INFLUENCE OF ANAEROBIC PRE-CONDITIONING ON PHOSPHORUS REMOVAL IN SWINE MANURE BY AERATION

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Abstract. A feasibility study with three pre-conditioning times was carried out to examine the effect of anaerobic pre-treatment on phosphorus removal by aeration. The results showed that soluble phosphorus could be decreased from 120 mg L^{-1} to about 40 mg L^{-1} within one-day aeration. However, soluble phosphorus concentration was found not affected by pre-conditioning, but more pH dependent. Anaerobic pre-treatment significantly increased the uptake of phosphorus by microbes indicated by the increase in concentration of organic phosphorus fraction. The presence of a large proportion of inorganic insoluble phosphorus considerably eclipsed the biological removal of soluble phosphorus, which suggested that the solids/liquid separation before aeration could be important to improve the biological uptake of soluble phosphorus.

Keywords: aeration, anaerobic pre-conditioning, phosphorus removal, swine manure

1. Introduction

Field application is often a relatively economical and convenient way to dispose of agricultural wastes. However, this method is limited due to the high concentration of nutrients in manure such as nitrogen and phosphorus that are considered to be the main elements causing water pollution. Various nutrient removal techniques have been studied to treat swine manure so large quantities of manure can be applied to the field without adversely impacting the environment. Among such efforts, much attention has been geared towards phosphorus (P), the most important element responsible for water eutrophication (Vollenweider, 1985). The insoluble portion of phosphorus in swine manure can be removed by physical processing such as screening and natural sedimentation. However, the soluble fraction, which is readily bioavailable, can only removed by biological processes or physico-chemical treatment. A number of researchers reported that phosphorus could be removed by aeration with relatively high efficiency (Ten Have, 1980; Osada *et al.*, 1991). Biological removal of phosphorus is theoretically accomplished by the luxury accumulation of phosphorus by microbes subject to cyclic anaerobic/aerobic conditions. Many species of microbes are involved in this process, such as *Acinetobacter calcoaceticus* that is reckoned as the most efficient with ability to accumulate 8–10% of its dry weight (Fuhs and Chen, 1975; Barnard, 1984).

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In anaerobic phase of this process, the bacteria hydrolyze polyphosphate to get energy for poly-β-hydroxybutyric acid (PHB) synthesis using short chain fatty acids, whereas in aerobic phase, oxygen is used as electron acceptor and the PHB reserve is consumed as carbon source together with soluble substrates (Converti *et al.*, 1995). Therefore, the anaerobic phase before the aerobic phase is essential in order for microbes to accumulate enough energy to be used in acquiring phosphorus in the aerobic process. A similar study done by Gerrish *et al.* (1975) reported that the rates of chemical oxygen demand (COD) and total solids (TS) reduction in the aerobic stage were enhanced by the anaerobic pretreatment. To date, little information is available about the extent to which the anaerobic pre-conditioning influences biological phosphorus removal in swine manure. The research proposed here is to investigate the variations of soluble phosphorus, inorganic phosphate, and organic phosphorus during aeration to reveal the effect of anaerobic pre-conditioning on biological removal of phosphorus and to provide information that may be vital for improving the biological removal of phosphorus from swine manure.

2. Materials and Methods

2.1. Experimental setup

The manure used in this experiment was collected from a finishing barn in the University of Minnesota Southern Research and Outreach Center at Waseca. The barn was equipped with a pull-plug manure handling system with a shallow pit underneath the slatted floor and the manure on the floor was hydraulically flushed into the pit once a day using fresh water and vacated every two weeks under normal operating conditions. There was a collection pit at one end of the barn that received manure when the plug was pulled. Manure samples in this study were collected from the collection pit during rigorous agitation of manure in the pit to ensure a representative sample to be obtained. The manure samples used for this project were about one week old. The characteristics of the raw manure are presented in Table I.

The apparatus for anaerobic incubation and aeration was schematically shown in Figure 1. This apparatus consisted of an air pump that introduced air into the manure at an airflow rate of 1.0 L min^{-1} controlled by an airflow meter, and a column that was filled with 15 L liquid manure, leaving 7.5 cm headspace to keep manure from spilling and to allow stirring during sampling. The whole system was placed in a room with temperature maintained around 20 °C. Three anaerobic pretreatments were tested, i.e. 0, 2, and 4 weeks so three columns were run simultaneously. Aeration was initiated at the end of each anaerobic period. For comparison, an extra column with no treatment was used as control. Since this is a feasibility study with no replications, comparisons between treatments were made based not on statistical analysis but on the general trends observed from

TABLE I

Chemical characteristics of used manure

pH		6.47
TKN	(g L^{-1})	2.88
Total P	(mg L^{-1})	710.0
Soluble ortho-P	(mg L^{-1})	116.5
Organic P	(mg L^{-1})	74.2
Insoluble inorganic P	(mg L^{-1})	530.3
Organic carbon	(g L^{-1})	14.6
Total solids	(g L^{-1})	26.06
Total volatile solids	(g L^{-1})	20.69
Temperature of manure in pit		1.0 °C

the available data. The information obtained from this study can provide guidance to design further experiments involving statistical analysis to show if there exist significant differences among different treatments.

The manure was sampled every 24 hr for the first 7 days and every 48 hr for the next 7 days. The sampling was performed by stirring manure with a motorized paddle-stirrer for 5 min to reach uniformity, then a volume of 100 mL of manure was drawn at a depth 40 cm below the surface. The samples, after pH measurement, were stored in a freezer at –20 °C, and were thawed before laboratory analysis.

2.2. Chemical analysis

The pH of each sample was measured by a pH meter (Fisher, Model 720A) immediately after sampling. Total Kjeldahl nitrogen was measured using the Kjeldahl method (APHA, 1998). Organic carbon was measured by the tube digestion method recommended in determination of soil organic carbon (SSSA and ASA, 1996). Total solids and total volatile solids were determined using standard laboratory methods (APHA, 1998).

Total P was measured after digestion with H_2SO_4–H_2O_2 and diluting to 100 mL. Soluble phosphate was determined in the filtrate after filtering 10 fold diluted manure using a Whatman filter paper. The total inorganic P was measured using the acid extraction method (SSSA and ASA, 1996). Insoluble inorganic P was expressed as the difference between total inorganic P and soluble P. Organic P was taken as the difference between total P and total inorganic P. Orthophosphate was determined colorimetrically as the phosphomolybdate complex after reduction with ascorbic acid (APHA, 1998).

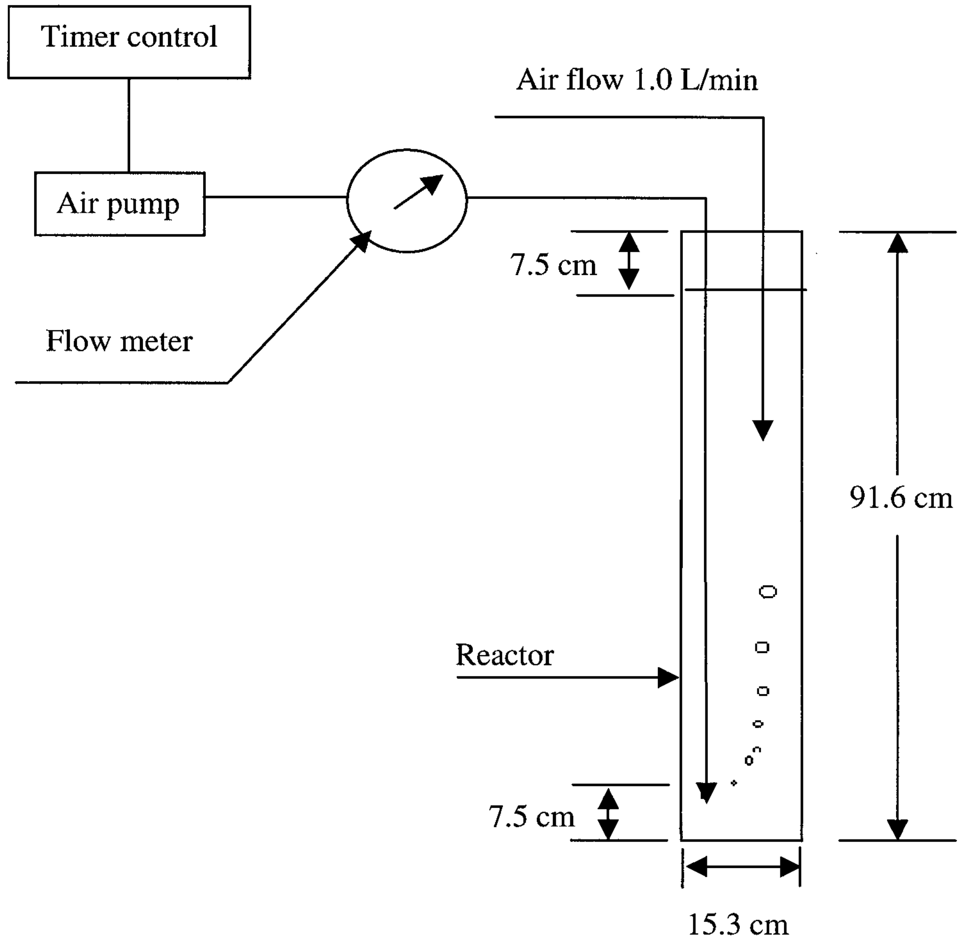

Figure 1. The schematic of aeration apparatus used in this experiment.

3. Results and Discussion

3.1. SOLUBLE PHOSPHORUS

The variation of soluble phosphate in swine manure for the four treatments is shown in Figure 2. The result indicates that the soluble phosphate in the treatment without aeration increases slightly in the first three days and then gradually decreases in the rest of experiment. It is also seen that aeration sharply decreases the soluble phosphate to about 40 mg L^{-1} within day one (about 67%), and that further aeration does not promote the soluble phosphorus removal. The initial reduction in soluble phosphate was most likely caused by precipitation of insoluble metal phosphates formed at the raised manure pH (Figure 5) due to aeration, as reported by previous workers (Moore and Miller, 1994; Campbell *et al.*, 1997).

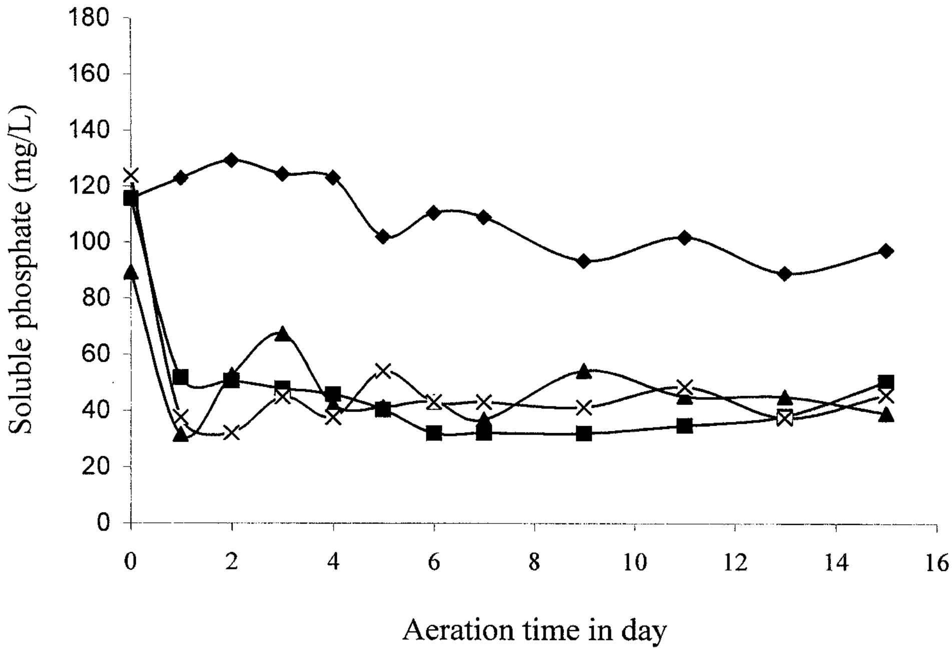

Figure 2. The effect of pre-conditioning on the concentration of soluble phosphate in swine manure during the aeration process. ♦ without aeration (control); ■ aeration without preconditioning; ▲ aeration with 2-week preconditioning; × aeration with 4-week preconditioning.

No substantial differences in soluble phosphate reduction are observed among the three different anaerobic pre-conditioning treatments. This result suggests that anaerobic pre-conditioning would not lead to a higher efficiency of soluble phosphate removal.

3.2. Organic phosphorus

The variation of organic phosphorus in the manure with different anaerobic pre-conditioning times during the 2 week aeration is shown in Figure 3. Aeration results in a considerable increase in organic phosphorus compared to the treatment without aeration in which very slight increase of organic phosphorus is observed. Most significant increases of organic phosphorus are observed after aeration for 7 days, 3 days, and 1 day in the treatments without pre-conditioning, with 2 week pre-conditioning, and with 4 week pre-conditioning, respectively. The results also show that a great difference in organic phosphorus occurs only in the first week among the three pre-conditioned treatments. The difference gradually becomes less and approaches the same level after two-week's aeration. In the first week, it is seen that the longer the pre-conditioning time, the higher the content of organic phosphorus. Considering the fact that organic phosphorus is synthesized by microbes, it thus can be inferred that pre-conditioning promotes the biological uptake of phosphorus.

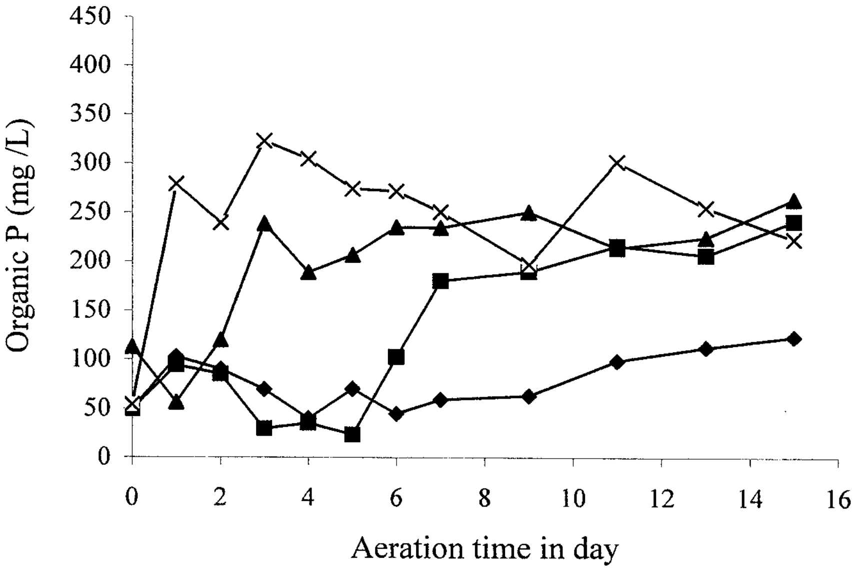

Figure 3. Organic phosphorus in the manure during aeration with different pre-conditioning time. ♦ Without aeration (control); ■ aeration without preconditioning; ▲ aeration with 2 week preconditioning; × aeration with 4 week preconditioning.

This observation further suggests that anaerobic pre-conditioning reduce aeration time for biological accumulation of phosphorus so that the most efficient biological removal of phosphorus can be accomplished within the first week of aeration.

The effect of anaerobic pre-conditioning on the phosphorus accumulation may be attributed to the accumulation of fatty acids and other reduced intermediate compounds during anaerobic storage. It is widely accepted that the essential prerequisite to induce bio-P removal is the presence of an anaerobic zone upstream of the standard aerobic process (Comeau *et al.*, 1986), in which energy and carbon are accumulated as PHB in microbial cells using short chain fatty acids. Jones *et al.* (1987) reported that addition of short chain substrates such as acetate enhanced the release of phosphorus in the anoxic stages and subsequently led to increased biological uptake (removal) of phosphorus. Therefore, during the anaerobic storage, a process that will facilitate phosphate uptake by microbes in the following aerobic process should be the accumulation of short chain fatty acids. The evidence for this postulate can be found from past research, in which substantial amount of fatty acids was observed to accumulate from the degradation of the organic matter in manure during the storage (De La Torre and Goma, 1981; Rainville and Morin, 1985).

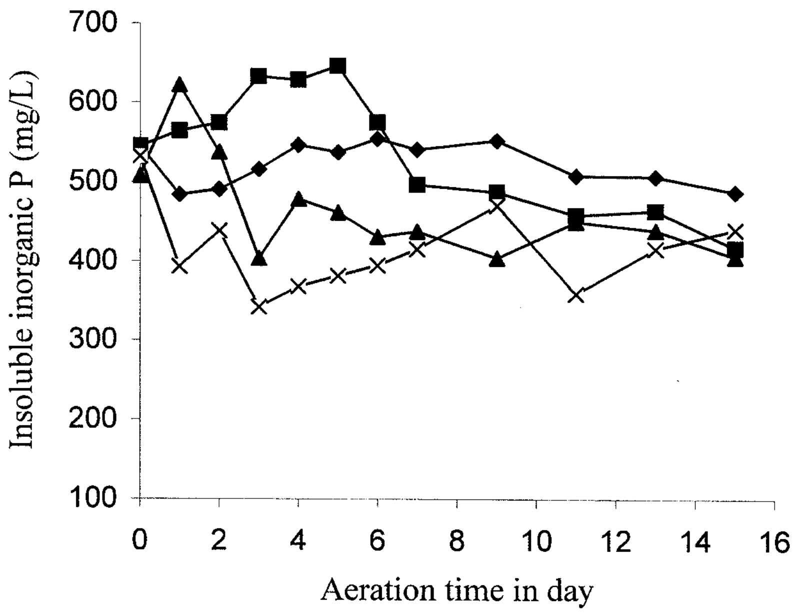

Figure 4. The variation of inorganic insoluble phosphorus in swine manure with different pre-conditioning time during the aeration process. ♦ Without aeration (control); ■ aeration without preconditioning; ▲ aeration with 2 week preconditioning; × aeration with 4-week preconditioning.

3.3. Insoluble Inorganic Phosphorus (IIP)

Significant differences in inorganic insoluble phosphorus among the three aerated treatments are observed during the aeration process (Figure 4). The treatment without pre-conditioning has higher IIP than the control treatment in the first week of the aeration, followed by a sharp reduction in the remaining aeration period. The treatment with 2 week pre-conditioning slightly accumulates IIP in the first two days followed by a decrease in the following period. A decrease in IIP is observed in the treatment with 4 week pre-conditioning during the whole period of aeration. The largest differences among the three treatments are observed in the first week and these differences gradually decrease thereafter. The fluctuation of IIP can be explained by two situations, i.e., the equilibrium occurring between chemical precipitation that results in the formation of IIP and biological assimilation that reduces the IIP by absorption. In the first situation, as observed by Stevens *et al.* (1974), aeration drastically and immediately increased the pH by purging carbon dioxide out of manure, under which phosphate could be precipitated out with metallic ions such as Ca^{2+} and Mg^{2+} that were found to be as high in concentration as phosphate in swine manure (Campbell *et al.*, 1997). The large increase in pH observed in this study obviously meets this requirement (Figure 5). In the second situation, the formation of IIP is probably due to the decomposition of organic matter. Since aeration accelerates the microbial breakdown of organic

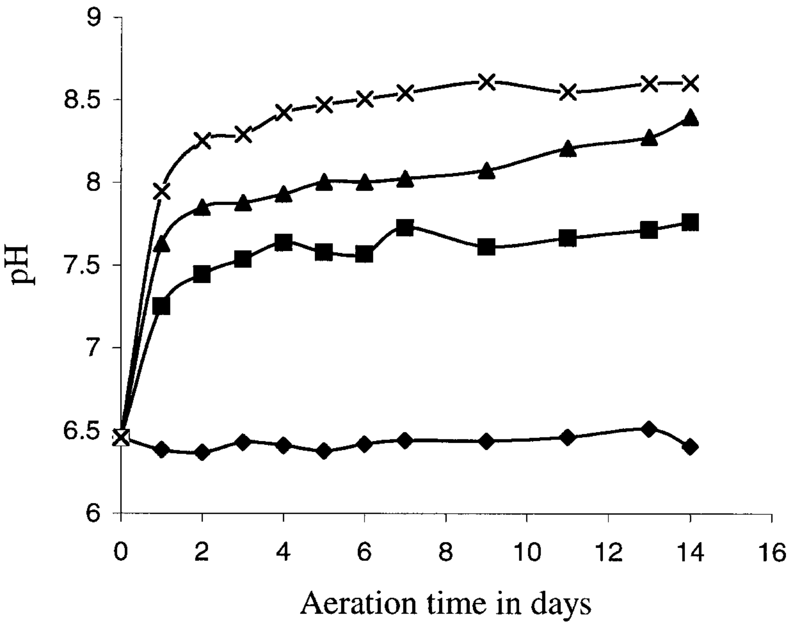

Figure 5. The pH changes during aeration in the treatments with three pre-conditioning times. ♦ Without aeration (control); ■ aeration without preconditioning; ▲ aeration with 2 week preconditioning; × aeration with 4 week preconditioning.

compounds into end products such as CO_2, H_2O, and NH_4^+ (Martinez *et al.*, 1995), the release of organic phosphorus into inorganic fraction will be expedited, which will be quickly converted into IIP under high pH. This is evidenced by the slight reduction in organic phosphorus observed in the treatment without pre-conditioning and with 2 week pre-conditioning in the first several days. Then, microbes will start to absorb inorganic phosphate to synthesize organic phosphate for growth, which will decrease the inorganic fraction. In the treatment with 4 week pre-conditioning, no obvious IIP accumulation is observed. Although the IIP fluctuates during the aeration process, the trend of decreasing is clear, which indicates that the microbial assimilation has outstripped the chemical precipitation.

3.4. PHOSPHORUS BALANCE

The mass balance of phosphorus for all the treatments is presented in Figure 6. First, it is interesting to note that the manure in all treatments is characterized by a large proportion of inorganic insoluble phosphates. This scenario greatly differs from that of municipal wastewater that contains much lower concentration of suspended solids, the major source for the insoluble inorganic phosphate. In the treatment without aeration, a smaller fraction of organic phosphorus but a higher fraction of soluble phosphate than in other treatments was observed and the respective levels among the three fractions did not change significantly in the two-week

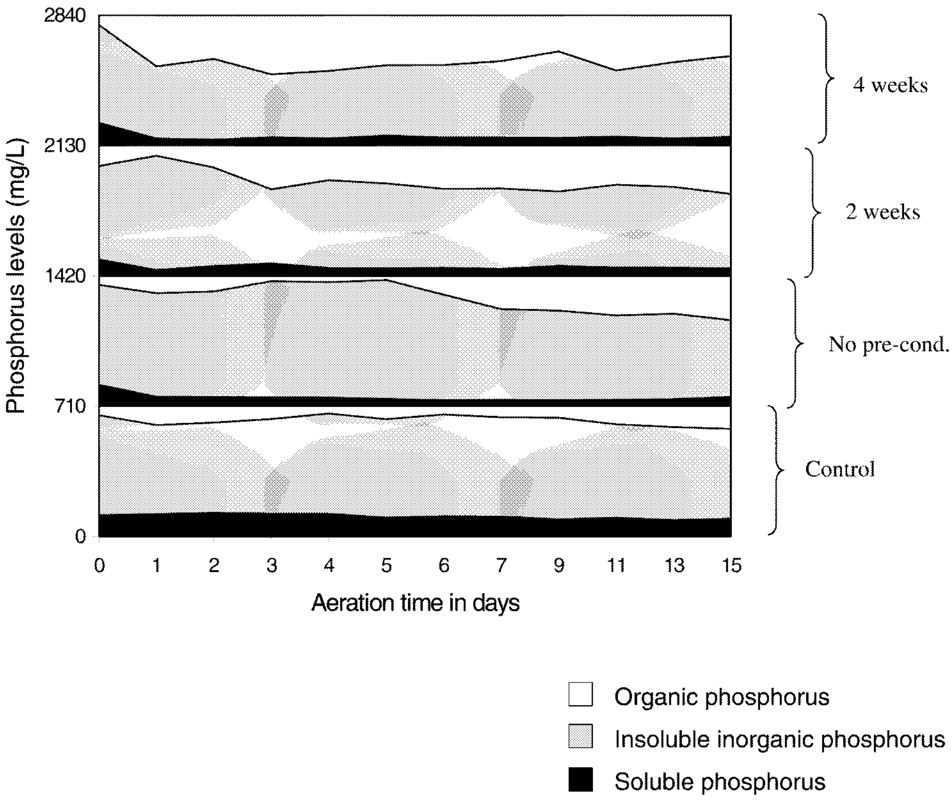

Figure 6. Phosphorus balance in the test manure for all the treatments.

anaerobic incubation. The mass balance of phosphorus in the aerated treatments shows that the proportion of organic phosphorus gradually increased after aeration was initiated, while the largest increase in organic phosphorus was observed in the treatment with the longest pre-conditioning time. The increase in organic P in the top three treatments in Figure 6 is mainly attributed to the biological conversion of the inorganic insoluble P into organic P facilitated by aeration (Fuhs and Chen, 1975).

Second, the results in Figure 6 also demonstrate that there is considerable variation in the three fractions of phosphorus in the treatments with different anaerobic pre-conditioning periods, which is characterized by the increase in organic phosphorus and the decrease in IIP at different levels. However, the reduction in soluble phosphorus brought about by aeration is much smaller than the increase in organic phosphorus, which indicates the role of microbes in this process. With soluble P being almost constant after the first day of aeration, the increase in organic phosphorus is obviously accompanied by a simultaneous decrease in inorganic insoluble phosphate. The correlations between pH and soluble P in Table II show the function of pH in the removal of soluble phosphate. These results show that sol-

TABLE II

The correlation between pH and soluble phosphorus concentration

Treatments	Correlation co-efficient
Without aeration	–0.556
Without pre-conditioning	–0.929**
Two-week pre-conditioning	–0.741**
Four-week pre-conditioning	–0.889**

The data followed by two stars indicate the significance at P = 0.01 level.

uble P concentration is significantly pH dependent. Considering the fact that swine manure contains as much calcium as phosphate, it is logical to say that the major part of the insoluble inorganic phosphate is calcium phosphates. Barber (1984) described in detail the species of phosphate anions in solution and the effect of pH on solubility of calcium phosphates. In a solution with a pH range of 4.0–8.5, the anion of phosphate is present either as $H_2PO_4^-$ or as HPO_4^{2-}, and the ratio of which depends completely on pH. When pH is above 7.2, HPO_4^{2-} dominates in the solution, suggesting that the major form of phosphate would be $CaHPO_4$, which has a decreasing solubility with the pH increase. Therefore, altering pH results in the change in the solubility of phosphates, hence changing the soluble phosphate level. It is clear that the soluble phosphate will be reduced in the way of precipitation as metal phosphates when pH rises, and vice versa. The presence of large quantities of insoluble fraction indicates that the manure solution is in a phosphate-saturated status, which means that the amount of soluble phosphate removed by microbes from the solution will always be replenished by dissolving the insoluble fractions unless all the IIP is exhausted. It is seen from this study that the accumulation of phosphate by microbes results in a corresponding decrease in IIP but little or no change in soluble phosphate, i.e., the presence of IIP in large quantities completely eclipsed the microbial uptake. It may therefore be inferred that any techniques of biological removal of soluble phosphorus will hardly be effective unless the amount of inorganic insoluble phosphates is significantly reduced. Based on the above discussion, the removal of soluble phosphate from raw manure by aeration in this study is most likely owing to the pH change instead of microbial incorporation. Although a large amount of phosphorus is removed by pH increase in this case, the metal phosphates may be unstable as compared to the phosphorus retained in the microbes because the precipitates can again be dissolved in the settling tank when the pH decreases due to the accumulation of respiratory carbon dioxide. Therefore, the biological retention of soluble phosphate in biomass should be more effective in reducing soluble P in manure than the chemical precipitation brought about by pH increase. The entrapped P in the biomass can then be removed through subsequent treatments such as settling or solid/liquid separation.

The above results strongly suggest that the anaerobic pre-conditioning treatment enhance the efficiency of biological phosphorus removal. Since the inorganic insoluble P contained in suspended solids may offset the biological P removal according to the P balance data from this study, it is obviously advantageous to perform solids/liquid separation prior to aeration to improve the biological uptake of soluble P by reducing IIP in the manure.

4. Conclusion

The soluble phosphate in swine manure is reduced from 120 mg L^{-1} to about 40 mg L^{-1}, by forming insoluble metal phosphates that settle, after one-day aeration with or without pre-conditioning. Further reduction of phosphate in the remaining aeration process is not observed. Therefore, using short-term aeration (such as one day) in conjunction with subsequent solid-liquid separation to remove the settled metal phosphates may be an effective way to reduce soluble phosphate in liquid swine manure.

The data indicate that the prolonged anaerobic pre-conditioning period will enhance the microbial accumulation of phosphorus. However, the presence of large quantities of inorganic insoluble phosphate is observed to be a major factor that interferes with the effective removal of soluble P by the microbes. Therefore, solids-liquid separation may be helpful prior to aeration to reduce the levels of inorganic insoluble phosphate, thus potentially increasing the efficiency of biological P removal.

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