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Review article

Parameters affecting biological phosphate removal from wastewaters

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Abstract

This paper reviews some of the key wastewater composition parameters, which influence the biological removal of phosphate from wastewaters, such as COD content, volatile fatty acid (VFA) content, cation concentration, phosphorus load, pH and food to microorganism ratio. The discussion also focuses on operational parameters affecting successful nutrient removal in wastewater treatment plants, such as temperature, sludge quality, sludge settlement, dissolved oxygen (DO) concentration, anaerobic P-release and secondary P-release. The aim of this review is to compile an updated document for researchers and operators of biological nutrient removal (BNR) systems. In addition, the article will provide a good foundation for readers with no prior knowledge of the process.

Keywords: Phosphate; Wastewater; Volatile fatty acid

1. Introduction

Limited water resources and increasing urbanisation require a more advanced technology to preserve water quality (Lee et al., 1996). One of the important factors affecting water quality is the enrichment of nutrients in water bodies (Romanski et al., 1997). Discharging wastewater with high levels of phosphorus (P) and nitrogen (N) can result in eutrophication of receiving waters, particularly lakes and slow moving rivers (Sundblad et al., 1994; Danalewich et al., 1998). Biological processes are a cost-effective and environmentally sound alternative to the chemical treatment of wastewater (Osee Muyima et al., 1997).

Currently, the majority of treatment plants incorporating P-removal utilise chemical precipitation using alum or lime. Biological removal systems are, however, increasingly being incorporated in sewage treatment works (Stratful et al., 1999). Biological phosphorus removal (BPR) from wastewaters is based on the enrichment of activated sludge with phosphate accumulating organisms (PAOs) (Brdjanovic et al., 1998; Wagner and Loy, 2002). BPR exploits the potential for microorganisms to accumulate phosphate (as intracellular polyphosphate) in excess of their normal metabolic requirements (Brdjanovic et al., 1998; Mino et al., 1998). The BPR process is primarily characterised by circulation of activated sludge through anaerobic and aerobic phases, coupled with the introduction of influent wastewater into the anaerobic phase (Wagner and Loy, 2002). In the anaerobic phase, sufficient readily degradable carbon sources, such as volatile fatty acids (VFAs), must be available, which induce the phosphate-removing bacteria to take up the acids and release phosphate into solution (Morse et al., 1998). In the aerobic phase, luxury P-uptake occurs, which results in overall phosphorus removal rates of as much as 80–90% (Morse et al., 1998). A high P-removal efficiency can be achieved by wasting excess sludge with high P-content (Mino et al., 1998). Incorporation of an anoxic phase permits the combined removal of nitrogen and phosphate from the process wastewaters (Metcalf and Eddy, 1991; Crites and Tchobanoglous, 1998). The microbiology and biochemistry of the BPR process has been extensively reviewed by Mino et al. (1998). In addition, the discovery of denitrifying P-accumulating organisms (DPAO) has been extensively reported and discussed (Kerrn-Jespersen and Henze, 1993; Rensink et al., 1997, Meinhold et al., 1999; Hu et al., 2002). Ekama and Wentzel (1999a) concluded that given the appropriate conditions, different species of PAO, which accomplish anoxic P uptake

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will find a niche in the systems but have a significantly low BPR performance and use influent readily biodegradable COD less "efficiently" compared with the aerobic P uptake PAO.

Having outlined the key biological processes underpinning phosphate removal, the key wastewater composition parameters and operational parameters affecting successful phosphate removal in wastewater treatment plants (WWTPs) will now be discussed.

1.1. Wastewater composition

For successful BPR, influent wastewater flows should be kept as stable as possible and minimal fluctuations with sudden drastic changes to the system being avoided. Increased loading rates should be applied by small increments over an extended period of time (Shehab et al., 1996). The BPR process is sensitive to disturbances, such as dilution of the wastewater, e.g. in times of heavy rainfall (Brdjanovic et al., 1998), with prolonged disturbances leading to recovery times of over 4 weeks (Okada et al., 1992). After periods of low organic carbon loads, the effluent phosphate is significantly increased on the following 1–2 days (Carucci et al., 1999a). This is of both quantitative and qualitative relevance, because the average phosphate load in the effluent is increased by about 60% due to this effect. Changes in the influent organic composition from VFAs to sugars, such as glucose, may induce accumulation of the glycogen accumulating organisms (GAOs) (Satoh et al., 1994).

Determination of the optimal COD loading rate is essential, as excessive COD loading rates can lead to deterioration of the BPR process. This was demonstrated by Morgenroth and Wilderer (1998) using a biofilm system where increasing the influent acetate concentration to 400 mg/l led to efficient anaerobic P-release (greater than 100 mg P/l) followed by improved P-removal. However, further increase of the influent acetate concentration to above 600 mg/l led to cessation of anaerobic P-release and deterioration of the P-removal capability. High influent acetate concentrations have also been shown to have a negative effect on BPR (Randall and Chapin, 1997). It has been reported that sludges with low COD-SS loading rates display a high P-uptake potential (Chuang et al., 1998), while sludges operating on a high COD-SS loading rate display lower P-uptake potential. At higher COD-SS loading rates, sludges appear to convert the influent organic matter to the storage product 3-hydroxyvalerate, which is the major storage form utilised by the GAO bacteria, resulting in a deterioration of BPR (Liu et al., 1996). A reduction in BPR can also occur with high influent COD/P ratios. If the COD is not totally sequestered in the anaerobic zone, residual substrate remains to support the growth of filamentous bacteria in the oxic zone (Chang et al., 1996). Furumai et al. (1999) demonstrated that, after attaining high BPR activities in a sludge, a decrease in the organic loading caused deterioration of BPR, with a simultaneous increase in effluent nitrate concentration. A subsequent increase in the organic loading rate restored the original effluent conditions.

The ratio of influent acetate/BOD/COD to P also exerts an influence on the BPR removal capability, with the general consensus being that efficient BPR, with effluent P levels of <1.0 mg/l require that the influent to the anaerobic zone of the BPR system should have a BOD_5: total P ratio of >20:1 or COD:P ratio >40:1 (Randall et al., 1992). Whenever the COD/P ratio drops below 50, BPR alone is inadequate to attain the desired level of P in the effluent (Pitman, 1991). It has also been shown that supplementation by each additional 7.5 mg acetate/l of influent contributed to an extra removal of 1.0 mg P/l (Manoharan, 1988).

1.2. Volatile fatty acids

Since volatile fatty acids are essential for effective BPR (Pitman, 1999; Ruel et al., 2002) process optimisation factors for phosphorus removal includes maximising VFA production in the equalisation tank of treatment systems (Comeau et al., 1996). Furthermore, Barnard (1993) reported that 7–9 mg of VFA are needed to remove 1 mg of phosphorus, while Oldham et al. (1994) have used VFAs to produce effluent phosphorus levels as low as 0.2–0.3 mg/l. VFAs can also be produced on-site with low operational costs and no storage or handling problems, making them an attractive choice as a nutrient removal carbon source (Maharaj and Elefsiniotis, 2001). Apart from VFAs, a wide range of organic compounds, including carboxylic acids, sugars and amino acids, have also been reported to be utilised anaerobically by PAO enriched sludges (Satoh et al., 1996). Carucci et al. (1999b) showed that BPR could be obtained with substrates other than VFA, such as glucose and peptone. These authors reported that anaerobic uptake of glucose was possible with and without BPR. These results are not consistent with the reported behaviour of PAOs and GAOs, indicating that different mechanisms of anaerobic organic compound uptake and storage can act either in favour or against BPR.

The effects of different influent carbon sources on BPR activity has been studied with maximum rates of anaerobic phosphate release being achieved with acetate and propionate, with decreased rates being observed with lactate, succinate, malate and pyruvate (Satoh et al., 1996). The concentration of PHA in the sludge was also found to increase with acetate and propionate as added electron donors, but to decrease gradually when the other acid substrates were supplemented. However, it should be noted that Canizares et al. (1999) reported that the presence of acetate in wastewaters can result in sludge settling problems.

In batch tests involving pre-fermentation of glucose, it has been reported that addition of VFA of two to five carbon chain length, including succinate but with the exception of propionate, resulted in greater P-removal efficiency (Randall et al., 1997b; Hood and Randall, 2001). Branched VFA have been shown to promote better P-removal than their linear counterparts with isovaleric acid promoting more consistent P-removal at lower influent molar concentrations, than other VFA. Studies by Rustrian et al. (1996) showed that acetate and butyrate were equally good carbon sources for P-removal, whereas propionate was the least efficient VFA studied. Glucose and amino acid rich synthetic wastewater are extremely detrimental to P-removal, with fructose or starch supplementation also being detrimental, but to a lesser extent than that observed with glucose. Randall and Khouri (1998) confirmed that, acetic and isovaleric acids not only enhanced removals during short-term experiments but also resulted in good BPR in long-term cultivation.

One of the major factors influencing the occurrence of DPAO and associated anoxic P uptake appears to be the nitrate load into the anoxic reactor, i.e., the nitrate load should be large enough or exceed the denitrification potential of ordinary heterotrophic organisms (OHO), i.e., non-PAO organisms in the anoxic reactor to stimulate DPAO in the system (Hu et al., 2002). In terms of this competition, if the nitrate load into the main anoxic reactor is less than the denitrification potential of OHO, then the OHO will outcompete PAO for use of the limited nitrate, while if the nitrate load in the main anoxic reactor exceeds the denitrification potential of OHO, then the PAO will use the "excess" nitrate and thus develop in the system (Hu et al., 2002).

1.3. Cations

The cation concentration and composition of the influent wastewater plays an important role in maintaining the stability of the enhanced BPR process and in the binding mechanisms of phosphorus in the activated sludge (Schönborn et al., 2001). The co-transport of both cations, magnesium and potassium, with P in the enhanced BPR process, is important in the intracellular stabilisation of poly-P (Rickard and McClintock, 1992). In batch experiments performed by Pattarkine and Randall (1999), phosphorus uptake by the sludge was affected by the availability of potassium, magnesium and calcium. Both potassium and magnesium were simultaneously required and neither was adequate by itself for EBPR. The artificial enhancement of the Mg-concentration in the influent from 15 to 24 and 31 mg/l, respectively, has been reported to result in an increase in the mean P-removal efficiency from 85% to 97% (Schönborn et al., 2001). In any poly-P chain, one positive charge is required to stabilise each phosphate group and the expulsion of each phosphate molecule from the cell needs one cationic charge from either K^+ or Mg^{2+} (Romanski et al., 1997). Jonsson et al. (1996) observed that, for each mole of P released in an anaerobic reactor, 0.27–0.36 mol potassium and 0.29–0.32 mol magnesium were simultaneously released from the sludge. Calcium, however, does not appear to be required for BPR and does not seem to be involved in biologically mediated chemical precipitation (Pattarkine and Randall, 1999).

It can been assumed that municipal wastewater contains an excess of K^+ and Mg^{2+} and that ion limitation of the enhanced BPR process is unlikely to occur (Randall et al., 1992). However, full-scale sewage treatment plants designed for BPR may periodically experience short- or long-term shortage of potassium in their influent. When these conditions of severe shortage of potassium in the influent were simulated experimentally it was found that (a) P-removal was absent; (b) polyphosphate concentration in the biomass decreased; (c) anaerobic P-release and related acetate uptake became negatively affected after several days of potassium absence. In contrast, the system achieved complete P-removal when potassium was present in excess amounts (Brdjanovic et al., 1996). Potassium also appears to strongly influence the settling, dewatering and effluent properties of activated sludge systems. The concentration of potassium required is not excessive with concentrations approaching the nutrient requirement of approximately 1% of cellular biomass being sufficient. An excess of potassium has also been found to be detrimental to the activated sludge process and has been associated with poor dewatering properties and effluent quality (Murthy and Novak, 1998).

1.4. Temperature

The temperature dependence of biological reaction rate constants is very important when assessing the overall efficiency of a biological treatment process. Temperature not only influences the metabolic activities of the microbial population, but also has a profound effect on such factors as gas-transfer rates and the settling characteristics of the biological solids (Metcalf and Eddy, 1991, Crites and Tchobanoglous, 1998). Temperatures below the optimum typically have a more significant effect on growth rate than temperatures above the optimum. It has been observed that growth rates double with approximately every 10 °C increase in temperature until the optimum temperature is reached (Metcalf and Eddy, 1991).

There are conflicting reports concerning the effects of temperature on BPR (Brdjanovic et al., 1997). For example, BPR efficiency has been reported to improve at higher temperatures (20–37 °C) (McClintock et al., 1993; Converti et al., 1995), while in contrast comparatively better P-removal efficiency has been observed at lower temperatures (5–15 °C) (Viconneau et al., 1985; Florentz et al., 1987). In studies performed by Panswad et al. (2003), the PAOs were found to be lower-range mesophiles or perhaps psychrophiles and predominated only at 20 °C or possibly lower. The GAO were somewhat mid-range mesophilic organisms with optimum temperature between 25.0 and 32.5 °C.

Helmer and Kunst (1998) have noted that, in the northern hemisphere, air temperatures are commonly below 0 °C during the winter months and these lower wastewater temperatures can affect plant efficiency. It has been reported that disturbances, such as rapid decreases in temperature or hydraulic shock loadings, may cause serious upset in plant BPR performance, with prolonged disturbances (such as for 10 days) resulting in a deterioration of BPR system performance for more than 4 weeks (Okada et al., 1992). Brdjanovic et al. (1997) observed that temperature has a large impact on the oxygen consumption rate in BPR systems. At 5 and 10 °C, incomplete P-uptake was observed in the aerobic phase. At 20 and 30 °C, complete P-uptake was observed. Temperature has also been shown to have a marked effect on nitrification. Krishna and van Loosdrecht (1999) reported that the accumulation of storage polymers is strongly dependent on temperature, with less PHB formation at higher temperatures. In the same paper, it was shown that, as the temperature increased from 15 to 35 °C, the rates of substrate uptake, ammonium consumption, oxygen uptake, CO_2 production and PHA formation all increased.

Converti et al. (1995) have reported that specific P-release is clearly reduced when temperatures are lowered, with sludge activity being strongly influenced by quick temperature changes, such as those that may occur in open wastewater treatment plants where no temperature control is provided. These authors reported that the greater the temperature fall the stronger the stress effect exerted with the time necessary to achieve a constant value for overall P-removal yield (>60%) increasing as the temperature decreased. Conversely, Marklund and Morling (1994) have reported effluent P concentrations of less than 1.0 mg/l at water temperatures as low as 5 °C using a BPR process, with a sharp increase in effluent P to >2.0 mg/l being observed at 4 °C.

It is clear from these investigations that P-removal can be negatively affected by low temperatures, particularly in a combined BNR system, since low temperatures can lead to higher nitrate concentrations in the return sludge, thereby impacting on BPR. It has been reported that 90% nitrification is possible at temperatures as low as 8 °C. However, denitrification becomes the limiting factor in the overall nutrient removal process leading to elevated levels of nitrate in the effluent (Choi et al., 1998). Many authors have also reported reduced nitrification at low temperatures (Helmer and Kunst, 1998; Rothman, 1998; Wagner et al., 1998; Louzeiro et al., 2002; Obaja et al., 2003).

One of the reasons that low temperatures exert an adverse effect on BNR systems relates to the low temperature selection for bacteria such as *Microthrix parvicella* that cause sludge bulking with optimum growth of this organism in activated sludge being induced at temperatures of $\leq$ 12–15 °C (Knoop and Kunst, 1998). Increases in temperature have been shown to reduce *M. parvicella* viable counts in a BNR system (Mamais et al., 1998). The seasonal occurrence of *M. parvicella* is well documented with a maximum in winter/early spring and a minimum during summer (Eikelboom et al., 1998; Rothman, 1998). Sludge settleability in systems with a low solids retention time (SRT) is significantly influenced by temperature, with an increased sludge volume index (SVI) being recorded at higher temperatures (Krishna and van Loosdrecht, 1999).

1.5. Sludge quality and settlement

Settling, or sedimentation, is the separation from water, by gravitational means, of suspended particles that are heavier than water (Metcalf and Eddy, 1991). A good settling sludge is necessary in treatment systems as it directly impacts on the effluent characteristics from the solids separation unit and, consequently, the capability of a WWTP to meet effluent standards (Scruggs and Randall, 1998). Wastewater cation concentrations have been shown to influence floc properties with changes in the cation concentrations such as the addition of calcium or magnesium to influent wastewater resulting in improvements in sludge volume index. The addition sodium, however, has been shown to impair sludge settleability (Sobeck and Higgins, 2002).

Filamentous bulking is a phenomenon common to activated sludge treatment facilities. It occurs when filamentous organisms proliferate to such an extent as to interfere with the proper compaction of settling sludge (Wagner and Loy, 2002). In general, the sludge settleability index declines with the introduction of nutrient removal, but among nutrient removal plants the best settling characteristics are found in plants with biological P-removal and the poorest in plants that perform simultaneous denitrification (Andreasen and Sigvardsen, 1996). Greater than 50% of activated sludge treatment plants in many different countries have suffered from bulking caused by filament production (Kristensen et al., 1994). Blackbeard et al. (1988) reported that 80% of BNR plants in South Africa had bulking sludge, while in Denmark, it was concluded that due to the implementation of P-removal processes, the percentage of plants with high sludge volume indices (SVI), indicative of settlement problems, had doubled (Eikelboom et al., 1998). In a study by Chang et al. (1996), high P-removal in a system treating a wastewater with a high COD/P ratio did not ensure a lower SVI value, with values fluctuating between 69 and 370 ml/g (high indices are indicative of sludge bulking) but at least 75% P-removal was achieved at all times. Despite the disruption created by the variety of filamentous microorganisms frequently implicated in activated sludge bulking problems around the world, relatively little is known concerning the factors affecting the growth of some of these filaments (Scruggs and Randall, 1998). However, it is widely accepted that *M. parvicella* is one of the most important filamentous species in nutrient removal plants (Eikelboom et al., 1998; Rothman, 1998, Wagner and Loy, 2002).

Settling characteristics vary throughout the year as outlined in the previous section, with organisms such as *M. parvicella*, predominating during the colder winter/early spring temperatures (Eikelboom et al., 1998; Rothman, 1998). However, Krishna and van Loosdrecht

(1999) reported that the SVI of a sequencing batch reactor sludge increased as the temperature increased from 15 to 35 °C.

Temperature is not the only parameter that selects for bulking sludge development. Other factors are involved such as wastewater characteristics including fluctuations in flow and strength, pH, influent staleness, nutrient content and the nature of the influent waste compounds (Metcalf and Eddy, 1991). Low food/microorganism (F/M) ratios always represent a critical condition for bulking, as does a system operating under alternating aeration conditions with the presence of oxidized nitrogen at the transition from anoxic to aerobic (Rossetti et al., 1994; Ekama and Wentzel, 1999b).

Recommended control strategies for inhibiting growth of filamentous organisms include: (1) addition of chlorine or hydrogen peroxide to the return activated sludge; (2) alteration of dissolved oxygen (DO) levels in the aeration tank; (3) increase the F/M ratio in the aeration tank; (4) addition of N and P; (5) addition of trace nutrients and growth factors; and (6) use of selectors (Crites and Tchobanoglous, 1998). A selector is a small tank (20–60 m contact time), or a series of tanks (typically three), in which the incoming wastewater is mixed with the return sludge under aerobic, anoxic or anaerobic conditions (Crites and Tchobanoglous, 1998). Chlorination of bulking sludge has been shown to only damage the filaments extending beyond the sludge flocs but not to kill the bulking organisms (Van der Waarde et al., 1998). The employment of selector systems, in which the substrate concentration and the metabolic pathways can be manipulated, has been successfully used to reduce bulking problems in conventional activated sludge systems without nutrient removal. However, initial experiences with nutrient removal activated sludge plants has shown that these plants have more bulking problems than conventional activated sludge plants, and the positive effect of selectors seems less pronounced (Eikelboom et al., 1998). It has been suggested by Scruggs and Randall (1998) that conditions in the selector environment (i.e., low DO/anaerobic conditions) may be a secondary growth requirement for some of the low F/M filaments, thereby explaining the poor success rate of selector methods. In selectors, premixing of influent and recycled sludge with contact times in the range of minutes has been shown to not only lead to decreases in the population of *M. parvicella* but also to lead to increase in the filament Type 0041 population (Eikelboom et al., 1998). Filamentous Type 0041, along with Type 0675 and *Nostocoida limicola*, have been detected in high abundance in plants suffering from bulking conditions (Lee et al., 1996; Rothman, 1998).

1.6. DO requirement

A combined BNR process has to satisfy many different oxygen demands from the bacterial populations present in the system. Activated sludge systems designed for carbon oxidation and nitrification typically require DO levels greater than 2 mg/l (Louzeiro et al., 2002). In BPR systems, the anaerobic zone must be kept devoid of oxygen (0.0–0.2 mg/l oxygen) as the presence of oxidising substances such as oxygen and nitrate will interfere with the BPR process (Shehab et al., 1996).

Maintaining an oxygen concentration of between 3.0 and 4.0 mg/l in the oxic zone has been recommended (Shehab et al., 1996). It has also been reported that, for successful BPR, a DO concentration of 2.0 mg/l is required, but when nitrification is also necessary a DO of 3.0–4.0 mg/l is essential. Since DO concentrations of >4 mg/l do not appear to further stimulate BNR, the maintenance of oxygen concentrations above this level represents a waste of energy for aeration purposes. A study by Brdjanovic et al. (1998) revealed that excessive aeration negatively affects the BPR process as cessation of P-uptake occurs due to depletion of poly-hydroxy-butyrate (PHB) in an over-aerated process. The DO concentration is a key factor affecting the growth of filaments in activated sludge systems (Scruggs and Randall, 1998). These authors showed that growth of some filamentous species, such as Type 1863, required low levels of DO (0.1 mg O_2/l or less). For other species, such as *Nocardia*, a near linear relationship between growth and DO concentration was observed as the DO concentration was increased from 1 to 5 mg O_2/l (Scruggs and Randall, 1998). Anammox-bacteria are reversibly inhibited by low (0.5% air saturation) concentrations of oxygen; therefore, this process must occur under oxygen limiting conditions, i.e., the aerobic ammonia oxidizers will have to remove virtually all of the oxygen from the liquid (Olav Sliekers et al., 2002).

1.7. Anaerobic phosphate release

According to current models of BPR, the uptake of phosphate in the aerobic zone is directly related to the quantity of phosphate released in the anaerobic zone at temperatures between 15 and 20 °C (Helmer and Kunst, 1998). Periods with low COD inlet load lead to a complete cessation of the anaerobic phosphate release and to a subsequent decreased capacity for phosphate uptake (Carucci et al., 1999a). At a temperature of 10 °C, aerobic phosphate uptake was increasingly independent of phosphate release in the anaerobic zone. At 5 °C, absolutely no correlation between anaerobic P-release and aerobic P-uptake was observed (Helmer and Kunst, 1998). These authors also reported a reduction in the rate of P-release as the operating temperature was lowered from 20 to 5 °C. Boswell et al. (1999) also showed that P-release increased with increasing temperature between 4 and 37 °C.

The presence of nitrate in the anaerobic zone has been reported to affect the BPR process (Shehab et al., 1996; Boswell et al., 1999). Residual nitrate in the anaerobic phase results in consumption of influent organic compounds by denitrifiers, thus decreasing the availability of organic matter for PAO. This reduction in PAO activity is evidenced by reduced anaerobic P-release rates (Furumai et al., 1999). Due to the recycle of approximately 25 mg NO_3-N to a 3.6-l working volume SBR, P-release in the anaerobic phase was reduced by 30% (Kuba et al., 1996).

The redox potential is also a key factor determining the rate of anaerobic P-release. In general, the lower the redox potential, the more phosphate that is released in the anaerobic phase (Rensink et al., 1997). The presence of even low levels of oxygen or other oxidising agents affects the redox potential and thus negatively impacts on the rate of phosphate release.

Intracellular P can be stored as "short chain poly-P" (SCP), "long chain poly-P" (LCP) or granular poly-P (GP), and it has been shown that movement of P between the surrounding medium and the biomass is consistent with an exchange of P between some of these pools (Lockwood et al., 1990; Lindrea et al., 1994). The bulk of this mobile P is generally held in the LCP fraction (Lindrea et al., 1994), which is the preferable storage fraction due to the stability it gives the BPR process. A redistribution can occur in the dominant storage fraction from LCP to SCP under conditions of higher nitrate return to the anaerobic phase and this is usually accompanied by an instability or loss of the P-removal process (Lindrea et al., 1998). P stored in the SCP form appears to be released at a faster rate than from LCP. LCP storage permits anaerobic release of only a portion of the mobile P fraction. It is believed that the greater stability of BPR processes associated with LCP storage is attributable to this slower and less variable rate of P-release and subsequent uptake (Lindrea et al., 1998).

The prevailing pH of the anaerobic phase also affects the rate of P-release. Bond et al. (1998) found that the rate of P-removal attained in an SBR reactor was improved when pH control was not practiced during the anaerobic phase. This was associated with an increase in the pH of the anaerobic stage. Under controlled pH conditions, Smolders et al. (1994) found that the ratio of P-release/acetate uptake, increased from 0.24–0.73 mg P/mg COD as the anaerobic pH was increased from pH 5.5–8.5. The anaerobic P-release rate has been shown to increase continuously as the pH is incrementally increased from 5.0 to 8.0 (Boswell et al., 1999). While above pH 8.0, the P-release rate has been shown to decrease and an optimum pH of 6.8 ± 0.7 has been proposed for anaerobic acetate metabolism (acetate uptake rate coupled with P-release rate) by Liu et al. (1996).

1.8. Secondary P-release

The phosphorus release that occurs in the absence of VFA is defined as secondary P- release and this form of P-release is detrimental to the BPR process (Barnard, 1994). Long hydraulic retention times (HRT) in the anaerobic zone, inadequate supply of organic compounds in the wastewater, and insufficient activity of the fermentative bacteria in the anaerobic zone can all lead to the secondary release of P and inefficient overall operation (Danesh and Oleszkiewicz, 1997). As outlined previously, nitrate can affect the BPR process if it is not consumed completely at the end of each treatment cycle. The following examples suggest a possible explanation for the role played by nitrate in the secondary release phenomenon.

In pilot scale studies, Rensink et al. (1997) observed secondary P-release in the anoxic zone of a modified Renphosystem for nutrient removal when all the nitrate had been denitrified before the end of the anoxic phase. Meinhold et al. (1999) reported a similar phenomenon with P-uptake occurring as long as nitrate was present in a system acclimated to higher nitrate concentrations. Once the nitrate had been consumed, a slow secondary release of phosphate took place. This secondary release phenomenon at low nitrate concentrations has also been observed in both pilot plant studies and at full-scale facilities. For example, Nicholls et al. (1986) reported an extensive secondary release of P in the presence of elevated nitrate concentration at full-scale in a South African WWTP. A WWTP in Wulkatal, Australia produced elevated effluent P-levels as the concentration of effluent nitrate increased (Eckenfelder and Grau, 1992). With effluent nitrate at a concentration of 1 mg NO_3-N/l, 80–90% BPR was obtained. However, with effluent nitrate at 3 mg NO_3-N/l, the BPR efficiency was reduced to 30% (Eckenfelder and Grau, 1992).

1.9. Phosphorus load

The majority of studies on BPR have been carried out on low-phosphate wastewaters, with few attempts to apply biological systems for influents containing >20 mg P/l. It has been proposed that limited phosphate loadings may suppress the development of PAO leading to the establishment of GAO with PAO establishing in an SBR under P-rich loading conditions, whereas GAO do- minate under limited P loading conditions (Sudiana et al., 1999).

The ratio of phosphorus to total organic carbon (P/TOC) in a system is important in the selection of the PAO bacteria and in giving them a competitive advantage (Liu et al., 1997, 1998). When a low P/TOC feeding ratio was used by Liu et al. (1998), growth of PAO was suppressed. In contrast, higher P/TOC feeding ratios encouraged the growth of PAO over GAO. Sludge with extremely poor P-removal was reported by Bond et al. (1999) when the influent P concentration to their system was reduced. The resulting sludge became dominated by bacteria resembling GAO.

Filauro et al. (1991), utilising a semi-industrialised pilot-scale PhoStrip system for biological nutrient removal (BNR) from a combined wastewater of municipal and pig farm origin (containing up to 100 mg P/l), obtained combined high N- and P-removal efficiencies while operating under these unusual process conditions. Converti et al. (1993) also obtained P-removal rates of >90% for wastewaters containing phosphate loads of up to 70 mg/l. At higher influent P-concentrations, the system proved capable of tolerating P overloading for not more than 10 days, with removal efficiencies subsequently falling rapidly to zero. In addition, a lab-scale SBR operated by Comeau et al. (1996) on a 12-h cycle treating an influent P concentration of 60 mg P/l for a 193-day period, achieved an average total P-removal of 53 mg P/l (i.e., 88%).

In a batch system operated by Shin and Jun (1992), the influent P concentration was increased threefold (to 39 mg P/l) over a 12-h period. All of the influent P was removed, indicating that the system had sufficient redundant energy to uptake the shock P load applied to the reactor (Shin and Jun, 1992). A long-term study carried out by Randall et al. (1997a) showed that doubling of the influent P (to 20 mg P/l) to an SBR resulted in a significant immediate increase in the P-removal rate, followed by a long-term downward trend in the efficiency of P-removal.

1.10. pH control

The pH of a combined BNR system requires careful monitoring since the various processes, such as nitrification, denitrification, P-release and P-uptake, all have specific pH ranges within which they can be optimised. Nitrification in particular appears to be sensitive to changes in pH (Wagner et al., 1998), with optimal nitrification occurring between pH 7.5 and 9.0 (Surampalli et al., 1997). If alkalinity is insufficient, the pH value may fall below pH 7 within which the rate of nitrification decreases as pH values fall, becoming zero at approximately pH 6.0 (Eckenfelder and Grau, 1992; Surampalli et al., 1997). The pH optimum for denitrification appears to be between pH 7.0 and 8.0 (Metcalf and Eddy, 1991; Sanchez et al., 1998). Nitrite inhibition of activated sludge denitrification has been found to be exacerbated at pH 7.0 at relatively low nitrite levels of 250 mg/l NO_2-N, probably due to the formation of the protonated species nitrous acid (Glass and Silverstein, 1998). Fluctuation in pH is one of the many characteristics, which lead to sludge ‘‘bulking’’ conditions in BNR systems (Metcalf and Eddy, 1991). Smolders et al. (1994) highlighted that the rate of P-release under anaerobic conditions was increased as the pH was increased. Similarly, Bond et al. (1998) showed that an SBR operated without pH control in the anaerobic phase exhibited an improved P-removal by comparison with an SBR, which had pH control. Absence of pH control resulted in an increase in the prevailing pH of the anaerobic phase. Liu et al. (1996) reported that an acidic pH had a negative effect on both acetate uptake and P-release in the anaerobic stage, whereas a more alkaline pH inhibited the uptake of acetate and stimulated more P-release than at acidic pH.

Maintenance of a stable, neutral pH was shown by Converti et al. (1995) to be essential for process stability in batch systems. When the influent pH was reduced from 7.2 to a weakly acidic value of 6.3, P-removal efficiency was adversely affected and 15 days were required to re-establish steady-state conditions.

1.11. Other practical parameters affecting P-removal

Three of the most important control parameters that must be optimised and monitored for successful operation of BNR systems are:

(i) The F/M ratio, which is the ratio between the organic loading rate to an activated sludge system and the mass of sludge in the system (Bitton, 1998).
(ii) The HRT, which represents the time that a liquid stays in a reactor (Bitton, 1998).
(iii) The SRT (otherwise known as the mean cell residence time-(MCRT) or sludge age), which is defined as the mass of organisms in the reactor divided by the mass of organisms removed from the system each day. In effect, it corresponds to the average time the microorganisms remain within the system (Bitton, 1998).

It is essential to BPR that sludge be wasted under aerobic conditions, as this sludge will contain the maximum amount of phosphorus (Surampalli et al., 1997). Over the past 20 years, there has been a marked improvement in the understanding of the process understanding. This has allowed the design of BNR plants such as those in Western Canada, that operate at SRTs of 9–13 days during the winter and 5–7 days during summer. The changes in design parameters are due to the increased knowledge of the factors, which affect BNR processes and smaller safety factors have been included in process design (Oldham and Rabinowitz, 2002).

In activated sludge systems, the sludge-loading rate controls the SRT. Broadly speaking, the lower the sludge loading rate and the longer the SRT, the better the quality of effluent, at least up to the point at which the loading is insufficient to sustain a coherent settleable sludge (Metcalf and Eddy, 1991; Eckenfelder and Grau, 1992).

The low-load concept of using F/M ratios of less than 0.10 kg BOD/kg MLVSS/day for the sizing of biological reactors has become quite common, especially when BNR is a process goal. Typical F/M values for combined carbon oxidation, nitrification and denitrification vary from 0.03 to 0.06 kg BOD/kg MLVSS/day (Crites and Tchobanoglous, 1998). However, most Italian activated sludge plants operate at low F/M values of up to 0.5 kg COD/kg MLVSS/day (Rossetti et al., 1994). Low F/M values always represent a critical condition for sludge bulking (Rossetti et al., 1994). Cuevas-Rodríguez et al. (1998) tested an activated sludge SBR with organic loading rates of 0.13, 0.25 and 0.35 kg COD total/kg TSS/day, respectively, and reported PO_4-P concentrations under 1.1 mg/l and COD between 37 and 38 mg/l being consistently achieved. Knoop and Kunst (1998) found that an increase in the F/M from 0.1 to 0.2 kg COD/kg MLVSS/day prevented growth of *M. parvicella*, even at lower temperatures.

The different populations involved in combined nutrient removal processes have different requirements in relation to SRT. Slow growing organisms that are generally lost at low SRTs include nitrifying bacteria and ciliated protozoa (which ingest freely suspended bacteria and other small non-settleable particles that give rise to turbidity in settled effluents) (Eckenfelder and Grau, 1992; Nam et al., 2000; Wagner and Loy, 2002). Anaerobic ammonium oxidation can occur in fully autotrophic systems with very long sludge retention times or biofilm systems (Van Loosdrecht and Jetten, 1998). Experience with European activated sludge systems shows that SRTs of approximately 15–30 days are economically feasible and remove up to about 95% of BOD and all but a few milligrams per liter ammonia (Eckenfelder and Grau, 1992). The SRT for low-loaded domestic wastewater systems is generally of the order of 20–40 days or more, resulting in a very stable waste activated sludge (Crites and Tchobanoglous, 1998). Rusten and Eliassen (1993) concluded that an SRT of 12–13 days, calculated only on the basis of the aerobic phase, was necessary to obtain complete nitrification in an SBR with suspended biomass. Garzon-Zuniga and Gonzalez-Martinez (1996) suggested that longer aeration times are required, in addition to longer SRTs, in order to achieve complete nitrification. By increasing the DO content from 2 to 4 mg/l, Rothman (1998) obtained nitrification at an aerated sludge age of 5 days, which is below the generally accepted limit for nitrification.

There are various reports of BPR plants operating at a range of different SRTs. Mamais and Jenkins (1992) obtained efficient BPR at SRTs>2.9 days, while anoxic and aerobic P-uptake was observed at a SRT of 10 days by Chuang et al. (1998). However, cessation of anoxic P-uptake occurred at a SRT of 5 days, followed by incomplete aerobic P-uptake in the subsequent aerobic stage (Chuang et al., 1998). Previous work by Chang et al. (1996) demonstrated that SRTs of 5, 10 and 15 days resulted in similar COD removal performances. P-removal was observed at all three SRTs, with 10-day SRT giving the best P-removal rate. Similarly, Choi et al. (1996) obtained higher P-removal efficiencies at an SRT of 10 days by comparison with SRTs of 5 or 20 days. BPR was tested at SRTs ranging from 11 to 65 days by Rodrigo et al. (1996) and the inference drawn was that the poly-P microbial fraction of the mixed culture decreased as sludge age increased. SRTs below 11 days were not tested by these authors, as sludge settlement difficulties would have been expected at these lower SRTs.

Furumai et al. (1999) operated two SBRs at SRTs of 7 (R1) and 15 days (R2), respectively, with excellent P-removal being achieved in both reactors after 21 days. However, P-removal declined in R2 due to the very high nitrification rates achieved at the longer SRT. This led to increased nitrate levels in the anaerobic phase due to incomplete denitrification in the previous cycle. For combined systems, the authors suggested that longer SRTs are beneficial for P-removal. However, if the target is P-removal, then the SRT should be reduced to decrease nitrification activity. In other studies on the effects of SRT on COD, nitrogen (NH_4-N, NO_3-N) and phosphate (PO_4-P) removal, a sludge age of 10 days was found to be optimal, resulting in maximum nutrient removal efficiencies and minimum SVI (Chang and Hao, 1996; Kargi and Uygur, 2002).

Monitoring the SVI is also necessary to provide information on the settling properties of the sludge. The mixed liquor suspended solids (MLSS) concentration has considerable influence on the SVI measurement, since high values of the former imply low values of the latter (Rossetti et al., 1994). The recommended MLSS for both N- and P-removal should be maintained between 1500 and 1700 mg/l (Shehab et al., 1996). Below 1500 mg/l, the nitrifiers will be low in concentration and the N concentration of the effluent will increase. Above 1700 mg/l, the concentration of P-removing organisms will be too high, leading to starvation and cell death. This can result in release of P from the cells (Shehab et al., 1996).

The phosphorus content of the sludge is also of primary concern to BPR plant operators as wasting of sludge is the method by which P is ultimately removed from the system. The poly-P content of sludge is considered an essential factor relating to energy storage in the BPR system (Chuang and Ouyang, 2000). The phosphorus content of sludge has been reported to be in the range of 4.1–15.6% under enhanced mixed-culture conditions (Mino et al., 1998). Randall (1988) reported a high P-content, in the biomass, of 8.4% (dry weight) for a BPR process treating a municipal wastewater containing 11 mg P/l, 250 mg COD/l and operated on a 14-day SRT. Comeau et al. (1996) obtained a similarly high P-content of 7.6% g P/g MLSS in a BPR system treating a wastewater containing 60 mg P/l, 1550 mg COD/l and operated on a 20-day SRT. In some cases, sludge phosphorus contents of up to 18% have been obtained with artificial, tailored substrates (Appeldoorn et al., 1992). Aerobic P-uptake is not only dependent on the poly-hydroxy-butyrate content of the biomass, but also on the maximum poly-P storage capacity of the cells (Brdjanovic et al., 1998). The P-content of the sludge also has an effect on acetate metabolism, as reported by Liu et al. (1996). It has been suggested that the microbial population of sludge with a lower P-content may consist of species other than PAOs, such as GAOs that do not release phosphate during acetate uptake. Liu et al. (2000) observed that a gradual increase in the P/C ratio from 20:100 to 2:100 did not affect the carbon uptake and storage under anaerobic conditions, but caused a drop in the sludge P content from ~ 12% of sludge dry weight (or 33% polyphosphate) to a cellular constituent level (~ 2% P content or ~ 0% polyphosphate). Panswad et al. (2003) observed that the phosphorus content of the biomass (mg P/mg MLVSS) decreased as the temperature increased, i.e., declining from 14.8% at 20.0 °C to 10.4%, 8.2%, 6.2% and 2.4% at 25.0, 30.0, 32.5 and 35.0 °C, respectively.

2. Conclusion

In order to operate a successful biological phosphate removal process, it is imperative that the incoming wastewater contain the correct balance of nutrient, carbon sources and pH, as discussed in this review. In addition, careful consideration must be given to operating the system at the correct F:M, HRT, SRT, temperature and dissolved oxygen concentration to ensure that a healthy sludge population prevails in the reactors, with good settling properties and ability to perform primary anaerobic release of phosphate, while at the same time preventing undesirable phenomenon such as secondary release of phosphate.

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