others have reported different microalgae in wastewater treatment, *Cyanobacteria*, *Phormidium* sp. (Abdel-Raouf et al. 2012), *Chlorella* (*Chlorella* sp.), *Scenedesmus dimorphus* (*S. dimorphus*), *Chlorella vulgaris* (*C. vulgaris*), *Scenedesmus quadricauda* (*S. quadricauda*) (Singh and Thomas 2012), *Spirulina platensis* (Lodi et al. 2003), *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* (Bhatnagar et al. 2011). Many people have reported the effective removal of nutrient in wastewater with the aid of microalgae culture (Table 6.4).

Table 6.4 List of microalgae culture used in nutrient removal

Sr. No.	Microalga culture	Efficiency of nutrient removal Nitrogen (%)	Efficiency of nutrient removal Phosphorus (%)	References
1.	*Chlorella vulgaris*	86	78	Pires et al. (2013)
2.	*Microalgae* (order Chlorococcales) and *Cyanobacteria*	67.2	97.8	Ruiz-Martinez et al. (2012)
3.	*Chlorella* sp., *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Scenedesmus dimorphus*	72 and 92	82 and 92	Singh and Thomas (2012)
4.	*Spirulina*	84–96	72–87	Olguin et al. (2003)
5	*Botryococcus braunii*	79.63	100	Sydney et al. (2011)
6.	*Neochloris oleoabundans*	99	100	Wang and Lan (2011)
7.	*Phormidium bohneri*	82	85	Pires et al. (2013)
8.	Microalgal consortium	70 ± 8	85 ± 9	Posadas et al. (2013)
9.	*Algal biomass*	83 ± 25	91 ± 12	Mulbry et al. (2008)

6.8.2 Current Advances in the Phosphorus Sequestration by Algae

Pires et al. (2013) have reviewed the recent improvement in the use of microalgae culture in the wastewater treatment with the three different culturing techniques, i.e. suspended cells, immobilized cells and microalgae consortia (microalgae and bacteria). In suspended cell culture, the microalgae were found to remove average nutrient concentrations, 35.5 mg/l of NH_4^+, 0.40 mg/l of NO_3^- and 3.89 mg/l of PO_4^{3-}, and it was observed that initial higher cell density gives better efficiencies of removal (Abdel-Raouf et al. 2012). For the immobilization of cells, alginate and carrageenan polymers were used (Pires et al. 2013), *Chlorella vulgaris* (Tam and Wong 2000), *Dunaliella salina* (Pires et al. 2013), *Scenedesmus obliquus* (Ruiz-Marin et al. 2010), *Scenedesmus rubescens* (Shi et al. 2007) and *Scenedesmus* sp. (Fierro et al. 2008; Zhang et al. 2008; He and Xue 2010). Various studies have been carried out using the microalgae and bacteria consortia. This proves to be an economical way to remove nutrients as microalgae provide O_2 and bacteria provide CO_2 by its action of degradation of organic compounds (Munoz and Guieysse 2006; Park et al. 2008; Subashchandrabose et al. 2011). De-bashan and Bashan (2004) reported 100% ammonium, 15% nitrate and 36% phosphorus removal from municipal wastewater by immobilizing the microalgae-bacterium consortium (*Chlorella vulgaris/Azospirillum brasilense* and *Chlorella sorokiniana/Azospirillum brasilense*) in alginate beads.

The expensive immobilizing material and its fragility during long-term operation use lead to the innovative idea of using biofilm photobioreactor (Guzzon et al. 2008; Boelee et al. 2011; Zamalloa et al. 2013). Posadas et al. (2013) studied the carbon, nitrogen and phosphorus removal with the efficiencies 91 ± 3%, 70 ± 8% and 85 ±

9%, respectively. A study of phosphorus removal under different light regime was carried out by using microalgal biofilm (biofilm photobioreactor), and it was reported that under continuous artificial illumination, this system can remove 97 ± 1% of total phosphorus from wastewater, while only 36–41% is removed when illuminated for 12 h. The species found to be present in biofilm were *Phormidium autumnale*, *Pseudanabaena* sp., *Chroococcus* sp., coccal green alga, *Monoraphidium contortum*, Diatoms, *Scenedesmus acutus* and *Cymbella minuta*; they were subject to seasonal variation in abundance, but some remains constant throughout the same like *Pseudanabaena* sp. remains always dominant and *Cymbella minuta* remains always rear (Sukacova et al. 2015).

Perspective

Huge amount of money is spent by the government for the construction and upgradation of wastewater treatment plants, but it did not achieve the appropriate goal of making wastewater fit for discharge. Phosphorus (nutrient pollution) in the waterbodies causes eutrophication which alters the water ecology. Wastewater treatment plants exploit the ability of PAOs for the phosphorus removal from wastewater. This proves to be effective, but still there is a scope of research for making these bacteria more efficient and achieve above the discharge limit. Metagenomics and genomic techniques revealed a lot of information about the community dynamics and give the insight about the molecular level which gives the idea of metabolic fluxes. For better understanding of this process, system biology approaches must be applied along with other fruitful strategies like metatranscriptomics, meta-metabolomics or community metabolomics, proteomics, etc.

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