The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal Part 1: Literature review

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Abstract

Simultaneous chemical precipitation of phosphate (P) is commonly used in activated sludge systems to supplement biological excess P removal (BEPR). This paper briefly reviews the use of metal salts (typically iron or aluminium) for this purpose and focuses on the question of possible interference with the BEPR mechanism arising from the addition of chemical precipitant. Some evidence of weakened BEPR has emerged in activated sludge systems in South Africa, based partly on observations from full-scale plants in which controlled studies were not satisfactorily carried out. In some cases, extrapolations have been made either from laboratory-scale systems in which unrealistically large doses of metal precipitant were used, or from systems which were not operated close to steady-state conditions over extended periods. In other cases where simultaneous precipitation has been applied, the systems studied were not designed for BEPR. It was concluded that there is room for further investigation of the reported negative effect of simultaneous chemical precipitation on BEPR. To this end, a review of methods for fractionating the phosphorus compounds in activated sludge is presented. It does not appear to be possible to tailor a crude fractionation procedure to suit *specifically* the extraction of biologically-formed polyphosphate (polyP) separately from chemically-formed phosphorus precipitates in a complex medium such as activated sludge. More powerful analytical techniques are required to determine the nature, chain-length and mass of stored polyP in activated sludge. Similarly, there is a need to carry out further fundamental research into the interaction between the biological polymers in the sludge matrix and chemical removal mechanisms. Nevertheless, the available basic chemical fractionation procedures do make it possible to obtain a broad classification and measurement of chemical vs. biologically accumulated forms of P in activated sludge.

Introduction

Eutrophication of natural and man-made impoundments has become a problem in many countries, including South Africa. Problems associated with eutrophication include profuse algal blooms, excessive growth of nuisance aquatic plants, negative aesthetic aspects, deoxygenation, and problems relating to water purification for potable use. Many limnological studies have been conducted into the phenomenon, its causes and effects (*inter alia* Walmsley and Thornton, 1982; Walmsley and Thornton, 1984; Twinch, 1986; Grobler 1988 (a; b); Chutter, 1990; Dillon and Molot, 1996). Such studies have indicated that the limiting nutrients in eutrophication of freshwater systems are usually phosphorus and nitrogen (in that order), and that eutrophication can be controlled by significantly reducing the phosphorus (P) load discharged to a catchment. Worldwide increasing awareness of this causative effect on eutrophication has led to the introduction of legislation controlling the discharge of P to receiving waters.

In South Africa, the special phosphate standard was introduced, restricting the concentration of phosphorus in wastewater discharges to 1 mgP/ℓ as dissolved orthophosphate (*Government Gazette*, 1984). To comply with the new effluent legislation, a number of existing wastewater treatment plants in South Africa were modified or new plants constructed to implement biological excess phosphorus removal (BEPR) *[Also known as enhanced biological phosphorus removal (EBPR)]*. The decision to opt for biological P removal was based partly on the emergence of local expertise and partly on cost considerations. BEPR processes typically involve higher capital investment than those using chemical P precipitation; however, BEPR processes have the potential to offer lower operating and maintenance costs than conventional processes with chemical dosing. Similar trends have emerged in several countries, such as Canada, Australia and Germany (Nutt, 1985; Yue et al., 1987; Hartwig and Seyfried, 1991; Peter and Sarfert, 1991; Barnard, 1995; Hartley, 1997).

Since its implementation, considerable practical experience has been gained with BEPR systems. However, biological phosphorus (P) removal tends to be sensitive and subject to many fluctuations, making it difficult to achieve full compliance with discharge standards (*inter alia* Osborn et al., 1986; 1989; Lötter, 1991).

In many cases, the practical solution to meet effluent standards has been to supplement biological P removal with chemical P removal. Nutt (1985) investigated the technical and economic feasibility of retrofitting wastewater treatment plants with biological P removal. To consistently achieve less than 1.0 mg/ℓ as total P, Nutt (1985) found that BEPR processes require effluent filtration and/or supplementary chemical dosing. Similarly, the IAWQ Nutrient Removal Tour to South Africa (1993) highlighted that supplementary chemical dosing into biological nutrient removal (BNR) activated sludge plants was being used in several cases. In some cases, concern for over process optimisation has led to the use of "back-up" chemical dosing at the tertiary stage, followed by clarification, filtration or dissolved air flotation (DAF), in preference to simultaneous chemical addition at the secondary (activated sludge) stage (e.g. Hartwig and Seyfried, 1991; De Wet et al., 1992; Hamilton and Griffiths, 1997). Alternatively, side-stream processes

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such as *Pho-Strip*, originally developed in the 1960s and early 1970s (Levin et al., 1975) have been further developed and integrated into BNR systems, thereby attempting to keep the chemical and biological sludges separate. Szpyrkowicz and Zilio-Grandi (1995) gave an example of the use of this type of design. Lilley et al. (1993) compared the economic merits of the *Pho-Strip* process with various other processes, including conventional activated sludge systems, BNR (Phoredox) processes and tertiary chemical phosphate removal following primary and secondary treatment by biofiltration. They found that the BNR (Phoredox) process was significantly cheaper to build and operate than the other processes with P removal capability. For cases where the BNR process alone cannot achieve the necessary effluent P standard, simultaneous chemical addition is economically attractive in that it avoids the capital expense associated with building tertiary dosing and solids separation facilities. However, there is evidence in the literature that simultaneous chemical addition may have a deleterious effect on biological excess P removal. It is clear that the economic benefit of building a BNR system could be lost if simultaneous addition of chemicals does result in significant inhibition of the biological P removal mechanism.

This paper is the first in a series which will examine the interaction between biological and chemical P removal in modified activated sludge systems with a view to determining whether or not simultaneous chemical dosing produces an "inhibitory" effect on biological P removal processes. Definition of the term "inhibition" may in itself pose a difficulty in this context, but is used here to broadly discern inhibitory effects from those which apparently arise from competition for available phosphate. To discern such effects, in this study it was necessary to operate parallel pilot plant BEPR activated sludge systems, with and without the simultaneous addition of chemical P precipitants, and to apply analytical methods for broadly distinguishing the P compounds accumulated in the mixed liquor. The aim of this paper is to provide an overview of the literature from which the study and methodology were developed. The second paper in this series (**Part 2**, De Haas et al., 2000) will examine the analytical methods used. Subsequent papers will deal with the results found for alum and iron precipitants in simultaneously dosed activated sludge systems, as well as modelling studies undertaken.

Chemical phosphorus removal in modified activated sludge systems

In reviewing chemical processes for phosphate removal, Jenkins et al. (1971) pointed out that iron or aluminium salts (with or without lime) were used for BOD and suspended solids removal since the early days of wastewater treatment in this century (ca. 1920s) and were also used at that time to improve the settling characteristics of activated sludge. However, because biological treatment processes were more economical and posed fewer sludge disposal problems, these early chemical treatment schemes became less frequently used (Jenkins et al., 1971). Chemical treatment for phosphate removal from wastewater was revived in the 1960s when eutrophication problems emerged in several countries, particularly the Great Lakes of North America.

Systems for chemical and biological P removal have been reviewed by Arvin (1985) and Yeoman et al. (1988). Chemical addition may take place at one of three stages of wastewater treatment, namely:

- Primary treatment (i.e. primary sedimentation, if present), for which the term "pre-precipitation" may be used.
- Secondary treatment (typically an activated sludge or biofilter system) for which the term "simultaneous precipitation" may be used.
- Tertiary treatment (chemical flocculation followed by sedimentation or flotation, sometimes followed by filtration) for which the term "post-precipitation" may be used.

Based on model treatment plants with idealised configurations for biological phosphorus removal, Nutt (1985) concluded that BEPR and chemical precipitation processes should ideally be applied simultaneously to optimise performance and minimise costs, particularly capital costs. Nutt (1985) also reported that simultaneous chemical precipitation in new or retrofitted conventional activated sludge plants may be more economically attractive than BEPR processes in some cases, depending on effluent nutrient limits, plant design and wastewater characteristics.

Iron salts

Oxidation state of iron and P precipitation

For chemical P removal, or a combination of chemical and biological removal, iron salts are widely used. In reviewing guidelines for chemical phosphate removal from municipal wastewaters, Wiechers (1987) stated that both forms of iron (Fe^{2+} and Fe^{3+}) combine with orthoP in precipitation reactions, and with hydroxide in a competing reaction. The iron hydroxide also participates in the removal of phosphate, setting up a slower exchange reaction of hydroxyl ions with orthophosphate (orthoP) ions (Rabinowitz and Marais, 1980).

From stoichiometry, ferric (Fe^{3+}) ions form $FePO_4$ (strengite) in the reaction with orthophosphate, while ferrous (Fe^{2+}) ions form $Fe_3(PO_4)_2.8H_2O$ (vivianite). Both Fe^{3+} and Fe^{2+} ions also react with hydroxide to form amorphous iron hydroxide flocs. The iron hydroxide can destabilise negatively charged iron-phosphate colloids, enmesh them and provide an adsorption capability for orthoP and polyphosphate (polyP) molecules (e.g. pyrophosphate and tripolyphosphate) which are commonly used as softeners in detergents (Wiechers, 1987). The stoichiometric mass ratio of Fe:P for $FePO_4$ (ferric) and $Fe_3(PO_4)_2$ (ferrous) is 1.8:1 and 2.7:1, respectively (Wiechers, 1987). Competition between hydroxyl ions and phosphate ions for the iron ions at the point of addition, the reaction of bicarbonate ions forming iron hydroxides, and the need to destabilise colloids (e.g. iron phosphate, dispersed micro-organisms or influent organics), probably account for the stoichiometric excess of ferric iron that is sometimes required for phosphate precipitation (Jenkins et al., 1971). To allow for a variable stoichiometry, in their precipitation model Luedecke et al. (1989) used a generalised formula for ferric hydroxy phosphate: $Fe_r\ PO_4\ (OH)_{3r-3}$.

Of the iron salts, iron (III) chloride (ferric chloride) is most commonly used for P precipitation, but iron (II) (ferrous) salts may also be used (Yeoman et al. 1988). Aspegren (1995) described operation of a full-scale plant in Sweden with pre-precipitation in the primary treatment stage using ferrous sulphate while Olesen (1990) gave an example of the use of ferrous sulphate for simultaneous precipitation on small sewage works in Denmark. Chaudhary et al. (1991) presented an evaluation of ferric chloride at the primary stage in one of the largest sewage treatment plants in the USA. Similarly, both ferrous and ferric chloride are widely used in South Africa to supplement biological P removal in BNR plants, mainly by simultaneous precipitation (Leopold, 1996).

Singer (1972) noted that under anaerobic conditions in primary sludge (i.e. where ferrous iron was dosed into raw sewage before primary sedimentation), phosphate was precipitated as crystalline

ferrous phosphate (vivianite) and little phosphate was released when this primary sludge was treated by anaerobic digestion. Similarly, Frossard et al. (1997), using X-ray diffraction, electron microscopy and ^{57}Fe Mössbauer spectroscopy, found direct evidence that most (67%) of the phosphate precipitated in anaerobically digested sludge is in the form of crystalline vivianite ($Fe_3(PO_4)_2.8H_2O$). *[These sludges were derived from works with (pre-)precipitation using ferrous sulphate, followed by primary sedimentation]*. Interestingly, by applying the same techniques to activated sludge receiving simultaneous addition of ferrous sulphate, Frossard et al. (1997) found that as much as 43% of the total phosphate in the sludge was also precipitated in the form of vivianite. They concluded that vivianite may be precipitated slowly and in anaerobic pockets in the activated sludge system; the balance of the iron in the sludge was expected to be in the form of Fe^{2+} or Fe^{3+} ions associated with organic compounds, as well as Fe^{3+} in the form of hydroxides. *[Frossard et al. (1997) used the term "oxy-hydroxides", probably meaning amorphous ferric hydroxides of the type FeOOH. Frossard et al. (1997) appear to have accepted that oxidation of ferrous ions to ferric ions is possible and likely in aerobic activated sludge systems, but considered it unlikely that the sludge would contain ferric ions from the oxidation of vivianite after precipitation]*. However, Yeoman et al. (1988) assumed that under aerobic conditions Fe^{2+} salts mostly act as phosphate precipitants after oxidation to the Fe^{3+} form. The reaction may be written as (Loewenthal et al., 1986):

$$Fe^{2+} + \tfrac{1}{4} O_2 + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2} H_2O$$

This oxidation reaction requires a neutral or weakly alkaline pH and has a significant oxygen demand (0.15 g O_2/g Fe^{2+}) (Singer, 1970, quoted by Yeoman et al., 1988). The reaction half-time for oxidation of Fe^{2+} to Fe^{3+} is about 16 min, at pH 7.0, 2 mg/ℓ dissolved oxygen (DO) and 25°C (Singer, 1972).

It is generally assumed that most Fe bound in activated sludge is in the oxidised (Fe^{3+}) state, probably as ferric hydroxide (Rasmussen and Nielsen, 1996). Using techniques that are commonly applied in soil science, Rasmussen and Nielsen (1996) were aimed to verify the oxidation state of Fe in activated sludge. Sludge samples were obtained from a Danish treatment plant with biological N and P removal in which P removal is also augmented chemically with ferrous sulphate. The average total iron content of the activated sludge samples was ca. 63 mg Fe/g dry solids. By means of extraction techniques involving iron reduction to Fe^{2+} and determination of the latter using ferrozine, they showed that fresh sludge contained very little Fe^{2+}; Fe^{2+} was also not detectable in the supernatant from fresh sludge. Fe^{3+} accounted for 70 to 90% of the total Fe in the sludge. Reduction of Fe^{3+} to Fe^{2+} commenced immediately after initiation of an anaerobic stage. Almost all the Fe^{2+} formed by reduction remained in the floc matrix. The rate of Fe^{2+} accumulation was somewhat higher in short-term than in long-term anaerobic experiments, suggesting that some form of substrate limitation (or depletion of easily available Fe pool) comes into effect. From unpublished data, Rasmussen and Nielsen (1996) concluded that the Fe reduction process was mainly biologically mediated. However, on the basis of their observed Fe^{3+} reduction rates, and assuming an Fe:P ratio of 2.5:1, Rasmussen and Nielsen (1996) concluded that relatively slow P release rates may be expected from this source. This, together with the fact that re-oxidation of any soluble Fe^{2+}, followed by P precipitation, is expected in the aerobic zone, suggests that Fe reduction is of secondary importance in the combined chemical-biological P removal mechanisms of activated sludge systems.

Rasmussen and Nielsen (1996) were not able to determine the form in which Fe^{3+} is precipitated in activated sludge (e.g. crystalline vs. amorphous precipitate), nor the extent to which it is organically bound. However, they did point out that extracellular polymeric substances are common in activated sludge and consist of humic substances, polysaccharides, proteins, and DNA, all of which are known to bind metal ions to some degree.

Nielsen (1996) studied the role of iron in oxidation-reduction reactions in several Danish activated sludge plants with chemical dosing, usually in the form of ferrous sulphate. The total amount of iron in these systems was relatively high, as expected (~65 to 190 mgFe/gVSS). By means of HCl extraction, Nielsen (1996) found that the concentration of Fe^{2+} was always lowest in the aerobic/anoxic tank of a Biodenipho plant (10 to 15 mgFe/gVSS), with little difference noted during the aerobic/anoxic cycle. In the return sludge and anaerobic tank, the Fe^{2+} concentration was a little higher (14 to 24 mg Fe/gVSS). Nielsen (1996) postulated that Fe^{3+} reduction occurs in activated sludge under anaerobic conditions as a result of iron-reducing bacteria (FeRB) using organic substrates as source of electrons and energy. Nielsen (1996) suggested that acetate production by FeRB could be a source of acetate for phosphorus-accumulating organisms (PAO) (i.e. polyP organisms) in the BEPR mechanism. However, from the rates of Fe(III) reduction in full-scale plants observed by Nielsen (1996) the rate of acetate production by FeRB would be relatively low. For practical retention times in the anaerobic tank of BEPR plants, the acetate from this source would be relatively insignificant (< 4 mg COD/ℓ). Nevertheless, if the reduction of Fe^{3+} to Fe^{2+} resulted in dissociation of iron phosphate precipitate with an hypothetical Fe:P molar ratio of 2.5:1 (Luedecke et al., 1989), then for the changes in Fe^{2+} during the anaerobic-aerobic cycle observed by Nielsen (1996), P release of up to approx. 10 mgP/ℓ in a typical anaerobic tank of a BEPR plant could result. P release from this source would be of chemical origin, mediated through biological reduction of iron, and may be significant when interpreting data from combined chemical-biological P removal plants.

Effect of pH on precipitation with iron salts

According to Benedek et al. (1976) (quoted by Yeoman et al., 1988), in an iron-orthoP system, removal of phosphate is independent of pH below an Fe:P molar ratio of 1.5:1. At ratios above this value, pH has an increasing influence. The optimum pH for phosphate precipitation with ferric iron is the range between pH 4.0 and 5.0, while that for ferrous iron is close to pH 8.0 (Wiechers, 1987). In practice wastewater treatment systems usually rely heavily on biological processes which have an optimum pH in the range ca. 6.8 to 8.0. Standards for discharge of treated effluent to rivers and lakes or dams also usually mandate a pH range close to neutral. Moreover, special corrosion-resistant construction materials would be required for reactors to tolerate a pH as low as 4.0, and pH correction to a neutral pH would incur major additional chemical costs. It is therefore seldom practical to operate a wastewater treatment process in the optimal pH range for ferric phosphate precipitation. Fortunately, in practical terms the low solubility products of ferric phosphate makes it chemically possible to achieve low effluent P concentrations (ca. 0.1 to 1 mgP/ℓ) at near-neutral pH with simultaneous iron dosing (Luedecke et al., 1989).

Observations with simultaneous iron dosing

Wuhrmann (1968) conducted a number of pilot experiments involving the addition of ferric chloride to the aeration basin of a conventional activated sludge plant (reviewed by Jenkins et al., 1971). Effluent total P concentrations were seldom less than 0.5

mgP/ℓ at a dose of 30 mg/ℓ as Fe^{3+} (Fe:P mole ratio = 3.1:1). Wuhrmann (1968) also reported that ferric chloride dosing caused the virtual disappearance of protozoa from the activated sludge culture. The poor phosphate removal observed was largely attributable to the failure of the secondary sedimentation process to remove fine phosphate-containing particles. It was not clear to Wuhrmann (1968) whether the turbid effluents obtained were due to poorly flocculated ferric-hydroxy-phosphate particles or as a result of dispersed activated sludge particles due to the absence of protozoa. Wuhrmann (1968) obtained better phosphate removal and less turbid effluent in tertiary chemical treatment experiments but commented on the poor settling and dewatering properties of the tertiary sludge (Jenkins et al., 1971).

In the late 1960s and 1970s concern over eutrophication led to widespread implementation of simultaneous chemical dosing (mainly with iron salts) for P removal in the USA, Canada and parts of Europe (e.g. Boyko and Rupke, 1973; Stepko and Shannon, 1974; Viitasaari, 1976; Sutton et al., 1978; Black, 1979; Rensink et al., 1979; D'Elia and Isolati, 1992). However, most of these plants were high-rate (short sludge age) or conventional (completely aerobic) activated sludge plants which did not make provision for BEPR. Therefore, the possible interaction between biological and chemical P removal mechanisms were hardly considered. BEPR process designs began to emerge in South Africa in the late 1970s and the first new BEPR plant in North America was built in Canada in 1980 (Barnard, 1995).

Rabinowitz and Marais (1980) were possibly the first to investigate the effect of simultaneous addition of ferric chloride and ferrous sulphate to modified activated sludge systems incorporating BEPR in the 3-stage Phoredox or UCT configurations. They drew several important conclusions:

- Chemical addition enhanced the P removal in the test systems. The iron phosphate chemical removal mechanism appeared to operate independently of the biological removal mechanism. This conclusion was drawn by comparing the observed system P removal with the theoretical biological P removal potential (based on an empirical model for BEPR).
- Chemical P removal was strongly pH dependent. For both $FeSO_4$ and $FeCl_3$ addition, if the process pH fell below 7.0, the P removal efficiency decreased, the effluent became yellow-green in colour, and turbidity increased. At a process pH $\geq$7.2, the P removal efficiency increased to a maximum, the effluent was virtually colourless with low turbidity.
- Using $FeSO_4$ addition at process pH$\geq$7.2, an estimated stoichiometric chemical removal efficiency of 80% was achieved. Using $FeCl_3$ addition, an estimated stoichiometric removal efficiency of 100% was achieved. There were indications that when the effluent phosphorus concentration fell to <1.5 mgP/ℓ, the removal efficiency decreased (i.e. $Fe_{dose}/P_{removal}$ ratio increased). Consequently, for low effluent P concentrations, the iron dose needed would be greater than the stoichiometric amount required for P removal.
- Simultaneous chemical addition exhibited a "persistence effect". This was evident from P removal behaviour under cyclic loading conditions, as well as continued P removal (in excess of the calculated BEPR removal potential) for several days after cessation of dosing. It was proposed that this was due to the accumulation of ferric hydroxide precipitate in the sludge mass, leading to phosphate removal through an ion exchange effect between hydroxide and phosphate ions.
- The point of iron dosing did not appear to have any significant effect on the system chemical P removal performance.
- "Iron leakage" into the effluent was measured when dosing $FeCl_3$, but effluent Fe concentrations were consistently low (<0.1 mg Fe/ℓ). It was concluded that virtually all the added Fe was adsorbed or precipitated in the sludge mass.

Lötter (1991) reported satisfactory plant performance (effluent <1 mgP/ℓ as orthoP) at Alexandra Works (Johannesburg) with ferrous sulphate dosed in the *mass* ratio of 2.1:1 to 2.4:1 for $Fe:P_{removed}$. At Northern Works (Johannesburg), satisfactory performance was achieved with one module dosed with ferric sulphate at a mass ratio of approximately 2:1 for $Fe:P_{removed}$ *[A mass ratio (Fe:P) of 2:1 corresponds to a molar ratio of 1.1:1].* However, Lötter (1991) also reported that partial inhibition of the biological P removal process may make it difficult to control simultaneous iron dosing where BEPR is required to make a major contribution to the system P removal performance.

Luedecke et al. (1989) developed a chemical model of ferric phosphate precipitation in activated sludge systems which described ferric hydroxy-phosphate precipitation either alone or together with ferric hydroxide. The model is based on equilibrium chemistry and additionally makes provision for adsorption of phosphate ions on ferric phosphate or ferric hydroxide precipitates. The application of this model to combined biological-chemical P removal activated sludge systems is limited by the fact that it takes no account of the biological processes for phosphate. In order to set up a combined model, the basis for dividing the available phosphate between the biological and chemical P removal processes would need to be carefully examined. A combined chemical-biological P removal model was proposed by Briggs (1996), based partly on the work of Luedecke et al. (1989). The IAWQ Activated Sludge Model No. 2 (IAWQ, 1995) also makes provision for simultaneous chemical precipitation in biological nutrient removal systems. However, there are major differences in the modelling approach followed by Briggs (1996) compared with that used in the IAWQ model. These differences will be examined in a subsequent paper as part of this series.

Alum and poly-aluminium chloride

Aluminium sulphate is used extensively for phosphate precipitation (*inter alia* Ulmgren, 1975; Klute and Hahn, 1992; Morales et al., 1991; De Haas et al., 1991). Hydrated aluminium sulphate (alum) is mainly used. Alum has the approximate formula of $Al_2(SO_4)_3.14H_2O$ (Wiechers, 1987). According to Wiechers (1987), two competing reactions are involved when alum is dosed into phosphate-containing waters. Neglecting reactions between condensed and organic phosphates, the main reactions involve competition between the formation of aluminium hydroxides ($Al(OH)_3$ ideally) and aluminium phosphate ($AlPO_4$ ideally). Equations for the formation of $Al(OH)_3$ and $AlPO_4$ may be written as:

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+$$

$$Al^{3+} + PO_4^{3-} \rightarrow AlPO_4$$

However, these equations are probably over-simplifistic. Firstly, numerous other hydrolysis products (ion pairs) of aluminium may form depending mainly on pH (Briggs, 1996). Secondly, the stoichiometry of Al:P is variable indicating formation of aluminium-hydroxy-phosphate precipitates. From an examination of the literature, Power et al. (1992) concluded that although a mechanism of adsorption/ion exchange of phosphate ions with aluminium hydroxide flocs has been proposed, there is also evi-

dence for direct precipitation of insoluble aluminium phosphates. However, the stoichiometric molar ratio of Al:P of 1:1 in $AlPO_4$ is never achieved in practice, and the actual ratio between aluminium dosed and P removed varies between 2:1 and 3:1 (Wiechers, 1987). This suggests that one or more of the hydrolysis products of Al^{3+} (e.g. $Al(OH)_2^+$, $Al_2(OH)_2^{4+}$) are involved in the precipitation of phosphate (Power et al., 1992). According to Jenkins et al. (1971), the formation of aluminium phosphate is thermodynamically and kinetically favoured over hydroxide formation. However, at low phosphate concentrations (<10 mgP/ℓ), the competition between hydroxide and phosphate is more significant (Stumm and Morgan, 1970, quoted by Yeoman et al., 1988). Hence, once formed, the precipitate probably has an amorphous composition intermediate between crystalline aluminium phosphate and hydroxide solids. The precipitation reactions are dependent on phosphate concentration and pH, with the optimum pH in the range 5.5 to 6.5, depending on the composition of the wastewater (Wiechers, 1987). To account for this variability, generalised formulae of the type $Al(PO4)_x(OH)_{3-x}$ are often used to describe the precipitate (Jiang and Graham, 1998).

The flocs resulting from aluminium salts are "lighter" (less dense) and slower to form than those from iron salts. D'Elia and Isolati (1992) reported that iron salts readily lead to the formation of well-developed flocs with good settling characteristics (i.e. low SVI, probably due to a "weighing down" effect of the iron atoms) but increased sludge production. On the other hand, the advantage of aluminium compounds is shown in a higher efficiency in the neutralisation of surface charges and hence in coagulation-flocculation processes (e.g. removal of turbidity) (D'Elia and Isolati, 1992).

Power et al. (1992) investigated the use of waste alum sludges (from water treatment works) for chemical phosphate removal in an activated sludge system at laboratory scale. The system selected for the experiment was of a modified Ludzack-Ettinger configuration. The alum sludges were found to have an ash content (or inorganic suspended solids, ISS) of 30 to 47%, and a COD/VSS ratio of 0.9 to 1.2 *[%ISS = (1-VSS/TSS) x 100]*. The alum sludge dosage used was 17 to 49 mg ISS/ℓ based on influent flow. After ashing, the ISS was considered to be virtually all aluminium oxide (Al_2O_3) *[Since these alum sludges originated from plants treating "soft" waters (low TDS, low alkalinity), the mass of inorganic precipitates such as calcium carbonate, magnesium carbonate and calcium sulphate in the resultant alum sludge was considered negligible]*. On this basis, and converting stoichiometrically from Al_2O_3, the aluminium dosage to the experimental reactors was approximately 9 to 26 mg/ℓ as Al. This would be equivalent to ~100 to 290 mg/ℓ as "fresh" alum ($Al_2(SO_4)_3.14H_2O$). Phosphate removal was measured by difference between parallel control and experimental systems, with alum sludge dosed into the latter. Biological P removal was discouraged (though not eliminated) by dosing nitrate to the anoxic reactor in order to prevent it becoming anaerobic. Neglecting experimental periods when the systems were considered to be far from steady-state, linear regression data suggested that the additional (chemical) P removal due to alum sludge addition amounted to ~0.34 mgP/mg Al dosed. If the stoichiometry for $AlPO_4$ precipitation is accepted (1.15 mgP/mg Al), then chemical P removal in the experimental system with waste alum sludge was just under one third of the stoichiometric amount. Steady-state VSS in the test reactor receiving alum sludge increased in direct proportion to the additional VSS dosed as alum sludge, indicating that the water works sludge is unbiodegradable. A marked improvement in DSVI was noted for the test system receiving alum sludge. However, the alum sludge tended to increase the turbidity and COD of the system effluent; as much as 50% of the alum sludge COD was estimated to have escaped in the effluent as unbiodegradable soluble COD. Dewaterability of the sludge from the test and control systems was very similar, although tending to be slightly better in the test system. This indicated that the poor dewaterability of the aluminium hydroxides significantly improved in the activated sludge system. Power et al. (1992) speculated that this improvement in dewaterability of the alum sludge was due to a reduction in aluminium hydroxide due to the formation of aluminium phosphate in the activated sludge reactor.

Poly-aluminium chloride (PAC), sometimes called poly-aluminium hydroxy-chloride, has also been tested for phosphate removal in wastewater treatment. According to Hahn (1992), "aluminium polymers" are more readily available for technical processes than pre-polymerised iron species. The advantage of pre-polymerised polynuclear hydroxo species such as PAC is that they are more efficient in destabilising colloids (therefore probably better for coagulation of organic matter) and not very dependent on mixing intensity compared to non-hydrolysed metal salts (e.g. alum). When dosed into water, the unhydrolysed metal salts need to hydrolyse and form metal hydroxides *in situ* before coagulating/precipitating properties are exerted. With decreasing mixing intensity, the metal salts become less efficient because optimal hydroxide formation requires rapid dilution of the metal ion before a pH change occurs (Hahn, 1992).

D'Elia and Isolati (1992) studied PAC in tandem with ferric chloride for simultaneous phosphate precipitation in a seasonally overloaded conventional activated sludge plant. Ferric chloride (50 mg/ℓ) dosed to the influent of an aeration basin, along with 30 mg/ℓ PAC in the return sludge, gave phosphorus reductions of approximately 85%, compared to 50 to 70% phosphorus reduction for 120 mg/ℓ ferric chloride alone in a previous year for the same plant. This suggested that the PAC+$FeCl_3$ system was more efficient in simultaneous precipitation.

Lime/calcium salts

The addition of lime (or other calcium compounds) for simultaneous P precipitation in activated sludge systems has been proposed (Jenkins et al., 1971). Phosphate precipitation with calcium results in the formation of apatites (e.g. $CaHPO_4$; $Ca_4H(PO_4)_3$; $Ca_3(PO_4)_2$) or hydroxyapatite ($Ca_5(PO_4)_3OH$) (Arvin, 1979). However, precipitation with lime or calcium salts is probably not as cost effective as the use of iron or aluminium salts (Arvin, 1985). One of the drawbacks of using lime or calcium salts is that P precipitation occurs at high pH (ca. 9), which would be outside of the optimal pH range for most biological processes. Moreover, relatively high doses of lime are required to achieve the necessary pH for calcium phosphate precipitation. According to Aspegren (1995), at pH 8.6 redissolution of calcium phosphate precipitate occurs at phosphate concentrations of <3 mgP/ℓ, while at pH 7.0, redissolution occurs at <90 mgP/ℓ (approximately). Special effluent discharge standards which restrict phosphorus usually call for maximum concentrations in the region of 0.5 to 2 mgP/ℓ. For these reasons, lime dosing was not considered as part of this investigation.

Advantages and disadvantages of simultaneous precipitation

Advantages

There is demand from treatment plant managers for a simple and flexible technology that combines nitrification, denitrification, and

simultaneous phosphate precipitation (D'Elia and Isolati, 1992). Wiechers (1987) pointed out that simultaneous phosphate precipitation offers the following advantages:

- Ease of operation (flow proportional dosing not necessary due to the retention of precipitate in the biomass, and continuous recycling from the secondary clarifiers back into the process).
- Flexibility to changing conditions (e.g. in reponse to influent characteristics and final effluent phosphate concentrations).
- Low capital costs, not requiring tertiary solids separation facilities, as with post-precipitation.
- Relatively small additional solids production.
- Improvements in sludge settleability and dewaterability.
- Low effluent phosphate levels are possible, and improved COD and suspended solids removal give a higher quality final effluent.
- Chemicals can assist in controlling activated sludge bulking and foaming.

Disadvantages

There may be certain disadvantages associated with chemical precipitation, and simultaneous precipitation in particular. These include the following:

- Increased dissolved solids (salinity) load on the receiving water, mainly in the form of either chloride or sulphate.
- Increased sludge production: For example, from a survey of fifteen conventional activated sludge plants (probably high rate, or short sludge age plants) in Canada, Schmidtke (1985) reported an average increase in sludge production as a result of chemical dosing of 26% on a mass basis and 35% on a volume basis. There appears to be a paucity of similar data for BNR plants (which usually operated at intermediate to long sludge ages) but an increase in sludge production in proportion to chemical dose may also be expected for such plants.
- Inhibitory effects on the biological process: Direct toxicity to the biological reactions at typical metal salt doses for normal domestic/mixed domestic-industrial wastewaters has not been reported at pH values close to neutral (Arvin, 1985). The most likely negative effect of chemical addition is pH depression, with nitrification processes being particularly pH-sensitive (Bliss et al., 1994). Most metal salt solutions are acidic with a free acid content as high as 1% m/m for some ferric chloride solutions (Reynolds, 1996). Furthermore, the chemical reactions of hydroxide formation and precipitation consume alkalinity (Loewenthal et al., 1986).
- Need for pH and alkalinity correction: The loss of alkalinity due to chemical dosing may necessitate alkalinity supplementation in areas with low alkalinity wastewater. Lime addition is most often used for this purpose. Minimum residual total alkalinity in the treated effluent of around 75 mg/ℓ as $CaCO_3$ has proved suitable for sustaining nitrification (Bliss et al., 1994). WRC (1984) recommended a minimum alkalinity in the region of 40 to 50 mg/ℓ as $CaCO_3$ to keep the pH of the system above 7.0. and minimise corrosion of concrete structures.
- Effluent turbidity: Simultaneous precipitation has been reported to produce turbid effluents in some cases (Wuhrmann, 1968; Barth and Ettinger, 1967; Eberhardt and Nesbitt, 1968). This may be due to inhibition of protozoa (scavengers for free bacteria in activated sludge) or pH effects where chemical addition depressed the reactor pH to the range 5.5. to 6.5 in some cases.
- Inhibition of biological P removal: Evidence has emerged that simultaneous dosing of metal salts into BNR activated sludge plants exhibiting BEPR results in partial inhibition of the BEPR mechanism. This evidence is reviewed in the next section.

Effect of simultaneous chemical dosing into full-scale BNR plants

There is anecdotal evidence to suggest that sustained iron dosing to an activated sludge plant diminishes the plant's capacity to remove phosphate, necessitating higher and higher doses (Lötter, 1991). For this reason, Lötter (1991) carried out a number of batch tests with iron salts added to mixed liquor from BNR plants in the Johannesburg area (South Africa). From these batch tests, as well as observations made at full-scale, Lötter (1991) concluded that:

- Continuous iron dosing to an activated sludge plant appeared to produce a "progressive inhibitory effect …. on the biological as well as the chemical process".
- The results of fractionation studies "demonstrated clearly that polyphosphate storage is inhibited by the addition of iron salts. The exact mechanism involved here requires further study".
- Ferric phosphate precipitation is inhibited by "prior iron treatment", as suggested by laboratory batch tests, and to some extent borne out by performance of two full-scale plants.
- From full-scale data, a beneficial effect apparently resulted from reducing the iron dose for a short time. Again, the underlying mechanism was unclear, but it was hypothesised that ferric phosphate build-up in the sludge inhibits the precipitation process. This posed an operational dilemma since effluent quality would not comply with the required standard while the sludge apparently recovered its precipitation propensity.
- The addition of iron to an area of high phosphate concentration (e.g. anaerobic zone) "clearly had a beneficial effect on the precipitation process. The effect of this on the biological process still has to be determined".
- In view of the apparent inhibitory effect of iron dosing on the chemical and biological processes, addition of iron salts in the primary treatment stage (prior to primary sedimentation or sludge fermentation) was advocated for further investigation.

From a re-interpretation of the data of Lötter (1991), a degree of uncertainty exists over the actual extent to which the biological mechanism is inhibited by continuous simultaneous chemical addition. Lötter's postulate that such inhibition can arise appears to have been heavily based on the fractionation studies, which will be examined in detail in the next section of this paper. Lötter (1991) discarded the results of anaerobic batch tests for measuring the magnitude of biological P fractions in activated sludge because samples from a control system (without iron dosing) showed less P release (i.e. weaker BEPR) than those in the presence of iron dosing. Apparently these results contradicted Lötter's postulate and were rejected mainly on that basis. Similarly, Lötter (1991) gave little or no information on the history of the activated sludge systems (e.g. the actual P removal performance) from which the samples for batch tests were taken. Lötter (1991) postulated that chemical precipitation efficiency itself is reduced by repetitive chemical dosing, but this postulate was heavily based on aerobic batch tests with only two repeat steps of chemical addition. Furthermore, the concentrations of both phosphate and iron added in the batch tests were much higher than those typically encountered in full-scale BEPR plants. The possible effect of aeration of the

control mixed liquor in these experiments was also not considered (e.g. CO_2 stripping and resultant pH effects - Wentzel et al., 1986; or so-called secondary P release - Barnard, 1984). In closing, Lötter (1991) did note that her results could not be considered unequivocal and advocated further study into the mechanism of combined chemical and biological phosphate removal.

Lötter (1991) also presented evidence from monitoring of full-scale plants which indirectly appeared to support the hypothesis that chemical addition negatively affects the biological P removal mechanism. For example, with ferric sulphate dosing to the return sludge lines of parallel modules at Northern Works (Johannesburg), it was observed that P removal was weaker and more erratic in a module with the Johannesburg (pre-anoxic zone) configuration, compared with the older 5-stage Phoredox configuration. This was contrary to expectations of better biological P removal performance with the Johannesburg configuration (Osborne et al., 1986; 1989; Pitman, 1991), and was taken by Lötter (1991) to be caused by chemical dosing, without detailed explanation. Similarly, ferric chloride dosing at Olifantsvlei Works was successful over a period of approximately 2.5 years after which it apparently became increasingly subject to upsets in terms of the effluent failing to comply with the 1 mgP/ℓ orthoP standard. The dose was increased to a Fe:P mass ratio of >1.8:1 (up to 3:1), reportedly without success. However, all the possible reasons for the plant failure were not discussed. Two relevant points in this regard (briefly mentioned by Lötter, 1991) were that the load on the plant increased prior to one period of unsatisfactory compliance; and that cessation of dosing "for a few days" produced a recovery. However, for the principal event recorded in this context, the recovery in effluent P concentrations was dramatic (from 6 mgP/ℓ to <0.5 mgP/ℓ), leaving the question whether changes in influent load did not influence the observations. Supporting data, such as nitrate recycles to the anaerobic zone, influent load and flow, were not given. According to Lötter and Pitman (1992, cited by Boyd and Lötter, 1993), success at Olifantsvlei was subsequently achieved with a ferrous/ferric chloride blend and refined dosing control.

Boyd and Lötter (1993) hypothesised that inhibition of the BEPR mechanism by iron salts is caused by ferric hydroxide precipitate "using up" hydroxyl ions necessary for the hydroxyl mediated transport process for phosphate across bacterial cell membranes. To investigate this hypothesis, Boyd and Lötter (1993) operated two pilot plants of unspecified size in 3-stage Phoreodox configuration at a 14 d sludge age. Ferrous sulphate was dosed to the anaerobic reactor of one pilot plant while the other plant served as control. Samples of mixed liquor were subjected to fractionation according to De Haas (1991), as well as microbiological tests on enrichment culture isolates from each of the aerobic reactors. According to Boyd and Lötter (1993), the experimental reactor (iron dosed) gave average effluent orthoP concentrations of 1.1 (± 0.3) mgP/ℓ, while the control reactor performed better with 0.7 (± 0.3) mgP/ℓ in the effluent. There was evidence of a lower intracellular polyP (IPP) concentration in the aerobic reactor of the test system relative to the control. However, a large unexplained decrease in biomass IPP concentration occurred in both the test and control systems in the middle of the experimental period, which diminishes confidence in these results.

In general terms, the work of Boyd and Lötter (1993) appeared to confirm the conclusion of Lötter (1991), namely, that BEPR is inhibited by continuous simultaneous chemical (iron) dosing. Similarly, from fractionation studies, Röske and Schönborn (1994a) concluded that in cases with addition of (iron) precipitants to an anaerobic-aerobic system, the biological P-elimination by bacteria is substantially reduced. Röske and Schönborn (1994b) found that the rate of P release (to the supernatant) under anaerobic conditions is lower in systems with simultaneous addition of ferric ions but it is not clear whether this was due to a purely biological effect or to a chemical effect in which biologically released P is adsorbed to (or complexed with) iron hydroxide bound in the sludge mass.

Chemical fractionation methods

The objective of this study was to attempt to define more clearly whether the BEPR mechanism is inhibited in the presence of chemical addition and if so, under what conditions. Clearly, this objective requires that the chemically-precipitated P content of activated sludge liquor be measured distinctly from the biologically-stored phosphate (or polyP) fraction.

Methods using cold perchloric acid

It is possible to study the composition of phosphate compounds stored or bound in a wide range of starting materials by means of chemical extraction. Such techniques are generally termed fractionation methods and have been applied to soils, sediments, sludges and other forms biomass originating from animals, plants, fungi and bacteria. Many different chemical solutions and extraction methods have been applied in a variety of fractionation procedures. Kulaev (1979) reviewed a number of these in so far as they relate to phosphate compounds of biological origin (notably polyphosphates and nucleic acids). De Haas (1989a, 1991) and Christensson (1997) reviewed fractionation methods which have been applied specifically to activated sludge and found that such methods may offer a useful tool for quantifying the P compounds which accumulated via chemical vs. biological P removal mechanisms. De Haas (1989a, 1991) also pointed out certain weaknesses of the available chemical fractionation methods when applied to activated sludge, such as the type of acid used as extractant and hydrolysis of polyP even at cold temperatures with commonly used acids. However, De Haas (1989a) was able to show that provided extraction times were kept to a minimum and cold extraction temperatures adhered to, the contribution of polyP hydrolysis to the orthoP fraction was relatively insignificant when extracting with cold perchloric acid. Good solubilisation of chemical precipitates (as orthoP) was obtained with cold PCA (De Haas (1989a). De Haas and Greben (1991) applied a procedure which incorporated a cold PCA step to a number of samples taken from full-scale BEPR plants in the Johannesburg area, noting an apparent increase in the "chemical" fraction and a decrease in the "biological" (polyP) fractions in response to iron salt dosing (see also Lötter, 1991). One of the weaknesses in the results of De Haas and Greben (1991) was that the two activated sludge modules were not subjected to parallel fractionations on the same day. Part of the reason for this was that the fractionation procedures (De Haas, 1989a) tended to be too tedious for fractionation of more than one mixed liquor sample on the same day. Storage of mixed liquor samples by freezing prior to fractionation had proved to be unreliable (De Haas and Dubery, 1989; Blonda et al., 1994).

De Haas (1989a) also drew attention to other limitations of the fractionation procedure used. Batch tests indicated that *in vitro* addition of relatively small amounts of phosphate and ferric sulphate to mixed liquor from a BEPR plant immediately before fractionation, gave a disproportionately large increase in the orthoP content of the sludge, compared with the control. Surprisingly, most of this additional orthoP was extracted in a dilute alkaline fraction, and not in the acid fraction. Furthermore, the polyP fraction decreased after the addition of ferric sulphate, relative to

the control. A similar batch experiment with *in vitro* ferrous sulphate addition did not produce the same result (De Haas, 1989a). With ferrous sulphate, the added P could be accounted for more closely by the increase in the orthoP of the PCA extract, although an over-recovery was still noted. Compared to the control, small shifts in other P fractions also occurred: some orthoP shifted from the alkaline extracts to the perchloric acid extract, and a form of complex P appeared in the supernatant. These fractionation results could not be satisfactorily explained (De Haas, 1989a; De Haas and Greben, 1991).

De Haas (1989a) speculated that complexes between iron, phosphate and biomass components (e.g. carbohydrates or proteins) may form upon chemical addition. Such complexes could explain extraction of phosphate under alkaline rather than acidic conditions in the fractionation procedures used. Proteins tend to precipitate in ice-cold acid solutions but dissolve more readily in alkaline solutions at room temperature (Munro and Fleck, 1966). Similarly, Gehr and Henry (1983) found that extracellular "biopolymer" could be stripped from mixed liquor solids by chemical (and physical) means in the presence of 0.04 M dibasic potassium phosphate (K_2HPO_4), which has an alkaline equivalence point of pH 9.75 (Loewenthal and Marais, 1976). It was further suggested by De Haas (1989a) that addition of reactive iron species (particularly Fe^{3+}) may cause membrane disruption and result in rapid enzyme-catalysed hydrolysis of polyphosphate closely linked to the cell membrane(s).

Given the above uncertainties, the work of Lötter (1991), De Haas and Greben (1991) and Boyd and Lötter (1993) did not indicate conclusively that simultaneous chemical addition inhibits the biological P removal mechanism. De Haas (1989a) concluded that it is not possible to tailor a crude chemical fractionation procedure to suit specifically the extraction of polyP separately from chemical P precipitates in a complex medium such as activated sludge. The need to develop more powerful analytical techniques to determine the nature, chain-length and masses of stored polyP from activated sludge was highlighted, as was the need to carry out further fundamental research into the interaction between biological and chemical P removal mechanisms. Nevertheless, with careful interpretation of the results, chemical fractionation procedures of the type used by De Haas (1989a) can give a broad classification and measurement of chemical vs. biologically accumulated forms of P in activated sludge.

Methods without cold perchloric acid

Witt et al. (1994) studied the interactions between biological and physico-chemical mechanisms in biological phosphate removal of activated sludge systems, applying a chemical fractionation procedure developed by Psenner et al. (1984) for lake sediments. The work was based on a laboratory-scale Phoredox plant fed raw sewage supplemented with acetate but not phosphate. The raw sewage was "soft", containing 15 mg Ca/ℓ and 11 mg Mg/ℓ. Phosphate was not added to the sewage. Unfortunately, Witt et al. (1994) did not report a control system to determine whether background fluctuations in the influent cation composition could have explained changes in the supposed physico-chemical fractions. Nevertheless from the data collected, Witt et al. (1994) suggested that the chemical and biological mechanisms are antagonistic (i.e. competitive). In the absence of added metal ions, the biological mechanism maybe expected to dominate, as observed from the experimental results (Witt et al., 1994).

It is worth noting that during batch experiments, Witt et al. (1994) were able to show that most of the anaerobic P release (increase in supernatant soluble reactive P, SRP) was associated with a decrease in the NaOH-non reactive P (NRP) fraction (i.e. polyP fraction). However, the bicarbonate-dithionite (BD) orthoP and NaOH orthoP fractions did show detectable increases also, suggesting that some 'entrainment' of phosphate in chemical precipitates did occur. The BD-NRP fraction (i.e. proposed Ca or Fe-bound polyP) showed a decrease during the anaerobic period. It is surprising that these changes in the physical-chemical fractions, although relatively small, were reversible during the aerobic period. Witt et al. (1994) did not attempt to explain the mechanism by which the chemical and biological fractions interact. Nevertheless, they concluded that:

- Anaerobic breakdown of NRP (mainly polyP) fractions is connected not only with P release to the soluble phase, but to a lesser degree also with an increase in the particulate (sludge) orthoP fractions which are considered to be chemically bound.
- Aerobic increase in the NRP fractions is caused not only by a transition of orthoP from the soluble phase into the particulate (sludge) phase, but also by transfer within the particulate phase from the orthoP to the NRP fractions.

Röske and Schönborn (1994a) applied the modified Psenner fractionation procedure to activated sludge samples from bench-scale and full-scale plants. A two-stage bench-scale (anaerobic-aerobic) process was operated at short sludge age to inhibit nitrification. P was not added to the influent and almost complete P removal was achieved on average. Ignoring the soluble (supernatant) phase, the mixed liquor typically contained about 27 mg P/g MLSS. Of this, around 24% was extracted into the bicarbonate-dithionite (BD) fraction and 66% into the NaOH fraction. NMR spectroscopy confirmed that polyP was present in the NaOH extract. The BD fraction was interpreted as containing chemical phosphate species solubilised under strictly reducing conditions, namely: Fe-phosphate or Fe-hydroxide complexes (extracted as orthoP); and Fe-polyP complexes (as NRP). About two-thirds of the TP in the BD extract was in the form of SRP, i.e. orthoP, and by difference, the other one-third was taken as NRP. Röske and Schönborn (1994a) pointed to the low influent iron concentration (ave. 0.2 mg Fe/ℓ) as the reason for only a small proportion of the phosphate being bound in complexes extracted in the BD fraction. However, with such low iron concentrations in the influent, it was surprising that as much as 24% of the mixed liquor TP occurred in the form of (supposed) iron complexes. Furthermore, no removal of iron across the system was noted (influent = effluent = 0.2 mg Fe/ℓ). Cation analysis of the fractions from the bench-scale plant were found by Röske and Schönborn (1994a) to contain principally calcium in the BD, HCl and NaOH extracts, in that order of decreasing concentration. This suggested that chemical precipitation of calcium phosphate (apatite or hydroxyapatite) had occurred in the system. This was supported by the comparatively high pH (7.9) and an average removal of 6 mg Ca/ℓ normally measured in the bench-scale plant (Röske and Schönborn, 1994a).

Röske and Schönborn (1994a) compared the results from their bench-scale plant to two full-scale activated sludge plants: Berlin-Münchehofe, in which iron was dosed for chemical P removal, and Berlin-Marienfelde, which is operated for biological P removal supplemented by simultaneous precipitation using a mixture of aluminium and iron salts. Röske and Schönborn (1994a) found that for the Berlin-Münchehofe sludge, most (65%) of the mixed liquor phosphate was extracted in the BD fraction, with only ca. 30% in NaOH extract. By contrast, the mixed liquor phosphate from Berlin-Marienfelde was similar to the bench-scale plant, with

about 15% of the TP extracted in the BD fraction and 72% in the NaOH fraction. In the case of the Berlin-Marienfelde sludge, iron was predominantly extracted in the BD fraction (70%) and to a lesser degree in the HCl fraction (20%); most of the aluminium (75%) was extracted in the NaOH fraction, with the remainder mainly in the HCl extract. Unfortunately Röske and Schönborn (1994a) did not report the percentage recovery of mixed liquor Total P and cations in the fractionation procedure. Nevertheless, their results suggest that the modified Psenner fractionation procedure was able to distinguish the degree of biological P removal from chemical P removal in activated sludge systems with simultaneous chemical addition. Although parallel test and control systems were not operated, the comparison between bench-scale (biological) and full-scale (combined chemical-biological) systems suggested that the P fractions of biological origin were "substantially smaller" in the presence of simultaneous chemical dosing (Röske and Schönborn, 1994a). To assist with interpretation of the fractionation data in such cases, it appears that knowledge of the extent of "background" chemical P fixation in the activated sludge without chemical dosing would be useful.

Röske and Schönborn (1994b) carried out further work with chemical dosing on a bench-scale plant. In sequential experimental periods, Röske and Schönborn (1994b) added respectively 3 and 6 mg Fe/ℓ as ferric chloride (based on influent flow rate) to the aerobic reactor of a 2-stage (anaerobic-aerobic) bench-scale system. Unfortunately, a full comparison of results before and after addition of precipitants was not presented by Röske and Schönborn (1994b). For example, it is not clear whether the systems were operated with "surplus" phosphate in the effluent, or under potentially P-limiting conditions in which the chemical and biological mechanisms would "compete" for available orthoP. The extent of P release in the anaerobic reactor (on a mass basis) of the system was also not reported, nor was the total P content of the mixed liquor before and during iron dosing given. This makes it difficult to interpret the fractionation results presented. However, Röske and Schönborn (1994b) did report from batch experiments that the rate of P release and P uptake was inhibited with the addition of ferric chloride to the bench-scale plant from which the mixed liquor samples were taken. Compared to the period without addition of ferric iron, the addition of 3 mg Fe/ℓ caused the rate of P release to be inhibited by 50%, while the rate of uptake was inhibited by 30%. An increase in the ferric iron dose to 6 mg Fe/ℓ, resulted in approximately the same degree of inhibition of the P release rate, but the rate of P uptake was inhibited by a further 30%.

Röske and Schönborn (1994b) were not able to fully extrapolate the results of the batch rate tests to their bench-scale plant. They reported that in the presence of Fe addition, "the release of P in the anaerobic tank" and the "proportion of polyP" (NaOH-NRP fraction) were not as high as prior to iron addition. Careful examination of the pie charts presented by Röske and Schönborn (1994b) suggests that the mass of P in the NaOH-NRP fraction did not decrease with iron addition and may even have increased by 5 to 10%, depending on the degree to which the system was operating at steady state. An increase in the BD-SRP fraction in the presence of iron addition was clearly discerned (Röske and Schönborn, 1994b), confirming the likely extraction of Fe-P complexes in this fraction. The BD-NRP fraction, which could contain Ca-polyP or Fe-polyP complexes (Witt et al., 1994), appeared to decrease to a variable degree in the presence of iron addition. This finding may be linked to the observation by Röske and Schönborn (1994b) that calcium was much less prominent in X-ray spectra of sludge samples taken during iron dosing, compared to those without metal addition.

In summary, the results of Röske and Schönborn (1994a, b) still leave room for doubt over the actual extent of inhibition of the biological P removal mechanism in the presence of simultaneous chemical dosing. It seems clear, however, that chemical fractionation procedures of the type used by Röske and Schönborn (1994a, b) offer the possibility of crudely quantifying the chemical versus biological P fractions accumulated in activated sludge mixed liquor.

Lindrea et al. (1994) applied the fractionation method of Clark et al. (1986) to activated sludge samples from full-scale and laboratory-scale BEPR systems. In the majority of samples tested, P storage occurred mainly in the "long chain polyP" (LCP) fraction extracted with phenol-chloroform solvent. The other important fractions, namely, the "short chain polyP" (SCP) fraction extracted with cold trichloroacetic acid (TCA)/TCA-acetone solvent, and the "granular P" fraction extracted with phenol-chloroform solvent, were numerically smaller on a P/VSS basis and showed smaller changes during batch anaerobic-aerobic tests as well as full-scale plant monitoring. Lindrea et al. (1994) did not attempt to distinguish differing physiological roles for the respective polyP fractions observed. Unfortunately, they did not report fractionation results for sludge from the full-scale plant during a period when major deterioration in BEPR was recorded due to a suspected influent Mg limitation. However, laboratory-scale trials showed that during Mg limitation, both the SCP and LCP fractions were depressed relative to the control (Lindrea et al., 1994).

Müssig-Zufika et al. (1994) compared various chemical fractionation methods with a view to isolating intact polyphosphate chains from activated sludges associated with biological P removal and a pure culture of *Acinetobacter*. It was not specifically stated whether pH control was applied to the culture. The growth phase during which the cells were harvested was also not stated. Apparently working from the assumption that the true orthoP content of the biomass is negligible, Müssig-Zufika et al. (1994) reported that using the method of Mino and Matsuo (1985) (which includes cold PCA extraction), 24% of the polyP was hydrolysed to orthoP. The methods of Psenner et al. (1984) and Fitzgerald and Nelson (1996) gave approximately 5% and 10% polyP hydrolysis to orthoP respectively, while the method of Clark et al. (1986) gave only 1% hydrolysis. They concluded that only the method of Clark et al. (1986) was acceptable for further investigation. They pointed out the limitation that the method of Clark et al. (1986) is unsuitable for Gram-positive organisms since it failed to extract polyP from a culture of Gram-positive organisms which apparently contained polyP granules when examined by electron microscopy.

Müssig-Zufika et al. (1994) developed a new method partly by combining various steps of published procedures. Their modified method involved the formation of three fractions from the sludge pellet obtained after removal of the supernatant of the original sample:

- **Fraction I** - The combined extracts obtained in series from extraction with EDTA (pH 7), followed by trichloroacetic acid (TCA) and TCA-acetone-water.
- **Fraction II** - The combined extract of two steps (including a freeze-thaw cycle) involving extraction with methanol, chloroform, sodium hypochlorite and an unspecified amount of ammonium sulphate at pH 7 to 8.
- **Fraction III** - The pellet resuspended in distilled water, frozen until needed for analysis, thawed, centrifuged and the supernatant taken. Any residue was discarded.

Müssig-Zufika et al. (1994) also used a novel analytical technique that allowed on-line detection of polyP after separation according

to molecular weight by HPLC (using either an ion exchange column for polyP chain lengths of P1 to P4, or a gel column which separates high molecular weight polyP of >P45). Phosphate emerging from the column was determined by means of an automated vanado-molybdate colorimetric method, with post-column hydrolysis of polyP to orthoP in the presence of 6.5% (ca. 1.4 M) nitric acid at 140°C in an oven. According to Müssig-Zufika et al. (1994), organic phosphates are not hydrolysed under these conditions, since such compounds require the presence of an oxidising agent.

Evaluation of fractionation methods

Müssig-Zufika et al. (1994) were critical of fractionation procedures other than that of Clark et al. (1986) and their new method, when applied to pure cultures of *Acinetobacter*. Appraisal of the work of Müssig-Zufika et al. (1994) hinges on the envisaged purpose of the fractionation procedure. The following main observations may be made:

- The objective of Müssig-Zufika et al. (1994) was to attempt to isolate polyP from activated sludge or pure culture samples in as intact a state as possible. They did not report an objective of attempting to determine the relative importance of chemically precipitated phosphate in the biological samples examined. Their work clearly met the stated objective. *[It was of interest too that aerobic sludge from the full-scale works tested (Berlin-Ruhleben) contained only a modest amount of stored total P (around 22 mgP/g MLSS) and showed about equal quantities of polyP in the chain length range P5 to P40 as in the range P70 to >P100. On the other hand, the sample of aerobic sludge from a pilot-plant demonstrating stronger P removal (total P in sludge = 50 mgP/g MLSS) showed about 90% of the polyP in the higher molecular weight range (P70 to >P100)].*
- With respect to organic P (by their analytical definition given above), Müssig-Zufika et al. (1994) found that the sludges from the full-scale plant contained about 10 mgP/g MLSS and the pilot plant sludges contained about 18 to 20 mgP/g MLSS. No comment on this difference was given. Müssig-Zufika et al. (1994) did not report the %VSS content of their sludges. This makes comparison of the organic P content difficult, particularly where the sludges contain large variations in total P content. For sludges with a total P content in the range 39 to 55 mgP/gMLSS from three full-scale plants in South Africa, De Haas (1989a;b) found a nucleic acid P content (based on determination of the pentose sugar content of RNA and DNA) of around 16 to 20 mgP/g VSS (10 to 15 mgP/g MLSS), which is in general agreement with the results of Müssig-Zufika et al. (1994) for Berlin-Ruhleben. For two pilot plants fed a synthetic sewage (using acetate or propionate as main substrate, similar to those of Müssig-Zufika et al., 1994) and producing a sludge of about 150 to 180 mgP/ gMLSS, De Haas (1989a;b) found a nucleic acid P content of 9 to 12 mgP/g VSS (6 to 7 mgP/g MLSS). On the one hand, this suggests that where the sludges contain relatively large masses of polyP, the method of Müssig-Zufika et al. (1994) may have over-estimated the organic P content to a degree; on the other hand, De Haas (1989a,b) measured nucleic acid sugar directly and assumed other organic phosphates to be negligible.
- With respect to orthoP, using a fractionation method which did not include PCA, Müssig-Zufika et al. (1994) reported 20 to 23% of the sludge total P as orthoP for sludges from the full-scale plant, and 2 to 5% as orthoP for their pilot plant. These results are not dissimilar to those reported by De Haas (1989a, 1991) using a fractionation procedure including cold PCA for samples of sludge from four different full-scale biological P removal plants in South Africa not receiving chemical supplements. These results do not support the finding by Müssig-Zufika et al. (1994) that much of the polyP extracted into cold PCA is hydrolysed to orthoP. This finding is also contradictory to a large number of other studies in which a fractionation procedure including cold 0.5M PCA has been used for extracting large quantities of polyP from activated sludge and pure cultures (*inter alia* Harold, 1962; 1963; Kulaev, 1979; Lötter, 1985; Murphy and Lötter, 1986; Mino and Matsuo, 1985; Mino et al., 1985; 1987; Ohtake et al., 1985; Miya et al., 1987; Van Groenestijn, 1988; Appeldoorn et al., 1992).
- A critical issue is whether or not pH control is applied during the growth of pure cultures for P removal studies. Amongst others, Wentzel et al. (1988) and De Haas (1989a) drew attention to the importance of pH control in pilot plants fed large amounts of fatty acids in order to prevent chemical P precipitation. This point was not mentioned by Müssig-Zufika et al. (1994). Several researchers (*inter alia* Gerber et al., 1987; De Haas, 1989a, 1991; Streichan and Schön, 1991; Appeldoorn et al., 1992) have reported the importance of controlling pH close to neutrality when growing pure cultures of *Acinetobacter* in order to prevent the chemical precipitation of P from the liquid growth medium. For example, Appeldoorn et al. (1992) were able to grow cultures of *Acinetobacter* (under pH controlled conditions) containing up to about 20% of their total P content (or about 17 mgP/g dry weight) in the form of cold PCA-extractable polyP (or LPP) determined by difference between total P and orthoP content of the extract. The orthoP content of these cold PCA extracts never exceeded 3 mgP/ g dry weight and the organic P content (determined by adsorption to powdered activated carbon) never exceeded 2-3 mgP/g dry weight (Appeldoorn et al., 1992). Again, these results are not consistent with the results of Müssig-Zufika et al. (1994).
- It is entirely plausible that cold 0.5 M PCA will not extract polyP in an intact state. This conclusion was also reached by De Haas (1989a, 1991) from attempts to use gel chromatography to characterise the chain length of polyP extracted from activated sludge. However, several studies have shown that the contribution of acid hydrolysis of polyP to the orthoP result in cold PCA extracts is relatively insignificant, provided the samples are kept cold and orthoP analysed immediately (De Haas, 1989a; Blonda et al., 1994; Kerdachi and Roberts, 1985). These results also do not support the conclusion of Müssig-Zufika et al. (1994) concerning polyP hydrolysis in cold PCA.
- Müssig-Zufika et al. (1994) recommended a fractionation procedure which used 2 mM EDTA, 2% TCA and 0.7% TCA in acetone-water for the extraction of orthoP complexes of from the biomass. De Haas (1991) compared fractionation procedures in which the first step (following preliminary removal of the supernatant and washing on the sludge pellet) was either: (A) extraction with 50 mM EDTA at room temperature followed by 1% (61 mM) TCA at 0°C; or (B) 0.5M PCA at 0°C. The two procedures were applied in parallel to the same activated sludge sample showing good BEPR activity without chemical supplements. For Procedure A, the orthoP content of the sludge was found to be higher than for Procedure B. This contradicts the findings of Müssig-Zufika et al. (1994) which suggested that an EDTA-TCA based procedure would minimise hydrolysis of polyP and give a lower orthoP result.
- De Haas (1989a) discontinued the use of TCA-based

fractionation procedures for activated sludge on the basis that neither 1 to 2% TCA nor 0.7% TCA in acetone-water solvent at 0°C satisfactorily dissolved chemical precipitates of ferric (hydroxy) phosphate, calcium phosphates and magnesium ammonium phosphate. On the other hand, 0.5M PCA at 0°C gave >98% recovery of phosphate for the same chemical precipitates in three steps, and usually >80% recovery in the first one or two steps. The same conclusion was reached by Appeldoorn et al. (1992), both in the presence and absence of pasteurised sludge containing at least 60 mgP/g dry weight. Kerdachi and Roberts (1985) reported similar results and drew attention to the fact that metal perchlorates are generally considered very soluble in water.

In summary, it follows that the procedure used by Müssig-Zufika et al. (1994) was developed for optimal extraction of intact polyP chains from activated sludge or pure cultures. It could not be reliably used to determine the relative contents of sludge orthoP (largely of chemical origin) and polyP (biological origin), nor for estimating the interaction between these two broad groups of compounds when chemical precipitants are deliberately dosed into the activated sludge system.

Iron hydroxide and the possible role of extracellular polymers

In reviewing the development of P fractionation procedures for activated sludge, reference was made to speculation by De Haas (1989a) that complexes between phosphate, metal ions (especially iron), and cell membranes (or some form of extracellular biopolymer) may occur in activated sludge. In this context, it may be significant that Bark et al. (1992) reported considerable adsorption of phosphate to extracellular polymers (ECP) of a pure culture of *Acinetobacter*. ECP would comprise polysaccharides, proteins and similar macromolecules with numerous sites for complexing metal ions.

Brown and Lester (1979) reviewed the role played by extracellular polymers in the removal of metals by activated sludge. They pointed out that a considerable proportion of metals entering a wastewater works are generally removed in the activated sludge process. Metal removal efficiency in this process is usually >60% and sometimes >90%. Specifically, in the case of iron, Brown and Lester (1979) noted a high removal efficiency (87 to 98%). These results are in agreement with the those of Rabinowitz and Marais (1980) in this respect.

From the review by Brown and Lester (1979), there appears to be good evidence that bacterial cell flocs in pure cultures, as well as extracellular polymers from these cultures or from activated sludge, can adsorb large quantities of metal ions from solution. Extracellular polymers in activated sludge are produced by the bacteria which make up the biomass and play an important part in its flocculation. These polymers are composed mainly of polysaccharides, although proteins and nucleic acids from autolysis (cell death and lysis) may also be constituents of the polymer matrix (Brown and Lester, 1979). Different metal adsorption sites appear to exist on neutral and anionic polysaccharides. Neutral polysaccharides may bind metal cations at the hydroxyl groups of hexose or pentose sugars, exchanging with hydrogen bonds from water molecules "bound" by the polymer. Where polymers are anionic, carboxyl groups may be the metal binding sites. This type of bond is largely ionic and is much stronger than the hydrogen bonding between neutral polysaccharides and metal cations. There is some evidence to suggest that in activated sludge, the former type of complexation may occur to a greater degree than the latter since the carboxyl groups on the sludge surfaces appear to be already occupied (Brown and Lester, 1979). Nevertheless, it seems that if cations such as calcium and magnesium normally form part of the floc structure, other metal ions, including the heavy metal ions, may replace these alkaline earth metals in the sludge flocs (Brown and Lester, 1979).

He et al. (1996) studied the three-dimensional structure of newly formed ferric hydroxide. They found that the structure resembles a highly porous sponge made up of cross-linked chains. When iron salts are used in water or wastewater treatment, with the progress of hydrolysis, the iron hydroxide forms linear aggregates of colloidal polycations with a ramified cross-linked chain-like structure. As anions or negatively-charged "contaminant" colloids make contact with these aggregates, they are immediately trapped on the extensive surface provided by the three-dimensional network of polycations; this step takes a few seconds to accomplish. Depending on the nature and concentration of the trapped micro-components, one of several mechanisms might follow: adsorption, ion exchange or surface complexation.

Using X-ray coupled transmission electron microscopy (TEM), He et al. (1996) also studied the incorporation of phosphate by iron hydroxide derived from mixed liquor of a full-scale conventional activated sludge plant dosed with pickle liquor as the iron source. In summary, this work showed a close association between iron hydroxide, precipitated phosphate and activated sludge biomass, highlighting that there is a large capacity for adsorption of iron (hydroxide) to polymers of biological origin, notably extracellular polymers, which are abundant in activated sludge. On this basis, it seems inevitable that the biological and chemical P removal mechanisms of such systems will be linked. However, interaction between the two mechanisms and possible links between them are still not well understood.

Conclusions

To secure reliable compliance with low effluent phosphate standards in wastewater treatment, simultaneous chemical precipitation in modified activated sludge systems designed for BNR offers several advantages. Considerable research has been conducted into the interaction between biological and chemical P removal mechanisms in such combined systems. Some findings indicate that simultaneous dosing of metal salts at small to moderate dosages does not interfere with the biological processes. Anecdotal information from full-scale applications, as well as the results of chemical fractionation techniques, suggests that the biological P removal mechanism is inhibited by simultaneous dosing of metal salts. This would have serious implications for full-scale applications. The higher capital cost of biological P removal plants is usually justified on the basis of lower chemical consumption, in comparison with conventional activated sludge plants which depend heavily on chemical P removal. Yet many BNR plants world-wide continue to operate with simultaneous chemical addition. There is a paucity of information in the literature on the extent to which the biological mechanism continues to be viable in such plants. Accordingly, a thorough investigation of the influence of simultaneous chemical addition on the biological P removal mechanism seems warranted.

From a review of published procedures for chemical fractionation of the P compounds in activated sludge, it appears that methods based partly on extraction with a cold perchloric acid (PCA) are among the simplest and have been widely applied with reasonable success. The PCA solution allows most metal phos-

phate precipitates to be dissolved and determined as orthoP, apparently without significant interference from co-extracted "complex" phosphate species (typically polyP and organic P compounds). These complex P species can be broadly determined by difference between the total P and orthoP content of the extracts.

It is apparent that an extraction procedure incorporating exposure of the biomass to a strong acid such as PCA (even at 0°C) does not allow intact chains of polyP to be extracted. More complicated fractionation and analytical procedures would be needed to extract largely intact polyP for chain-length analysis by chromatography. Nevertheless, there is convincing evidence that, provided suitable care is taken with sample handling and that analysis of the extracts for orthoP is not delayed, the degree of hydrolysis of polyP to orthoP during extraction with cold PCA is relatively insignificant. By this means, P compounds accumulated in activated sludge by biological means can be broadly distinguished from those originating from chemical adsorption or precipitation. The purpose of the next paper (**Part 2**) in this series (De Haas et al., 2000) is to refine and test a fractionation procedure based on extraction with PCA to meet this objective, whilst at the same time keeping the procedure as easy to perform as possible.

It appears to be over-simplistic to think of chemical phosphorus removal in wastewater treatment systems as involving the direct precipitation of metal phosphate. It is most likely that a major part of the chemical P removal at relatively low concentrations in such systems involves the formation first of amorphous metal hydroxide, followed by some form of ion exchange between hydroxide and phosphate ions, probably incorporating phosphate as "bridging" groups between metal hydroxide chains. There is also some evidence from electron microscopy of direct interaction between the biological polymers (e.g. extracellular polysaccharides or related polymers) in the activated matrix and chemically precipitated metal hydroxide or metal hydroxy-phosphate. It appears improbable that the chemical and biological components operate entirely independently of each other but the methods available for measuring this interaction are very limited at present.

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