Measuring metal and phosphorus speciation in P-rich anaerobic digesters

C.M. Carliell-Marquet* and A.D. Wheatley**

* School of Civil Engineering, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
** Water Research Group, Department of Civil and Building Engineering, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK

Abstract High concentrations of soluble orthophosphate, magnesium and potassium are released during anaerobic digestion of biological phosphorus removal (BPR) sludge. This research was undertaken to investigate the effects of phosphorus enrichment on digester performance, metal and phosphorus speciation. High concentrations of soluble PO₄-P (> 250 mg/l) were found to have a retarding effect on anaerobic digestion, reducing the rate of volatile solids digestion and methane production in comparison to control digesters. This was found to be reversible after a period of time, which was related to the amount of PO₄-P added to the digesters, higher concentrations of PO₄-P requiring more time for digester recovery. Addition of magnesium and potassium to the digesters, together with PO₄-P, reduced the inhibitory effect of phosphorus enrichment but these digesters still showed lower rates of volatile solids digestion and methane production in comparison to the control digesters. Phosphorus enrichment resulted in extensive precipitation of calcium, magnesium and manganese, markedly reducing the soluble and easily available fractions of these metals. Other trace metals such as copper, zinc, chromium, nickel and cobalt actually showed increased levels of solubility as a result of phosphorus enrichment. This was thought to be caused by high levels of soluble organic carbon in the phosphorus-rich anaerobic digesters, which acted as organic ligands for metal complexation.

Keywords Calcium; fractionation; magnesium; manganese; phosphorus; sequential extraction; speciation; trace metals

Introduction
Removing soluble phosphorus from sewage by means of BPR processes, transfers the phosphorus load of a sewage treatment works (STW) from the soluble fraction to the sludge fraction (where it is held intracellularly as polyphosphate) and ultimately to the anaerobic digesters. BPR sludge digestion results in high concentrations of soluble orthophosphate being released during anaerobic digestion (Jardin and Popel, 1994; Nyberg et al., 1994; Wild et al., 1997; Sen and Randall, 1998). In addition to orthophosphate, cations such as magnesium and potassium are also released, the concentrations being determined by the molar ratio of uptake of these ions during BPR, in relation to phosphorus uptake (Jardin and Popel, 1994). Digesters treating BPR sludge commonly contain 100 to 500 mg/l of soluble PO₄-P, depending on the type of phosphorus removal process, the amount of BPR sludge being digested and the availability of metals such as calcium and magnesium for phosphorus precipitation. As anaerobic digesters usually operate at soluble phosphorus concentrations between 10 and 30 mg/l, it was hypothesised that digestion of BPR sludge could change the inorganic balance of an anaerobic digester, in turn affecting nutrient bioavailability and ultimately, digester performance.

This research was undertaken to investigate the effect of high concentrations of soluble phosphate on the anaerobic digestion process and the speciation of phosphorus and metals within these phosphorus-rich anaerobic digesters.

Materials and methods
Batch laboratory digestion studies were conducted in 100 ml serum bottles, sealed with
butyl rubber stoppers and aluminium crimp seals (Aldrich Chemical Co.). Each batch digester contained: 80 ml of digested sludge inoculum; 10 ml of synthetic sludge feed with a total organic carbon (TOC) content of 20 g/l (starch 7 g/l; toilet paper 10 g/l; coffee creamer 11 g/l; high fibre bran 12 g/l; peptone 1 g/l; yeast extract 3 g/l; NaHCO₃ 3 g/l); soluble phosphorus (as Na₂HPO₄.2H₂O) to give concentrations of soluble PO₄-P of 100 mg/l, 500 mg/l, 1,000 mg/l and 2,000 mg/l; in some cases soluble magnesium (as MgCl₂.6H₂O) and soluble potassium (as KCl) to simulate the molar proportions of Mg and K released with PO₄ during BPR sludge digestion (assuming 0.26 mol Mg/mol P and 0.3 mol K/mol P). Six replicate digesters were set up for each experimental condition at the beginning of the batch tests, these being destructively analysed in sets of two on days 3, 8 and 18, the batch tests being carried out for 18 days in total.

The following analyses were performed on these batch anaerobic digesters: biogas volume and percentage methane; total solids (TS) and volatile solids (VS) (Standard Methods, 1989); soluble organic carbon (SOC) and inorganic carbon (SIC); pH; soluble sulphide (ion-selective electrode, Orion); soluble ammonia (ion exchange chromatography); phosphorus and metals (ICP). The sequential chemical extraction methods used for metal and phosphorus fractionation were based on those of Stover et al. (1976) and Uhlmann et al. (1990), the latter being adapted so that the bicarbonate-dithionite extraction was replaced with two sequential acetate buffer (pH 5.2) extractions of 45 min each (the Stover extraction has been previously described in Carliell and Wheatley (1997), full details of the adapted Uhlmann method are given in Carliell-Marquet (2001), which also includes details of the model compound extractions used to facilitate assignment of metal species to metal fractions generated by the sequential extraction methods).

**Results**

The batch anaerobic digesters were supplemented with either soluble orthophosphate or soluble orthophosphate plus soluble magnesium and potassium, the latter to more accurately simulate the inorganic conditions that occur when digesting BPR sludge. Results are presented on the effect/s of increasing concentrations of soluble PO₄-P on the anaerobic digestion process, the effect(s) on phosphorus speciation and the effect(s) on metal speciation in the anaerobic digesters.

**The effect of increasing concentrations of soluble PO₄-P on the anaerobic digestion process**

The results in Figures 1 and 2 show that less methane was produced in the P-rich anaerobic digesters, with a decrease in methane production seeming to be related to the increasing
concentrations of soluble \( \text{PO}_4^- \text{P} \). The *inhibitory* effect was, however, not directly proportional to the concentration of soluble \( \text{PO}_4^- \text{P} \) in the digesters.

In the digesters containing soluble \( \text{PO}_4^- \text{P} \) only (Figure 1), an immediate reduction in methane production was observed, in comparison to the controls. After a period of time, however, the *inhibitory* effect appeared to be overcome. This is illustrated for the 500 P digesters, which showed an increase in methane production on day 11 and went on to produce 85% of the total methane produced by the control digesters in the 18 day period. The 1000 P and 2000 P digesters (Figure 1) showed no recovery in methane production, however, in an additional experiment it was noted that batch digesters containing 1,000 mg/l \( \text{PO}_4^- \text{P} \) recovered so that near normal methane production levels were suddenly attained on day 46 of the batch test, this occurring on day 71 for the batch digesters containing 2000 mg/l \( \text{PO}_4^- \text{P} \).

Adding soluble phosphate to the batch digesters in combination with Mg and K resulted in a lessening of the *inhibitory* effect (Figure 2). Less soluble \( \text{PO}_4^- \text{P} \) was present in these digesters in comparison to those in Figure 1 (refer to Table 1) due to increased precipitation of magnesium phosphate compounds, however, the decrease in soluble phosphate was relatively small compared to the increased level of performance. The 1000P + Mg + K digester (Figure 2) contained 527 mg/l \( \text{PO}_4^- \text{P} \) in comparison to 298 mg/l \( \text{PO}_4^- \text{P} \) in the 500 P digesters (Figure 1) and yet the performance of these two digesters was similar.

The *inhibitory* effect was not thought to be related to methanogenic inhibition, as the methane content of the biogas was similar for control and P-rich digesters (ranging between 58 and 65% over the batch test). Volatile solids measurements showed that decreased methane production was linked to decreased volatile solids reduction. Ammonia concentrations were markedly decreased in the P-rich digesters, also indicating reduced digestion of organic matter. Other parameters such as pH and alkalinity were similar in all digesters and, therefore, did not indicate a build-up of volatile fatty acids (VFA) in the P-rich digesters. SOC measurements showed that P-rich digesters contained high concentrations of soluble organic carbon, SOC levels in the P-rich digesters being 2-fold higher on average than those in the control digesters.

### Speciation of phosphorus in the P-rich batch anaerobic digesters

Phosphorus was added to the batch anaerobic digesters in the form of soluble di-sodium phosphate. Phosphorus fractionation using the adapted Ulmann sequential extraction method showed that most of the phosphorus remained in the soluble fraction throughout the batch test (Table 1). In the digesters containing only \( \text{PO}_4^- \text{P} \), 36% of the total P content was soluble in the 500 P digesters, increasing to 56% in the 1000 P digesters and 72% in the 2000 P digesters (results not shown). When Mg and K were added with \( \text{PO}_4^- \text{P} \), soluble phosphorus concentrations were decreased due to increased precipitation with magnesium. The digester containing 1,000 mg/l \( \text{PO}_4^- \text{P} + \text{Mg} + \text{K} \) contained 44% soluble phosphorus in comparison to 56% in the equivalent digester without Mg and K.

<table>
<thead>
<tr>
<th>Phosphorus measured in each extraction, reported as mg/l of P in digested sludge</th>
<th>Soluble P</th>
<th>Water-extractable P</th>
<th>Acetate-extractable P</th>
<th>1 M NaOH-extractable P</th>
<th>0.5 M HCl-extractable P</th>
<th>Residual P</th>
<th>TOTAL P recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (avg)</td>
<td>4 (± 2)</td>
<td>8 (± 1)</td>
<td>59 (± 9)</td>
<td>128 (± 28)</td>
<td>136 (± 22)</td>
<td>10 (± 1)</td>
<td>345 (± 13)</td>
</tr>
<tr>
<td>500 P</td>
<td>298</td>
<td>42</td>
<td>123</td>
<td>136</td>
<td>210</td>
<td>14</td>
<td>823</td>
</tr>
<tr>
<td>500 P + Mg + K</td>
<td>259</td>
<td>42</td>
<td>171</td>
<td>164</td>
<td>150</td>
<td>9</td>
<td>795</td>
</tr>
<tr>
<td>1000 P</td>
<td>741</td>
<td>64</td>
<td>111</td>
<td>158</td>
<td>243</td>
<td>9</td>
<td>1327</td>
</tr>
<tr>
<td>1000 P + Mg + K</td>
<td>527</td>
<td>60</td>
<td>286</td>
<td>204</td>
<td>187</td>
<td>10</td>
<td>1275</td>
</tr>
</tbody>
</table>

Table 1 Fractionation of phosphorus in anaerobic digesters containing 500 and 1,000 mg/l of \( \text{PO}_4^- \text{P} \) and \( \text{PO}_4^- \text{P} + \text{Mg} + \text{K} \).
In the control anaerobic digesters not supplemented with soluble PO$_4$-P, the phosphorus fractionation profiles were dominated by the HCl and NaOH fractions, which comprised 39 and 37% of the total phosphorus. This correlated well with fractionation tests on the full-scale anaerobic sludge digester (from which the inoculum digested sludge for the batch digesters was taken) in which the HCl fraction comprised on average 39% and the NaOH fraction comprised 35% of the total P. Model compound testing (Carliell and Wheatley, 1997; Carliell-Marquet, 2001) has shown that most HCl-extractable phosphorus in digested sludge is solubilised from calcium phosphates such as CaHPO$_4$ or Ca$_3$(PO$_4$)$_2$. NaOH-extractable P is solubilised from organic phosphorus compounds and iron/aluminium phosphate compounds.

Phosphorus enrichment of the batch anaerobic digesters caused an increase in the water-, acetate- and HCl-extractable phosphorus fractions. Water-extractable P was found to be unrelated to solubilisation of an inorganic P compound, instead being derived from phosphorus adsorbed to the sludge matrix. Acetate-extractable P should be related primarily to struvite (magnesium ammonium phosphate) formation in digested sludge, however, high concentrations of calcium in the extracts indicated that this was more likely to be related to the formation of amorphous (easily soluble) calcium phosphate compounds. Comparing these results with the digesters containing P + Mg + K, it can be seen that Mg addition (K was found to remain in the soluble fraction and have a limited effect on phosphorus speciation) increased the concentrations of acetate-extractable and NaOH-extractable P. The acetate-extractable P was found to be related to struvite formation, particularly in the 1000 P digesters. The increase in NaOH-extractable P was also found to be related to struvite formation, the high concentrations of struvite having resulted in saturation of the preceding acetate extractions and leading to struvite extraction occurring in the NaOH fractions. The relative importance of calcium in phosphorus insolubilisation (HCl-extractable P) was decreased in the magnesium-containing digesters.

**Metal speciation in the P-rich batch anaerobic digesters**

As discussed in the previous section, P-enrichment resulted in increased precipitation of calcium and magnesium in the batch anaerobic digesters. This caused lower concentrations of soluble Ca and Mg in the P-rich digesters compared to the control digesters. In the digesters supplemented with P only, soluble Ca concentrations decreased approximately 10-fold in comparison to the control digesters (217 to 25 mg/l) and soluble Mg decreased from 35 to 14 mg/l. In digesters containing additional Mg, soluble calcium was still decreased to approximately 50 mg/l but soluble magnesium concentrations were greater than those in the control digesters (43; 51; 88; 73 mg/l for the control; 100P; 500P; 1000P digesters).

Iron and aluminium speciation in the P-rich digesters was unchanged from that in the control digesters, indicating that neither of these metals played an important role in precipitating phosphorus, unlike digesters treating chemically precipitated phosphorus sludge (Carliell and Wheatley, 1997; Carliell-Marquet, 2001).

Fractionation of trace metals in the batch anaerobic digesters was performed using the Stover sequential chemical extraction method, Cu, Zn, Mn, Cr, Ni and Co being measured. Similar results were obtained for digesters containing phosphorus only and those containing P + Mg + K. Concentrations of soluble trace metals in the anaerobic digesters were not decreased as a result of phosphorus enrichment, with the exception of manganese. Fractionation of manganese showed that soluble concentrations were decreased on average 5-fold (Figure 3) in all P-rich digesters, this occurring within the first 3 days of digestion and remaining stable throughout the 18 day digestion period. In fact all labile Mn fractions (soluble, KNO$_3$-extractable and KF-extractable) were decreased as a result of P
enrichment, causing Mn to be shifted upwards in the Stover fractionation profile, being recovered in the EDTA fraction. Although it is possible that these results were due to precipitation of a manganese phosphate compound, other experiments indicated that manganese was more likely to be co-precipitated with other compounds such as calcium and iron phosphates (Carliell-Marquet, 2001). The tendency of Mn to be strongly co-precipitated with other compounds in an anaerobic digester was previously reported by Ginter and Grobicki (1997).

An unexpected effect of P enrichment on trace metal speciation was to increase the soluble metal concentrations in the digesters. This was particularly marked for chromium (Figure 4), with soluble Cr concentrations increasing 5-fold in the digesters containing 2000 mg/l of PO4-P. Soluble Cr concentrations were found to be linearly correlated with increasing soluble P concentrations in the digesters, which in turn linearly correlated with increasing concentrations of SOC in the anaerobic digesters. It is thought that increasing SOC in the P-rich digesters resulted in more soluble organic ligands being available for complexation of the trace metals, which resulted in increased trace metal solubility. Increased Cr solubility, although statistically significant, was not thought to have contributed to decreasing digester performances, as these increases were relatively small, the maximum soluble Cr concentration recorded being 0.05 mg/l.

The speciation profiles of zinc, nickel and cobalt did not show any major changes (apart from a slight increase in metal solubility) as a result of P enrichment, although, the extremely low concentrations of nickel and cobalt in these digesters and the lack of selectivity of the Stover method for nickel could mean that these fractionation methods are not sensitive enough to detect small changes that might be significant with respect to digester performance. Copper in the digested sludge was found to shift from the last 2 fractions (HNO3 and residual) down the fractionation profile to be recovered in the EDTA fraction. This could represent an increase in copper availability.

Discussion
There are few reports in the literature on the effects of soluble orthophosphate on the anaerobic digestion process. Pfeffer and White (1964) and Alphenaar et al. (1993) showed that low levels of soluble phosphate (= 1 mg/l) were essential for efficient anaerobic digestion. It was also found that high concentrations of soluble phosphate (> 75 mg/l) caused instability in an anaerobic system (Pfeffer and White, 1964). Rudolfs and Stahl (1947) found that addition of a range of phosphorus compounds (soluble and insoluble) to batch anaerobic digesters caused inhibition of the digestion process, affecting volatile solids reduction more than methane production. The extent of inhibition seemed to be related to the solubility of the phosphorus compounds added, but a direct relationship between
soluble phosphorus concentrations and the extent of inhibition could not be established. Rudolfs and Stahl (1947) also noted that the retarding effects of the added phosphorus were particularly noticeable during the early stages of digestion but could be overcome if enough time was given for volatile solids reduction to be completed, as was noted during this research.

An objective of this research was to investigate whether changes in metal speciation, which occurred as a result of phosphorus enrichment, could be related to changes in digester performance, the hypothesis being that phosphorus enrichment could result in increased precipitation of metals, rendering them biologically unavailable. The concept of bioavailability of metals in anaerobic digesters is, however, a poorly understood issue. Soluble metal concentrations are generally considered to be indicative of the bioavailable metal fraction, although some studies have shown that soluble metals complexed with a strong chelating agents such as EDTA will be as biologically unavailable for uptake as insoluble precipitates (Callander and Barford, 1983; Speece, 1986). Metal precipitates are generally considered to be unavailable but Alphenaar et al. (1993) found evidence to suggest that phosphorus in precipitates was available to anaerobic microorganism under phosphorus-limited conditions in a UASB reactor. With respect to the results obtained from the sequential chemical extraction methods, bioavailability is generally thought to decrease as a metal moves up the fractionation profile, i.e. increased resistance to extraction is thought to be indicative of decreased bioavailability.

The methanogenic group of bacteria is known to have stringent requirements for trace metals such as nickel and cobalt and should be the microbial group most sensitive to changes in bioavailability of these metals. The results from this research indicated, however, that inhibition was not occurring at the methanogenic stage but earlier in the digestion process. Moreover, phosphorus enrichment was found to have little marked effect on the trace metals except to increase their solubility, and iron speciation was largely unchanged as a result of phosphorus enrichment. The metals that were significantly affected by soluble phosphorus enrichment were calcium, magnesium and manganese. All these metals are usually present in relatively high soluble concentrations in an anaerobic digester, but were markedly precipitated by phosphorus enrichment. Moreover, not only the soluble fractions but also the KNO₃-extractable (readily exchangeable metals) and KF-extractable (loosely adsorbed metals) were affected by phosphorus enrichment, being stripped from the sludge and precipitated as phosphates. Thus, calcium, magnesium and manganese fractionation was shown to shift towards the upper (unavailable) end of the fractionation profiles.

Calcium and magnesium have both been cited as important metals for anaerobic digestion, particularly methanogenesis (Takashima and Speece, 1990), however, very little information exists on the minimum concentrations of calcium and magnesium required in anaerobic digesters. Calcium is known to be an important growth regulator for many microorganisms (Hughes and Poole, 1989); prior to microbial cell division there is a rapid increase in intracellular Ca²⁺ followed by an efflux to the medium, thus, high concentrations of soluble calcium might be required for cell growth in anaerobic digesters. Magnesium is the most abundant element in methanogenic bacterial cells (Takashima and Speece, 1990) and has been shown to stimulate the acetate utilisation rate and the rate of methane production in an UASB digester (Schmidt and Ahring, 1993). In batch digesters with added magnesium, the inhibitory effects of phosphorus enrichment were lessened. This might have been related to the levels of soluble magnesium in these digesters, which remained higher than the control digesters, whereas the digesters supplemented with phosphorus only were operating at soluble magnesium concentrations well below those of the control digesters. Further research is required to establish the importance of calcium and magnesium in the anaerobic digestion process and to clarify the relationship between metal
speciation and metal bioavailability in digesters. Manganese does not form very insoluble sulphide or carbonate compounds (little information exists on manganese phosphate compounds) in anaerobic digesters and hence is usually available in relatively high proportions to other trace metals in an anaerobic digester. Manganese has not traditionally been considered to be an essential metal for methanogenesis, however, some acidogenic bacteria such as the lactic acid bacteria have shown high requirements for Mn (Hughes and Poole, 1989). It is possible, therefore, that reduction of the bioavailable Mn fractions as a result of phosphorus enrichment could have a negative effect on digester performance, although more research is required to clarify the role of Mn in the digestion process.

Conclusions and recommendations

• High concentrations of soluble orthophosphate (> 250 mg/l) were found to decrease volatile solids digestion and methane production in batch anaerobic digesters.
• Addition of soluble magnesium and potassium, in addition to soluble orthophosphate, was found to reduce the retarding effects of phosphorus enrichment.
• Phosphate was removed from solution in the anaerobic digesters by precipitation, primarily with calcium. In digesters with additional magnesium, phosphate was also precipitated as magnesium phosphate and/or struvite, particularly in the digesters with high concentrations of added phosphate (1,000 mg/l).
• Calcium, magnesium and manganese speciation was markedly affected by phosphorus enrichment, with bioavailable metal fractions (soluble, KNO₃- and KF-extractable) decreasing and metals being recovered instead in fractions towards the upper (unavailable) end of the Stover fractionation profile.
• Phosphorus enrichment increased the solubility of trace metals such as chromium, nickel, cobalt, copper and zinc, although this was most marked with respect to chromium. It was concluded that this phenomenon was related indirectly to phosphorus enrichment, through increasing concentrations of soluble organic matter being liberated into the digester supernatant, increasing the availability of ligands for metal complexation.
• It is recommended that further research is carried out to investigate the importance of calcium, magnesium and manganese in relation to digester performance, taking into account metal bioavailability and speciation.

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References


