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Influence of Temperature and Time on Phosphorus Removal in Swine Manure During Batch Aeration

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ABSTRACT

This study was conducted to investigate the effects of temperature and time on the mechanisms of phosphorus removal in swine manure during aeration. Removal of soluble orthophosphates significantly increased with aeration time and temperature. Successive significant ortho-P removals were observed between days one and nine but no significant additional removals were recorded thereafter. Removals were significantly higher at temperatures of 20 and 25°C than at temperatures of 5, 10, and 15°C and ranged between 22.9 to 31.0%. Insoluble inorganic phosphorus also changed significantly with aeration time and temperature and with a similar trend as soluble orthophosphates. The pH of the manure explained 92 and 87% of the content of insoluble inorganic phosphorus at lower temperatures (5, 10, 15°C) and at higher temperatures (20, and 25°C), respectively. Organic phosphorus and aerobes growth patterns were similar to that of soluble orthophosphates removal. The rapid growth of aerobes was most probably the principal factor behind a rapid soluble ortho-P removal above 15°C. The contribution of inorganic phosphates to the removal of soluble orthophosphates was approximately 61% while that due to organic P was approximately 35%.

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Precipitation was found to be the principal mechanism governing removal of soluble ortho-P in swine manure during aeration treatments.

Key Words: Batch-aeration; pH; Temperature; Swine manure; Phosphorus.

INTRODUCTION

For sustainable animal agricultural practices it is usually encouraged that animal excreta, which is rich in nutrients and organic matter, be recycled for production of animal feeds or fertilizers. This indeed has been the traditional approach for many centuries. In modern times, however, economic reasons have led to an increase in the size of animal facilities and regional concentrations of similar enterprises. Although this trend has invariably achieved economic successes, these systems produce huge amounts of liquid manure because of the methods of collection and handling employed. Even if it is economically advantageous to collect and handle the manure as liquid, the consequence is amplified problems in their utilization and recycle. In addition, because of the introduction of more stringent legislation to protect the environment, simple land spreading is becoming increasingly less acceptable as a utilization method and some kinds of pretreatments are invariably needed to adjust levels of organic carbon, nitrogen, and phosphorus.

Aerobic microbial metabolism is a very efficient means for reducing organic carbon loading while simultaneously reducing inorganic forms of nutrients by microbial biomass immobilization and conversion to other forms. Two possible mechanisms of microbial uptake of phosphorus are: 1) uptake and incorporation into microbial biomass for growth and energy transfer between Adenosine Tri-Phosphate (ATP) and Adenosine Di-Phosphate (ADP), and 2) uptake followed by storage in form of polyphosphates as phosphate reservoir for growth and energy metabolisms.

Improved removal of phosphates in wastewater treatments has been accomplished by simultaneous adjustment of pH and addition of salts of iron, calcium, or aluminum to form sparingly soluble phosphates, which are subsequently removed by settling and separation processes.[1−3] On the other hand, substantial accumulation of phosphates in activated sludge has been observed on aeration without addition of such salts. The latter observation has explained by noting that calcium from naturally hard water forms an insoluble phosphate on the activated sludge floc during aeration because of a rise in pH resulting from purging carbon dioxide from the activated sludge.[4] Swine manure contains Ca^{+2} and Fe^{+3} ions due to mineral supplements in feeds.[5] It therefore follows that similar aeration treatments of swine manure previously stored under anaerobic conditions (usually acidic) even without additions of Ca or Fe salts are likely to have some effects on the amounts of soluble ortho-P in the manure liquid.

In summary, total phosphorus in swine manure is sum of total of organic and inorganic phosphates. The organic P is found in both microbial biomass and in other non-microbial organic compounds in manure. Inorganic phosphates are distributed between soluble and insoluble orthophosphates and polyphosphates. During aeration, phosphorus removal can be effected by any or a combination of three mechanisms: 1) uptake and immobilization into microbial biomass, 2) uptake and storage as polyphosphates in the microbial cells, and 3) precipitation into insoluble orthophosphate complexes. Past studies have shown that the solubility of the complexes formed between ortho-P and the cations are affected by temperature.[3] Temperature also significantly influences the process rates of biochemical reactions and hence metabolism and growth of microorganisms.[6] The influence of temperature on the removal of soluble orthophosphates can therefore be better understood by studying effects of temperature on these three mechanisms.

The overall goal of this study was to determine the effects of temperature and time on the mechanisms of phosphorus removal in swine manure during aeration treatments. The specific objectives to achieve this goal were to investigate the effects of temperature and time on: 1) soluble orthophosphates, 2) pH, 3) aerobic microbial growth, 4) insoluble inorganic phosphates, and 5) organic phosphorus. The relationships between all the parameters that explained the contributions of each mechanism on the soluble orthophosphate removal were subsequently studied.

METHODS AND MATERIALS

Equipment and Instrumentation

A schematic of the equipment and instrumentation used in this experiment is shown in Figure 1. The reactors clear Plexiglas columns (91 cm tall and 15 cm diameter) were filled with liquid manure, leaving approximately 15 cm headspace to facilitate stirring and to provide room for any frothing created by aeration. A heating tape (Thermolyne BriskHeat®, model TP-FG-STD Barnstead Inc.) wrapped round the reactor was used to maintain the desired manure temperatures. The temperature of the

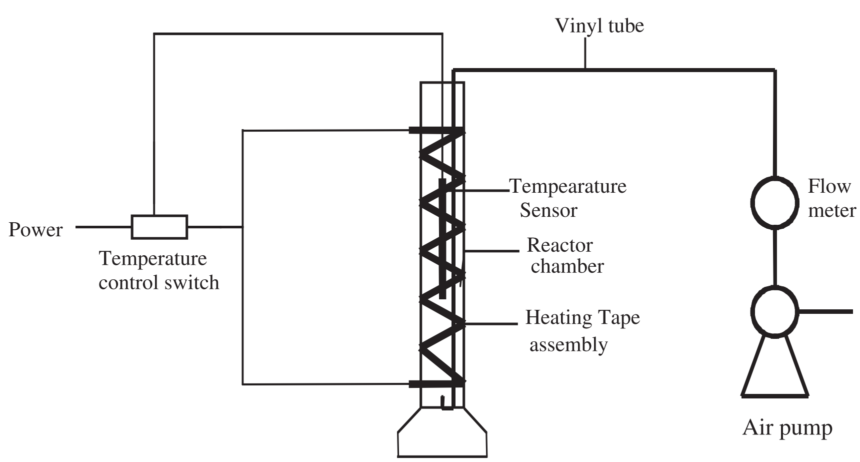

Figure 1. A schematic of the equipment and instrumentation used in the experiment.

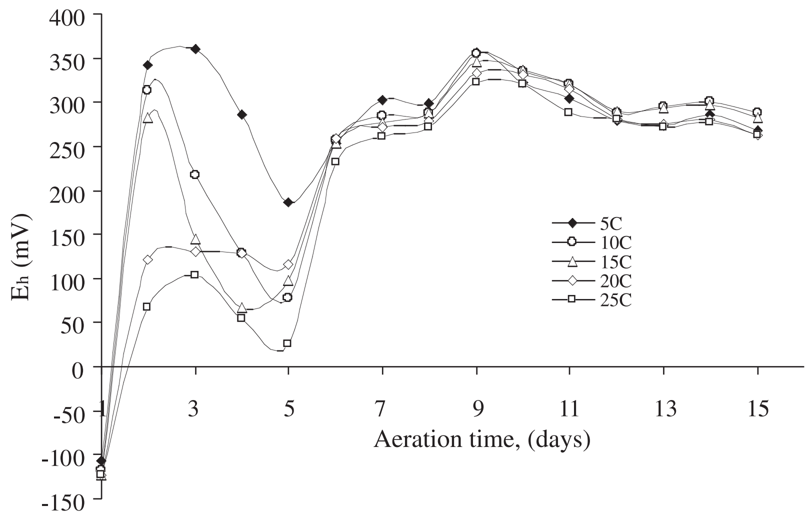

Figure 2. Changes in the oxidation–reduction potential of the manrue with time and temperature during aeration.

reactor was controlled using an Electronic Digital Thermometer (TRACEABLE®, model 11-463-47A, Fisher Scientific) with a temperature sensor located at approximately half the depth of reactor. The liquid manure temperature was maintained within ±1°C of the set temperature.

To aerate the manure, a positive pressure air pump (Emerson model 0623-V4-G180DX, Gast MFG Corp.) was used to introduce air into the manure through a vinyl tube (6.35 mm ID) at the bottom of each reactor. A perforated rubber boot was used to cap the delivery end of the vinyl tube to reduce the air-bubbles sizes or to act as a diffuser. A variable area flow meter (Model P-32461-64, Cole–Parmer Instrument Company) was used to regulate and maintain the flow of aeration air in each unit. Five such units were set up to provide manure temperatures of 5, 10, 15, 20, and 25°C, respectively. An airflow rate of 0.08 ± 0.01 L[air]·L^{-1}[manure]·min^{-1} was used in the first five days while a flow rate of 0.16 ± 0.01 L[air]·L^{-1}[manure]·min^{-1} was used for the rest of time to maintain aerobic status. An oxidation–reduction meter was used to monitor the aerobic status of manure (Figure 2). This study was conducted in a cold-room (below 0°C) to allow the establishment of the stated temperatures using the heating tapes.

Manure Collection, Loading, and Sampling

Swine manure from a pig-finishing barn located at the Southern Research and Outreach Center of the University of Minnesota was collected in plastic containers. Prior to filling the reactors, the manure slurry was thoroughly stirred to a uniform mixture. Before starting the experiment, the manure in each reactor was again thoroughly stirred using a motorized paddle-stirrer (Tline Laboratory Stirrer, Model

Table 1. Characteristics of manure used in this experiment (n = 5).

Total solids	29.11 ± 0.68 g/L
Total volatile solids	18.4 ± 0.49 g/L
Total suspended solids	18.58 ± 0.69 g/L
Total suspended volatile	14.51 ± 0.51 g/L
pH	7.72 ± 0.01
Total phosphorus	2092 ± 68 mg/L
Soluble orthophosphates	225 ± 8 mg/L
Ammonium nitrogen	1500 ± 90 mg/L
Redox potential (ORP)	– 119 ± 7 mV
Supernatant BOD_5	7870 ± 175 mg/L
Supernatant volatile fatty acids (VFAs)	9510 ± 581 mg/L

102, Talboys Engineering Corp.) and a sample was drawn from approximately the mid-depth of each reactor for laboratory analyses. The characteristics of this raw manure slurry are given in Table 1. For the rest of the test, samples were taken every two days. After the determinations of oxidation–reduction potential (ORP) and pH, and the completion of bacterial plating in the respective culture media, all the samples were kept in a deep freezer until they were analyzed for the other parameters. The frozen samples were then thawed and allowed to get to the room temperature prior to these laboratory analyses.

Laboratory Analyses

Oxidation–reduction potential of the liquid manure was determined electronically using an ORP meter (DIGI-SENSE, model 5938-52, Cole–Parmer Instrument Company). The pH of the manure was determined using a pH meter (Orion model 720A, Orion Research Inc.). Bacterial colony counts for aerobes were done using the Drop Count Method using a standard culture medium.[7] At least five drops from each of several dilutions of liquid manure were placed on the dry plates of the culture medium. The lids were replaced and the drops were allowed to dry before inverting the plates for incubation. Drops showing discreet colonies between 10 and 30 after 48 hours were selected for the final count. For determination of soluble ortho-Phosphorus, a well-mixed sample was diluted and then centrifuged at 4000 rpm for 30 minutes. The centrifuged sample was then filtered using GF/A Whatman filter papers. The soluble ortho-P in the filtrate was determined colorimetrically as the phosphomolybdate complex after reaction with ascorbic acid.[8] Total phosphorus was determined using the Persulfate Digestion Method, by which all the species of phosphorus in a sample were first converted to orthophosphates. The samples were then filtered and the phosphorus measured calorimetrically using the ascorbic acid method. Total insoluble inorganic phosphorus was determined by pipetting a 2 ml aliquot of each sample into a 50 ml volumetric flask followed by addition of 1.5 M HCl acid to make volume, for the extraction of total inorganic P (soluble and insoluble).[9] The extracted total inorganic P was determined by the ascorbic acid method. Insoluble inorganic P was then determined as the difference between total inorganic P and soluble ortho-P. Organic phosphorus was determined as the difference between total phosphorus and total inorganic phosphorus.

Experiment Design and Data Analyses

Effects or responses to be investigated in the manure were soluble ortho-P, organic-P, insoluble inorganic-P, pH, and cumulative aerobic microbial growth. The experiment's major factors were temperature at five levels (5, 10, 15, 20, and 25°C) and time at eight levels (1, 3, 5, 7, 9, 11, 13, 15 days). This scenario fits the classical two factor factorial design with multiple treatments at each factor. A two-way analysis of variance (ANOVA) among treatments was conducted for soluble ortho-P, organic-P, insoluble inorganic-P, pH, and cumulative growth of aerobes. The least significant difference (LSD) method was used to separate the means whenever there was significant variation with either time of aeration or temperature. A standard statistical analyses software was used for statistical analyses of the data in this study.[10] Test for significance between treatments means were at $\alpha = 0.05$ level unless otherwise stated.

RESULTS AND DISCUSSION

Removal of Soluble Orthophosphates

The analyses of variance of the cumulative removal of soluble ortho-P in swine manure during aeration with time and reactor temperature are presented in Table 2. Removal of ortho-P significantly ($p < 0.0001$) increased with time of aeration from day one to day nine and only marginal removals were observed thereafter. The maximum removal of approximately 35% obtained in this study compares well with

Table 2. Mean cumulative percentage soluble orthophosphates removal by day and reactor temperature.

Time days	Mean SP removed* (%)	Temperature °C	Mean SP removed* (%)
1	0.0^{a}	5	22.9^{a}
3	20.8^{b}	10	24.3^{a}
5	21.0^{b}	15	23.9^{a}
7	30.7^{c}	20	30.2^{b}
9	35.2^{d}	25	31.0^{b}
11	35.8^{d}		
13	34.6^{d}		
15	$33.6^{c,d}$		

*Means with the same letter in the same column are not significantly different; SP = soluble orthophosphates.

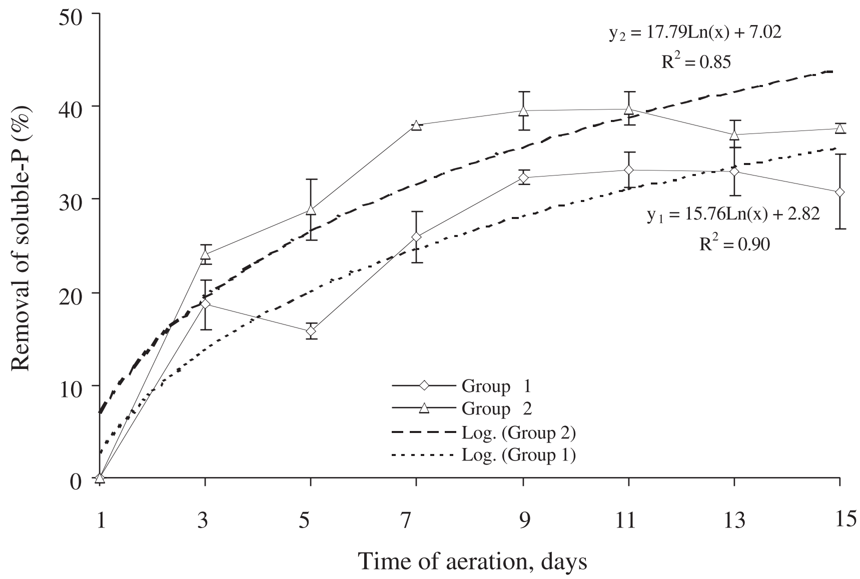

Figure 3. Variation of phosphorus removal with aeration time and bioreactor temperature.

levels reported in literature for most conventional biological treatment processes. Some researcher have reported phosphorus removals of below 50% while others have reported removal of between 30 and 40% in conventional activated sludge processes.[1,6]

Removal of ortho-P also significantly ($p < 0.0001$) increased with increase in reactor temperature. Upon separation of the means of ortho-P removal with respect to temperature using the LSD method, removal of soluble ortho-P was found to be significantly higher at temperatures of 20 and 25°C than at 5, 10, and 15°C. Such significant improvement of ortho-P removal at temperatures above 15°C in the activated sludge process has been observed in previous studies.[6] The data was, therefore grouped into two groups: 1) group 1 comprised temperatures of 5, 10, and 15°C, and 2) group 2 comprised temperatures of 20 and 25°C. This pooled data was used to develop the relationships between soluble ortho-P removal and duration of aeration for the two temperature groups as shown in Figure 3. It is evident from Figure 3 that at each temperature group, the removal of ortho-P varied logarithmically with respect to the duration of aeration, i.e. although the removal of soluble ortho-P increased with time, the rate of removal decreased exponentially with time. The correlation coefficients R for these logarithmic relationships were 0.92 and 0.95 for groups 1 and 2, respectively, indicating fairly good fits.

Insoluble Inorganic Phosphorus

The summary statistics of variation of insoluble inorganic P and pH with time and temperature of the bioreactors are given in Tables 3 and 4. Both insoluble inorganic P

Table 3. Mean insoluble inorganic phosphorus in the manure by time of aeration and temperature of reactors.

Time days	Mean insoluble inorganic-P content, mg/L*	Temperature °C	Mean insoluble inorganic-P content, mg/L*
1	791.40[a]	5	829.73[a]
3	846.42[b]	10	869.91[b]
5	877.10[c]	15	869.62[b]
7	892.91[c,d]	20	940.46[c]
9	905.41[d]	25	940.75[c]
11	917.62[d,e]		
13	943.76[e,f]		
15	946.12[f]		

*Means with the same letter in the same column are not significantly different.

and pH significantly (both with $p < 0.0001$) changed with time of aeration but only insoluble inorganic-P significantly ($p < 0.0001$) varied with temperature of the manure during aeration. The p-value of the variation of pH with temperature was 0.5218, which is not significant at the significant level α of 0.05. The contents of insoluble inorganic P in the manure were significantly less at the lowest temperature of 5°C than at the higher temperatures. In addition, the contents of insoluble inorganic-P were significantly higher at the higher temperatures of 20 and 25°C than at the middle temperatures of 10° and 15°C. The significantly higher amounts of insoluble inorganic P concentrations in the manure at 20 and 25°C correspond to similar reduction of soluble ortho-P at the same temperatures (see ''Removal of Soluble Orthophosphates'').

The insoluble inorganic P content in the manure during aeration as well as the respective changes in pH of manure are presented in Figures 4 and 5, respectively. These figures were developed by pooling together pH data with respect to temperature (pH not significantly affected by temperature) and grouping the insoluble inorganic P

Table 4. Mean pH of the manure by time of aeration and temperature of reactors.

Time days	Mean pH*	Temperature, °C	Mean pH*
1	7.72[a]	5	8.92[a]
3	8.69[b]	10	8.92[a]
5	7.75[b]	15	8.94[a]
7	9.14[c]	20	8.98[a]
9	9.24[c,d]	25	8.98[a]
11	9.28[d,e]		
13	9.36[e]		
15	9.39[e]		

*Means with the same letter in the same column are not significantly different.

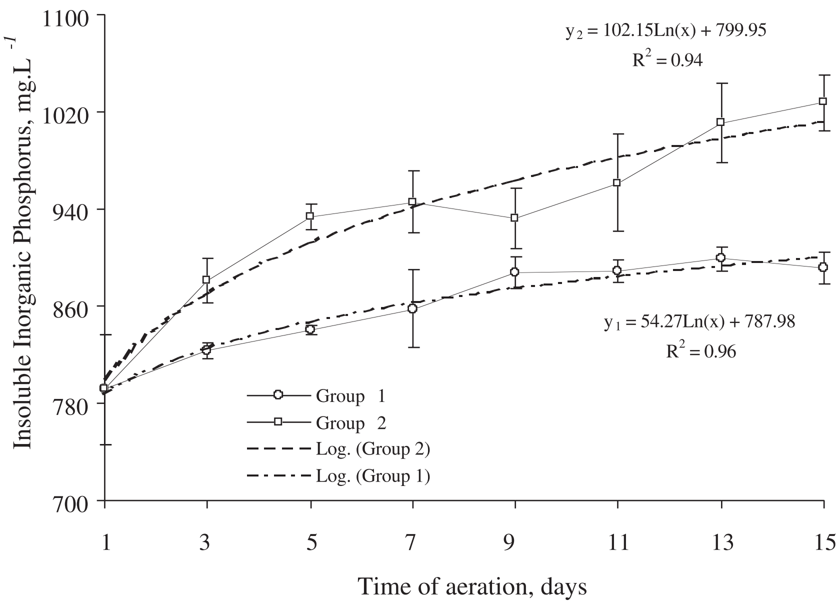

Figure 4. Variation of insoluble inorganic phosphorus with time and temperature of manure during aeration treatment.

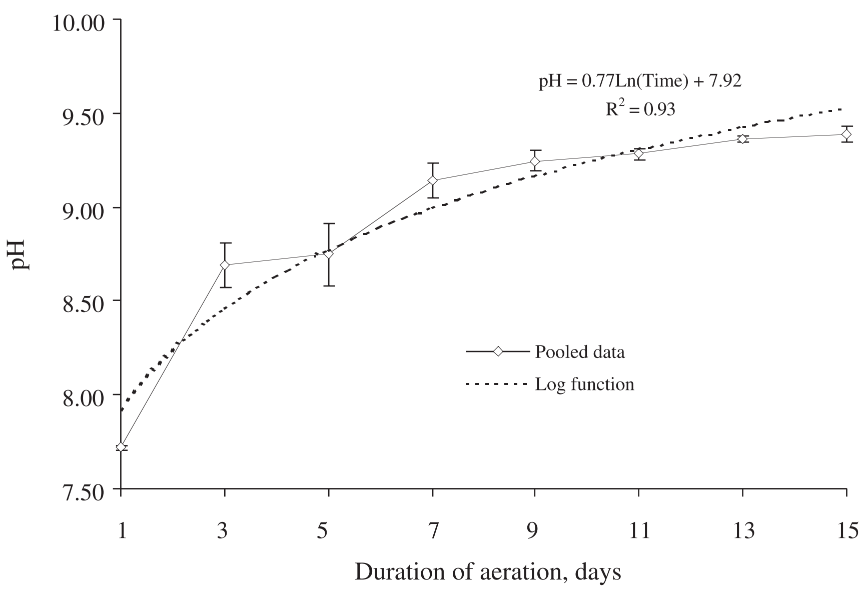

Figure 5. Change in manure pH with aeration time and bioreactor temperature.

data: group 1 comprising temperatures of 5, 10, and 15°C, while group 2 comprised of temperatures 20 and 25°C, to conform with data pooling in previous sub-section. The large increase in pH during aeration has also been observed by other researchers and is mainly attributed to purging of CO_2 out of liquid manure by the aerating air.[4,11–13] Based on Figures 4 and 5, it is evident that, the variations of insoluble inorganic P and the pH of the manure were logarithmic with time for the consolidated data similar to the variation of ortho-P. The corresponding correlation coefficients for these regressions were 0.98, 0.97, and 0.96 for group 1, group 2, and pH, respectively, indicating fairly good fits.

The relationships between insoluble inorganic P and pH for the consolidated data were also developed and are shown in Figure 6. The pH of the aerated manure explains better the content of insoluble inorganic P at the lower temperatures (higher value of coefficient of determinations R^2) than at higher temperatures. The pH explained 90 and 87% of the content of insoluble inorganic P at the lower temperatures (5, 10, 15°C) and at higher temperatures (20, 25°C), respectively. This is not surprising given that the solubility of inorganic P complexes decreases with increasing temperatures. Because there were no significant differences observed in pH at the different temperatures of the reactor during the aeration process, the difference in insoluble inorganic P with temperature cannot be attributed to pH but only to temperature or other factor(s). The observations above corroborate with past studies, in which it was observed that the formation of the complexes between orthophosphates and the Ca^{+2} and Fe^{+3} ions are chemically endothermic, i.e., higher temperatures are more favorable for their formation than lower temperatures.[3] Other possible mechanisms that could explain the trend of insoluble inorganic P are increased aerobes growth and increased uptake of soluble ortho-P and its subsequent storage in microbial biomass in form of polyphosphate with increased temperatures.

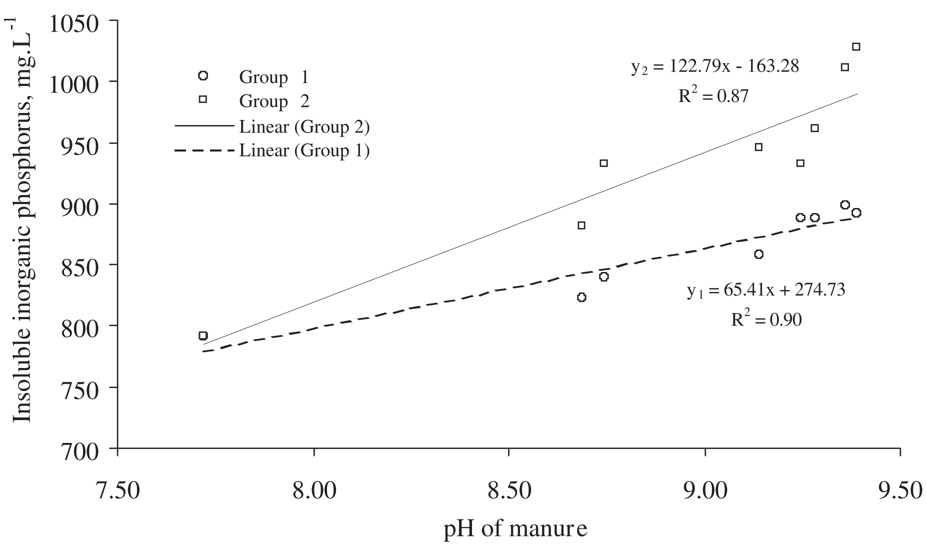

Figure 6. Relationships between manure pH and insoluble inorganic phosphorus during aeration at different bioreactor temperatures.

Table 5. Mean cumulative aerobic microorganisms count (5×10^4) in the manure by time of aeration and temperature of reactors.

Time days	Mean cumulative aerobes growth* (cfu/mL)	Temperature °C	Mean cumulative aerobes growth* (cfu/mL)
1	9.95[a]	5	29.35[a]
3	80.02[a,b]	10	46.98[a,b]
5	124.22[b,c]	15	108.43[b]
9	157.52[b,c,d]	20	279.50[c]
11	190.82[c,d]	25	262.54[c]
13	213.72[d]		
15	241.27[d]		

*Means with the same letter in the same column are not significantly different.

Organic Phosphorus

Relevant statistics for the variations of the cumulative growth of aerobic microorganisms and the content of organic P with time and temperature are summarized in Tables 5 and 6, respectively. Both aerobes growth and the content of organic P changed with both the time of aeration and the temperature of manure. The p-values for the variation of aerobes growth with time and temperature were 0.0001 and less than 0.0001, respectively, while those for organic P, were less than 0.0001 and 0.002, respectively. On separation of means using the LSD method, the changes in the organic P content and the growth of anaerobic microorganisms with reactor-temperature exhibited similar trends to the removal of soluble ortho-P (Figure 3 and Table 2), i.e., the cumulative growth at temperatures of 20 and 25°C were significantly higher than in at temperatures of 5, 10, and 15°C.

Similar consolidation of the data as in the previous sections for these two parameters was made to develop Figures 7 and 8 to present the cumulative growth of aerobic microorganisms, and the content of organic P with time of aeration and tem-

Table 6. Mean organic-P in the manure by time of aeration and temperature of reactors.

Time days	Mean organic-P* mg/L	Temperature °C	Mean organic-P* mg/L
1	1075.54[b,c]	5	1053.54[a]
3	1038.13[c,d]	10	1056.54[a]
5	1008.78[d]	15	1064.24[a]
7	1147.50[a]	20	1145.31[b]
9	1067.49[b,c,d]	25	1121.94[b]
11	1056.60[c,d]		
13	1182.07[a]		
15	1129.99[a,b]		

*Means with the same letter in the same column are not significantly different.

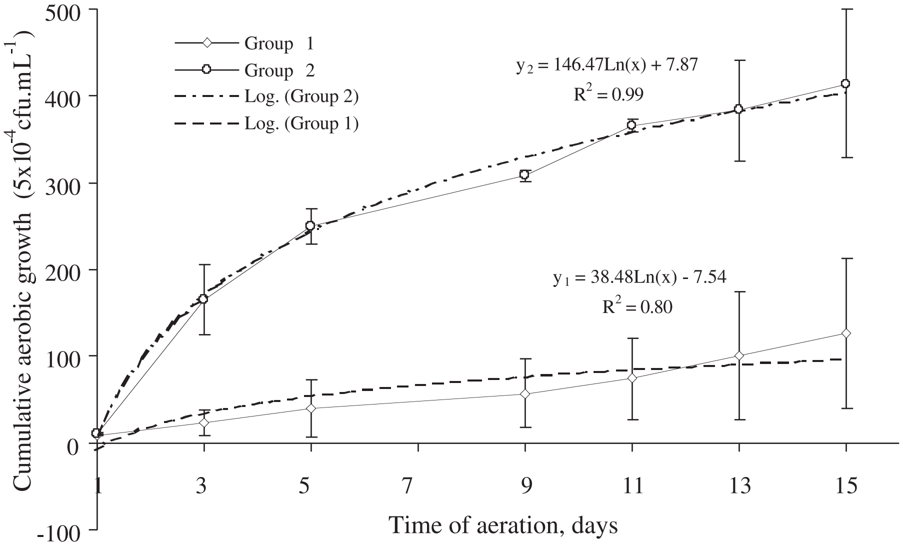

Figure 7. Cumulative growth of aerobes with aeration time and bioreactor temperature.

perature of the manure, respectively. It is clear from both Tables 5 and 6, and Figures 7 and 8 that, the variation of organic P with temperature resembles that of the variation of cumulative aerobes growth. This observation agrees with that of a comparable study, in which it was reported that an increased rate of phosphorus uptake was observed

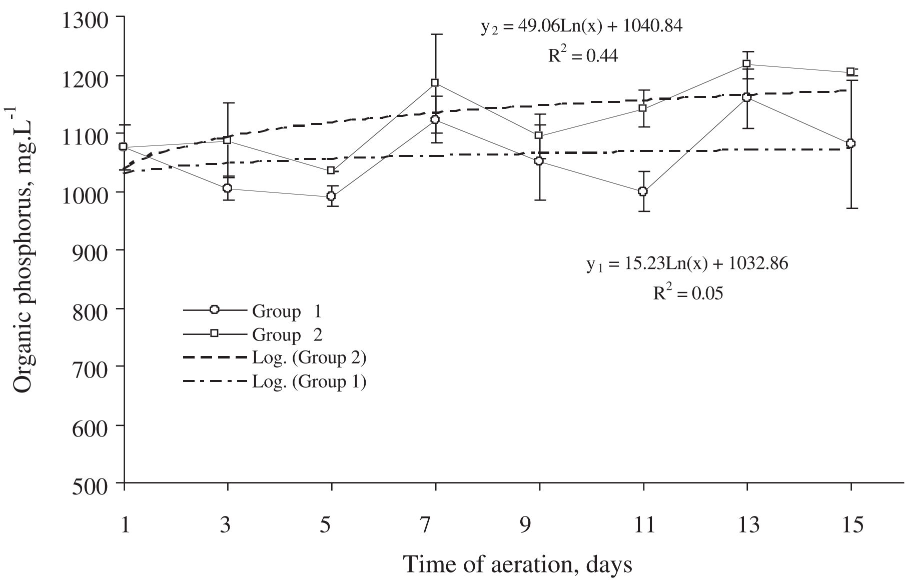

Figure 8. Variation of organic-P with time of aeration and temperature of the manure.

with increasing temperature between 5 and 30°C in activated sludge during aerobic incubation, with a significant uptake occurring above 15°C.[6]

The significant increase in the mean cumulative growth of aerobes between the higher temperatures of 20 and 25°C and the lower temperatures of 5, 10, and 15°C is comparable to that observed in the removal of soluble ortho-P. It can therefore be inferred that the two mechanisms of phosphorus uptake by microbes explain the significant differences observed between soluble P removal at 20 and 25°C and at 5, 10, and 15°C, better than the pH change.

Soluble Ortho-P versus Organic P and Insoluble Inorganic P

The relationships between insoluble inorganic P and soluble ortho-P, and between organic P and soluble ortho-P are both shown in Figure 9. The contributions of insoluble inorganic P and organic P to the content of soluble ortho-P denoted by the values of coefficients of determinations R^2 indicate that the contribution to ortho-P of insoluble inorganic P and organic P were approximately 61% and 35%, respectively. Of the 61% soluble ortho-P removal explained by insoluble inorganic P, the major contribution is probably the pH change during aeration as demonstrated by the high values of R^2 of 0.87 and 0.90 in the relationships between pH and insoluble inorganic P at the higher and lower temperatures, respectively, in the manure during the aeration process (Figure 6). It can therefore be inferred that, during aeration of swine manure at between 5 and 25°C, precipitation is the principal mechanism of soluble ortho-P

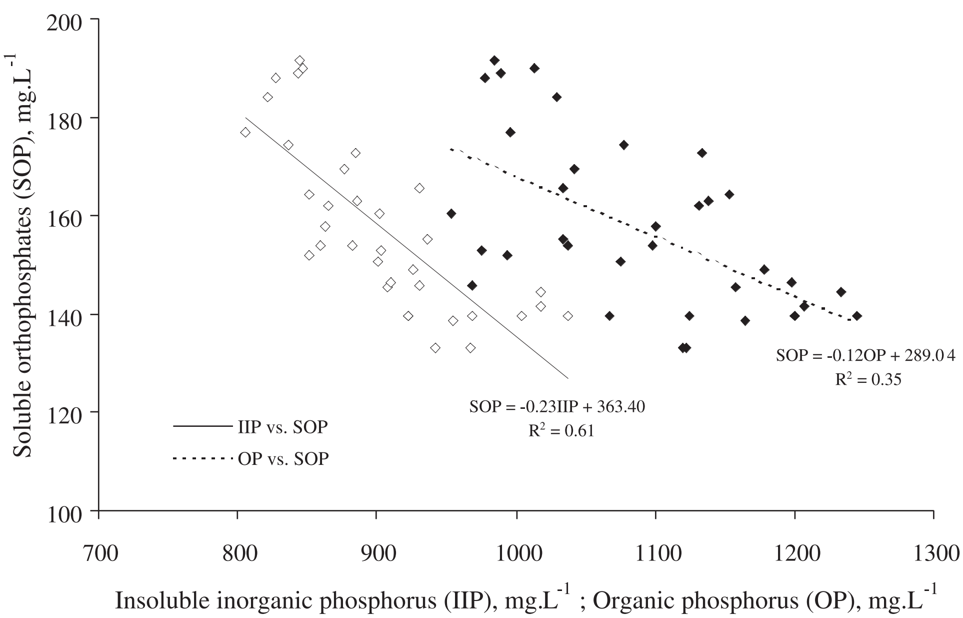

Figure 9. Relationships between insoluble inorganic phosphorus, organic phosphorus and soluble orthophosphates during aeration of swine manure.

transformation into insoluble inorganic P, accounting for 90 and 87% removal by this mechanism at the lower temperatures (5, 10, 15°C) and at the higher temperatures (20, and 25°C), respectively.

SUMMARY AND CONCLUSIONS

Cumulative removal of ortho-P significantly increased with time of aeration from day one to day nine and remained more or less constant thereafter. Removal of ortho-P also significantly increased with increase of bioreactor temperature with removal being significantly higher at the higher temperatures of 20 and 25°C than at the lower temperatures of 5, 10, and 15°C. A maximum removal of approximately 35% was achieved in this study.

Both insoluble inorganic P and pH significantly changed with time of aeration but while insoluble inorganic P significantly varied with temperature of the manure during the aeration process, pH did not. In addition, the contents of insoluble inorganic P were significantly higher at the higher temperatures of 20° and 25°C than at the middle temperatures of 10° and 15°C. The higher amounts of insoluble inorganic P in the manure at 20° and 25°C correspond similar higher removals of soluble ortho-P at the same temperatures. The pH of the aerated manure explained better the content of insoluble inorganic P at the lower temperatures than at higher temperatures. The pH levels explained 92 and 87% of the content of insoluble inorganic P at the lower temperatures (5, 10, 15°C) and higher temperatures (20, and 25°C), respectively.

Both aerobic growth and the content of organic P changed significantly with both the time of aeration and the temperature during the aeration process. The observed significantly higher growth of aerobes at the higher temperatures of 20 and 25°C than at the lower temperatures of 5, 10, and 15°C was similar the to observations made in regard to soluble ortho-P removal, insoluble inorganic P content, and organic P content. It was therefore inferred that the significant growth of aerobes beyond 15°C was probably more responsible for the significant removal of ortho-P observed above 15°C than the change in pH.

The contribution of insoluble inorganic P to the removal of ortho-P was approximately 61% while that of organic P was approximately 35%. Of the 61% soluble ortho-P removal explained by insoluble inorganic P, the major contribution was the pH change during the aeration treatment. It was inferred that, during aeration of swine manure at between 5 and 25°C, physical–chemical precipitation is the principal mechanism of soluble ortho-P conversion into insoluble inorganic P, accounting for 92 and 87% removal at lower temperatures (5, 10, 15°C) and at higher temperatures (20, and 25°C), respectively.

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