Relevance of a perchloric acid extraction scheme to determine mineral and organic phosphorus in swine slurry

Marie-Line Daumer a,*, Fabrice Béline a, Mathieu Spérandio b, Christian Morel c

a Environmental Management and Biological Treatment of Waste Research Unit, Cemagref, 17 ave de Cucillé, CS64427, F-35044 Rennes cedex, France
b INSA Toulouse, LIPE, France
c INRA, Transfert Sol Plante and Cycle Elements Mineraux Ec. UMR, Villenave Dornon, France

Received 8 May 2006; received in revised form 5 February 2007; accepted 5 February 2007

Abstract

To increase the phosphorus recycling potential from swine slurry, mineral phosphorus products which could be used as fertilizers should be obtained and new processes need to be investigated. A routine method is needed to better evaluate the dissolved and solid mineral phosphorus in swine slurry. Cold perchloric acid extraction method previously developed for wastewater or sludge analysis was adapted. Ionic chromatography was used to measure orthophosphate in extracts. Only one extraction step was needed to distinguish between mineral and organic phosphorus in slurry. Reproducibility of the method was high (less than 5% of variation on the measured fractions). Selectivity was assessed by adding several organic and mineral phosphorus sources in the slurry. Cold perchloric extraction followed by ionic chromatography was very selective in quantifying both the mineral and organic forms of phosphorus in swine slurry.

Keywords: Swine slurry; Phosphorus removal; PCA extraction; Phosphate

1. Introduction

Removing phosphorus (P) from manures and slurries in intensive animal operations is required to avoid excessive spreading on agricultural soils and resulting greater risk of P loss to surface waters (Coppenet et al., 1993; Leinweber et al., 1997; Sharpley and Moyer, 2000). This can be performed by centrifugation discarding solid P with organic matter. Due to its high moisture content and subsequent high transportation cost, the use of the product from centrifugation is limited. An alternative way is to treat swine slurry in order to form mineral P compounds such as calcium phosphate or struvite that can be sold as P fertilizers in replacement of traditional chemical phosphate (Ghosh et al., 1996; Greaves et al., 1999). Some successful attempts to recycle P as struvite from the liquid phase have been performed at laboratory and pilot scales (Burns et al., 2003; Kim et al., 2004; Nelson et al., 2003; Suzuki et al., 2005). However, recycling potential should be increased by increasing P concentration in liquid, dissolving P from the solid phase which is about 80% of the P in swine slurry (Barnett, 1994). To design a recycling process, it is important to know precisely how much P could react, the forms, amount of mineral and organic P in solid. Mineral orthophosphate groups (under ionic species, mineral precipitated Ca–P, Mg–P) and organic P (polyphosphate, inositol phosphates, phospholipids, DNA) have been identified in swine slurry (He and Honeycutt, 2001; Leinweber et al., 1997). Several schemes, more or less selective and complex, have also been proposed to analyse P speciation in soils (Tiessen and Stewart, 1983), sediments (Golterman, 2004) and other materials such as sludge produced by urban wastewater treatment plants (Appeldoorn et al., 1992). The chemical extraction schemes proposed for livestock effluent analysis

* Corresponding author. Tel.: +33 223 482 129; fax: +33 223 482 115.
E-mail address: marie-line.daumer@cemagref.fr (M.-L. Daumer).
have been developed to assess P availability to plants, or risk of loss of P by runoff and leaching (Barnett, 1994; Dou et al., 2000; Gerritse and Zugec, 1977; Sharpley and Moyer, 2000). Moreover, except for the analysis of some specific organic forms by enzymatic method, the selectivity of the method is rarely tested and the terminology for forms of P is often confusing and depends on the extraction scheme used (Greaves et al., 1999; Haygarth and Sharpley, 2000). Regarding the potential for producing a mineral P fertilizer from a given swine slurry, it is necessary to develop a simple procedure to distinguish mineral P (orthophosphate and their ionic combinations) which could easily enter a predictable physico-chemical process, from organic P.

A possible approach to differentiate among mineral and organic forms of P was that developed by Appeldoorn et al. (1992), following works of Mino and Matsuo (1985) and De Haas (1991). This extraction scheme is based on a cold perchloric acid (PCA) extraction to dissolve mineral P whereas organic P is assumed to remain in the non-extracted fraction of slurry or to be extracted without hydrolysis.

Our purpose was to assess the ability of a PCA extraction scheme to distinguish between mineral and organic P contents in swine slurry. Several synthetic P compounds, perfectly pure and identified were added to the slurry. The PCA extraction was performed and the distribution of the added P in each fraction, calculated by difference with the results of the same extraction on a control slurry. A mineral calcium phosphate, which has an extremely low solubility, a common polyphosphate, a calcium phytate, a DNA molecule and a phospholipid were tested. The variation of the P content in each extract was calculated for all P compounds.

2. Methods

2.1. Swine slurry

Three raw swine slurries were used, two of them (RS1 and RS2) were obtained from the homogenization pit of the same commercial growing swine farm in Brittany (farm 1) and the last one (RS3) sampled in a swine farm specialized in piglet production in Brittany (farm 2). Facilities are described in Table 1. The swine slurry RS1 was used for polyphosphate and phytic acid addition, RS2 for phosphatidylcholine and DNA addition and RS3 for hydroxyapatite addition. All the slurries were sampled after 30 min of homogenization by the device in place for daily use. In farm 1, a 50 L effluent was obtained by mixing 10 samples collected with a bucket in the tank. In farm 2, the slurry was sampled by a valve located between the homogenization tank and the separation device. Samples were stored at 4°C during the different experimental periods but the extractions were performed after those samples have reached the room temperature.

2.2. Fractionation method

The cold perchloric acid extraction method used by Appeldoorn et al. (1992) was adapted as described in Fig. 1 and performed as below. About 50 g of slurry were centrifuged (18000g, 20 min, 20°C). Supernatant was filtered.

Table 1
Swine farms characteristics (places)

<table>
<thead>
<tr>
<th></th>
<th>Sows</th>
<th>Piglets</th>
<th>Finishing pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td>356</td>
<td>1800</td>
<td>1592</td>
</tr>
<tr>
<td>Farm 2</td>
<td>428</td>
<td>900</td>
<td>800</td>
</tr>
</tbody>
</table>

Fig. 1. Cold perchloric acid fractionation method. TP, total phosphorus; DMP, dissolved orthophosphate; DTP, total dissolved phosphorus; PCATP, total phosphorus in PCA extract; PCAMP, orthophosphate in PCA extracts; RP, residual phosphorus.

(0.45 μm) and used to measure dissolved mineral P as orthophosphate (DMP) by ionic chromatography (DIONEX, Sunnyvale, USA). Dissolved total P (DTP) was measured by automatic flow injection spectrophotometric method (880 nm) (Lachat, Quickchem8000, WI, USA) after an acid–persulfate digestion followed by a coloring reaction with ammonium molybdate vanadium. Dissolved organic P (DOP) was calculated as the difference between total dissolved P and orthophosphate (DOP = DTP – DMP). Short chain polyphosphates, which are mineral P derived from a biological source, were included in the term DOP.

The cold PCA extractions were performed directly on the raw slurry. Increasing amounts of PCA were used to determine the ratio PCA/slurry needed to dissolve or desorb the mineral phosphate without biomass lyses. Four ratios were tested, 0.5/1; 1/1; 2/1 and 4/1. All the magnesium, 85% of P and 75% of calcium were released with a 1/1 ratio. The pH was 4.4. About 95% of phosphate and 90% of calcium were dissolved with the 2/1 ratio and the pH was 1.4. The 1.5/1 ratio was chosen to dissolve the mineral solids without excessive mineralization of the organic forms. With this ratio the pH was about 3. The 0.5 N PCA was added to slurry directly in a centrifuge bottle. Extraction was performed on a rotative stirrer (30 min, 4 °C). Samples were centrifuged (18 000 g, 10 min, 4 °C) and filtered on a filter cloth (35 μm). Orthophosphate (PCAMP) was measured in filtrate by ionic chromatography. Total dissolved P in filtrate (PCATP) was measured as described for DTP. The measured fractions are described in Fig. 1. Other fractions were calculated from the experimental results as following:

Sorbed mineral P (SMP) was calculated as the difference between orthophosphate in the PCA extract and dissolved orthophosphate (SMP = PCAMP – DMP). Organic extractable PCA (PCAOP) was calculated as the difference between total P in the PCA extract and the mineral forms (PCAOP = PCATP – PCAMP). From this fraction, sorbed organic P (SOP) was calculated by the difference between PCAOP and DOP (SOP = PCAOP – DOP). As for dissolved compounds, polyphosphate extracted by the cold PCA was included in the term SOP. The residual P (RP) fraction was measured as the total P in the solid pellet from the 35 μm filtration described above. It was measured after a persulfate and nitric/sulfuric acid digestion as described for DTP.

About 100 g of slurry were calcinated at 550 °C. Ashes were used to measure the total P (TP) content after an acid–persulfate digestion as described above. The ratio between the sum of fractions and the TP value was calculated to validate the extraction scheme.

2.3. Reproducibility and selectivity test

Both organic and mineral P compounds were added to evaluate the efficiency and the selectivity of the developed PCA extraction method. The amount of each product was calculated in order to add 400–500 mgP/kg. Additions were performed under continuous magnetic stirring, in a beaker containing 500 g of slurry. The following chemicals were added before the extraction: sodium polyphosphate (CAS: 68915-31-1; Riedel-de-Haen); hydroxyapatite (CAS: 1306-06-5; Fluka); phytic acid (CAS: 83-86-3; Fluka); deoxyribonucleic acid (CAS: 93384-16-8; Sigma) and l-α-phosphatidylcholine (CAS: 8002-43-5; Fluka), the main constituent of membrane cells phospholipids. Phosphatidylcholine is insoluble and it was previously suspended in water to allow micelles formation and homogeny dispersion in the beaker. For each addition trial, a control sample (slurry without addition) was tested. Three repetitions of each addition and two analyses of each samples were done, namely 6 values for each fraction. For each extraction, results between samples with added phosphorus and control were compared by comparing the means with the student test (P < 0.05) performed on Statgraphics® software (Sigmaplus, Statgraphics; v 4.1, 1999).

2.4. Phosphorus and cations analysis

In most of the previous works about fractionation, orthophosphate were measured by colorimetry using acid reagent. Interferences due to hydrolysis of polymers by the reactants have been previously described. Moreover, these methods are highly sensitive to pH or ionic strength variations (De Haas et al., 1990). Hydrolysis during storage could also contribute to overestimate the orthophosphate concentration, particularly in natural samples in which enzyme degradation could occur (Denison et al., 1998). Hydrolysis of P compounds was also observed with 4 °C storage and freezing (De Haas and Dubery, 1989). In order to avoid these problems, in this study, ionic chromatography, which is very selective for orthophosphate, was used just after sample preparation when both orthophosphate and polymers could be present in the samples. Ionic chromatography could not be used for the digested samples because of the large amount of sulfate and nitrate ions from the acids used for the digestion. As there was only orthophosphate after the persulfate–acid digestion, the colorimetric method was used on mineralized samples.

3. Results and discussion

3.1. Results on different swine slurries

Complete fractionations were performed for RS1, RS2 and RS3 (Table 2). Total P in PCA extract was 83, 86 and 88% of total P in RS1, RS2 and RS3 respectively. The P extracted was mainly as mineral (PCATP) in RS1 and RS2 (59 and 66% of total P respectively) but only 16% of the total P for RS3. The percentage of mineral P found for RS1 and RS2 was in agreement with previous studies (Barnett, 1994; Daumer et al., 2004). Sharpley and Moyer (2000) found 91% of P as inorganic in swine slurry. From these authors, the inorganic P could be overestimated by P hydrolysis induced by the colorimetric method used. The
low mineral P in RS3 could be due to the livestock activity (piglets and sows only).

To validate the fractionation method, the ratio between the sum of P in fractions and the TP value was calculated for all extractions tests. This ratio was 90 ± 1%. The lack of P calculated from the sum of fraction could be due to the underestimation of the RP. It was difficult to recover the entire solid from the 35 μm cloth filter used after the PCA extraction. Before ionic chromatography, the samples from the 35 μm filtration were filtered on a 0.45 μm single use syringe filter. Particles between 35 μm and 0.45 μm captured on this single use filter were not measured. A filtration on a filter that could be digested with the RP sample instead of the single use syringe filter should be better. However, the accuracy of the RP fraction measurement was only performed to establish the P balance of the extraction procedure and did not interfere with mineral P calculation. The experiments were not resumed.

The data from Table 2 were obtained from 9 extractions for RS1, 15 for RS2 and 3 for RS3. The coefficient of variation, calculated as the ratio between standard deviation and the average, was always less than 5% for the measured fraction. It was less than 15% in the organic fractions which was calculated for up to 4 measured fractions showing the good reproducibility of the extraction method.

3.2. Results on swine slurry amended with mineral compounds

The calculated amount of P added, as hydroxyapatite, was only 126 ± 29 mgP/kg against the 400–500 mgP/kg expected. Hydroxyapatite solubility is low. Lack of homogeneity and fast precipitation of the solid added in the beaker where mixing has been done could explain this result. However, as expected, P added as hydroxyapatite was recovered in the PCAMP fraction (+105 mgP/kg). The RP fraction was also increased (+33 mgP/kg). There was no more of either DMP or DOP detected after hydroxyapatite addition (Table 3). The P transfer from DOP and DMP to RP can be explained by the formation of complexes between the organic compounds from the DOP and the calcium released during the hydroxyapatite dissolution and by the sorption of the DMP on these complexes.

3.3. Results on swine slurry amended with organic P and polyphosphate

Orthophosphates measured by ionic chromatography after the cold PCA extraction are only from a mineral source precipitated and/or adsorbed. No organic P added was found in the mineral fractions (PCAMP and DMP) (Table 4). These results are in contradiction with hydrolysis of polyphosphate or other organic compounds by the PCA extraction previously described by De Haas (1991). Either hydrolysis did not occur or it was not complete. Ionic chromatography avoiding further acidic hydrolysis by reactants used for colorimetric dosage could explain the diverging results. Orthophosphates measured by ionic chromatography after the cold PCA extraction are only from a mineral source precipitated and/or adsorbed.

The amount of P recovered in OPCAP and RP fractions was higher than P added (up to 135%) showing a transfer from the mineral to the organic or residual fraction by PCA extraction. The MPCAP fraction was decreased by about 20% when polyphosphate, phytic acid or phosphatidylcholine were added. No decrease was observed with DNA addition. A part of mineral P dissolved by PCA extraction was probably adsorbed or included in complexes formed by the organic compounds or the polyphosphate added. In this case, PCA extraction could underestimate the mineral fraction. However, the amount of added P in the tests were considerably higher than the organic fraction in swine slurry.

Table 2

<table>
<thead>
<tr>
<th>Number of extractions</th>
<th>DMP (mg/kg)</th>
<th>DOP (mg/kg)</th>
<th>SMP (mg/kg)</th>
<th>SOP (mg/kg)</th>
<th>RP (mg/kg)</th>
<th>TP (mg/kg)</th>
<th>Sum of fractions (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS1 9</td>
<td>58 ± 2</td>
<td>24 ± 2</td>
<td>759 ± 34</td>
<td>298 ± 46</td>
<td>102 ± 5</td>
<td>1376 ± 42</td>
<td>1241 ± 38</td>
</tr>
<tr>
<td>RS2 15</td>
<td>74 ± 3</td>
<td>99 ± 8</td>
<td>802 ± 16</td>
<td>201 ± 20</td>
<td>64 ± 3</td>
<td>1359 ± 7</td>
<td>1240 ± 29</td>
</tr>
<tr>
<td>RS3 3</td>
<td>32 ± 0.3</td>
<td>31 ± 1</td>
<td>233 ± 16</td>
<td>606 ± 39</td>
<td>80 ± 2</td>
<td>1028 ± 5</td>
<td>982 ± 28</td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

DMP, dissolved mineral phosphorus; DOP, dissolved organic phosphorus; SMP, solid mineral phosphorus; SOP, solid organic phosphorus; RP, residual phosphorus; TP, total phosphorus.

Table 3

<table>
<thead>
<tr>
<th>P compound</th>
<th>Treatment</th>
<th>Liquid phase of slurry</th>
<th>Liquid and solid phase of slurry</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DMP</td>
<td>DOP</td>
<td>PCAMP</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>RS</td>
<td>32 ± 1</td>
<td>31 ± 1</td>
<td>265 ± 16</td>
</tr>
<tr>
<td></td>
<td>RS + hydroxyapatite</td>
<td>0</td>
<td>64 ± 3</td>
<td>370 ± 13</td>
</tr>
<tr>
<td>Variation (mg P/kg)</td>
<td>–32</td>
<td>+33</td>
<td>+105</td>
<td>–10 (ns)</td>
</tr>
<tr>
<td>Variation (% added P)</td>
<td>+83</td>
<td>–</td>
<td>+26</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

DMP, dissolved mineral phosphorus; DOP, dissolved organic phosphorus; PCAMP, PCA extractible mineral phosphorus; PCAOP, PCA extractible organic phosphorus; RP, residual phosphorus; TP, total phosphorus; ns, non significant (P > 0.05)
and the P adsorbed when extractions were performed in swine slurry would not be significant.

Phosphorus from polyphosphate was found mainly in the PCAOP fraction but 20% was found as residual phosphorus (RP). No increase was observed in the DOP. The chemical was used a mix of different length polyphosphates. These results are in accordance with Appeldoorn et al. (1992) and Mino and Matsuo (1985), which have shown that short and medium chain polyphosphates were extracted by the cold perchloric acid method but longer ones were not. De Haas (1991) has shown that long chain polyphosphate could also be extracted and partially hydrolyzed by the cold perchloric acid extraction. However, as in this study, about 20% of polyphosphate was remained in the residual fraction after PCA extraction.

Phytic acid is known to form insoluble salts with cations (Turner et al., 2000). In this study, the RP was corresponding to only 0.1 mmol of phytic acid i.e. about 4 mg/kg of calcium. However, nearly 200 mg/kg of calcium were still dissolved after phytic acid addition. All the P from phytic acid was recovered in the PCAOP fraction. Most of the swine diets contain phytase that is an enzyme that increases the digestibility of P from phytic acid (the main form of P in the vegetal of the diet), reducing the demand for a complementation by mineral P. In this study, the slurries came from facilities adding phytase in swine diets. A possible remaining phytase activity in slurries could hydrolyze phytate. Chemical hydrolysis of phytic acid by the perchloric acid could also occur. Acidic hydrolysis of phytic acid is slow; however the optimum hydrolyzing pH is 4–5 (Anderson, 1980). No increase was observed in the DOP fraction, therefore, only incomplete hydrolysis of phytic acid occurred during PCA extraction.

In contrast to the results of Appeldoorn et al. (1992) where P from DNA was supposed to be in the residual fraction, in this study, 66% of the P from DNA added was found in PCAOP fraction (42% in DOP and 24% in SOP) and 33% in RP fraction. The amount of P from DNA recovered in DOP was lower than the 70% of the DNA-like P from swine slurry in water obtained by He and Honeycutt (2001). However, in this paper, extractions were performed using a large amount of water which could change the chemical and adsorption equilibrium. DNA P in the PCA extract was previously described by De Haas et al. (1990) which found free ARN and DNA in PCA extract on freeze-dried sludge samples.

The distribution of P added in the different fractions shown that the organic P extracted by the PCA could be, not only the medium chain polyphosphate as described by Appeldoorn et al. (1992), but also organic compounds released by the cells (phospholipids and nucleic acids) or from the swine diets like phytic acid.

4. Conclusion

The results of this study have shown that the one step cold PCA extraction followed by P analysis by ionic chromatography is a selective method to quantify dissolved and solid mineral and organic P in slurry. Hydrolysis of organic compounds during the extraction/analysis step previously described by several authors was avoided by using ionic chromatography and shortening the storage. The quantification of the mineral P obtained will be useful to predict the P dissolution in a chemical process. As well as the polyphosphate, of which the extraction has been previously described, a part of nucleic acid, membrane cell phospho-

---

**Table 4**

<table>
<thead>
<tr>
<th>P compound</th>
<th>Treatment</th>
<th>Liquid phase of slurry</th>
<th>Liquid and solid phase of slurry</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DMP</td>
<td>DOP</td>
<td>PCAMP</td>
</tr>
<tr>
<td>Polyphosphates</td>
<td>RS</td>
<td>56 ± 1</td>
<td>23 ± 1</td>
<td>815 ± 8</td>
</tr>
<tr>
<td></td>
<td>RS + polyphosphate</td>
<td>51 ± 1</td>
<td>26 ± 4</td>
<td>651 ± 7</td>
</tr>
<tr>
<td>Variation (mg P/kg)</td>
<td>–5</td>
<td>ns</td>
<td>–164</td>
<td>+613</td>
</tr>
<tr>
<td>Variation (% added P)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+110</td>
</tr>
<tr>
<td>Na-phytate</td>
<td>RS</td>
<td>60 ± 1</td>
<td>26 ± 1</td>
<td>812 ± 4</td>
</tr>
<tr>
<td></td>
<td>RS + Na-phytate</td>
<td>34 ± 1</td>
<td>47 ± 3</td>
<td>651 ± 11</td>
</tr>
<tr>
<td>Variation (mg P/kg)</td>
<td>–26</td>
<td>+21</td>
<td>–161</td>
<td>+603</td>
</tr>
<tr>
<td>Variation (% added P)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+117</td>
</tr>
<tr>
<td>DNA</td>
<td>RS</td>
<td>73 ± 2</td>
<td>104 ± 9</td>
<td>866 ± 16</td>
</tr>
<tr>
<td></td>
<td>RS + DNA</td>
<td>80 ± 1</td>
<td>294 ± 6</td>
<td>896 ± 24</td>
</tr>
<tr>
<td>Variation (mg P/kg)</td>
<td>+7</td>
<td>+190</td>
<td>ns</td>
<td>+280</td>
</tr>
<tr>
<td>Variation (% added P)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+66</td>
</tr>
<tr>
<td>Lipid-P</td>
<td>RS</td>
<td>70 ± 1</td>
<td>94 ± 5</td>
<td>863 ± 56</td>
</tr>
<tr>
<td></td>
<td>RS + Lipid-P</td>
<td>62 ± 1</td>
<td>93 ± 9</td>
<td>706 ± 15</td>
</tr>
<tr>
<td>Variation (mg P/kg)</td>
<td>–8</td>
<td>ns</td>
<td>–157</td>
<td>+468</td>
</tr>
<tr>
<td>Variation (% added P)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+105</td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

DMP, dissolved mineral phosphorus; DOP, dissolved organic phosphorus; PCAMP, PCA extractible mineral phosphorus; PCAOP, PCA extractible organic phosphorus; RP, residual phosphorus; TP, total phosphorus; ns, non significant (P > 0.05).
lipid and phytic acid were also extracted by the cold PCA but measured only after mineralization of the extracts. These compounds can interfere with the chemical mechanisms and their quantification can be useful to understand the interactions between organic and mineral forms during the chemical process. The cold PCA extraction method could be used as a simple technique to assess the P recycling potential in swine slurry.

Acknowledgement

We thank Olivier Gaucher for his contribution to the experiments.

References


We thank Olivier Gaucher for his contribution to the experiments.

References


