

BACTERIAL RESPONSES TO TEMPERATURE DURING AERATION OF PIG SLURRY

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ABSTRACT

The temperature effect on total anaerobic and aerobic bacterial growth in pig slurry was studied using low level batch aeration treatments. Five bioreactors were built using Plexiglas tubes to perform five temperature treatments (5 °C, 10 °C, 15 °C, 20 °C, and 25 °C). An airflow rate of 0.129 L/min/L manure was used to aerate manure contained in all reactors. Data showed that temperature had a profound impact on the aerobic counts in pig slurry during the aeration process. When the temperature increased from 15 °C to 25 °C, the average oxidation-reduction potential decreased from +40 mV to –60 mV, accompanied by a 75% reduction of aerobic bacteria in the manure. At 25 °C, the anaerobic counts were consistently higher than aerobic counts for most of days. A quadratic relationship was observed between the aerobic counts and the oxidation-reduction potential with a correlation coefficient of 0.8374. To reduce odor generation potential, the oxidation–reduction potential in the manure should be maintained at +35 mV or higher.

Key Words: Aeration; Temperature; Pig slurry; Aerobes; Anaerobes

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INTRODUCTION

Tremendous research effort has been spent on using aeration to treat pig slurry for odor control and nutrient management (1-8). However, the main objectives of these studies have focused on testing aerators and developing aeration systems (such as intermittent vs. continuous, surface vs. full, with varying aeration rates, etc.) at both pilot and field scale in order to improve aeration efficiency and facilitate application. Little has been done in revealing the effect of temperature on the biological responses of the related bacterial flora in pig slurry under aeration. Only a few researchers have reported limited information with respect to the effect of temperature on aeration (9,10).

It has been established that both the solubility of air in liquids and the microbial metabolic activity are temperature dependent. It thus follows that under different temperatures, the oxygen content in the liquid, as well as the performance of aerobic bacteria, may vary with the temperature. High temperatures will lead to low solubility of oxygen in the liquid, which, in turn, may affect aerobic processes due to the biological constraints of the bacteria. Since aeration is a process that relies on aerobic bacteria to accomplish the treatment, any factors that can interfere with the aerobic decomposition process should be fully studied in order to properly utilize this technique. Some researchers have already observed the influence of temperature on the removal efficiency of carbonaceous materials from pig manure (11), but more information is needed to better understand these observations.

This paper provides information regarding the temperature effect on aerobic and anaerobic growth, manure pH, volatile fatty acids (VFAs) production, and oxidation-reduction potential (ORP) under low level aeration conditions. The relationships between these parameters are also discussed and the minimal ORP levels needed to maintain the aerobic environment and control odor are suggested.

MATERIALS AND METHODS

Experimental Design

The reactors used were made of clear plexiglas columns (91.44 cm in height and 15.3 cm in diameter) and one of such reactors was shown in Figure 1. The reactors were filled with test manure, leaving approximately 15.0 cm headspace to facilitate stirring and to provide room for any frothing created by aeration. The manure temperature in the column was maintained by a heating tape that wrapped around the column and was controlled by a controller (Catalog No. 11-463-47A, Fisher Scientific) that turned the heating tape on and off based on the feedback from a temperature sensor positioned approximately half way down the manure in the reactor. It was found that this configuration was able to maintain the manure temperature within 1 °C of the set temperature. The aeration of manure in reactors was realized by a positive pressure air pump (Emerson model 0623-V4-G180DX, Gast MFG) that introduced air into the manure through a vinyl tube (6.35 mm internal diameter) at the bottom of each reactor. A variable area flow meter (Model P-32461-64, Cole-Parmer Instrument) was used to regulate the flow in each reactor. A total of five reactors were built to accommodate manure

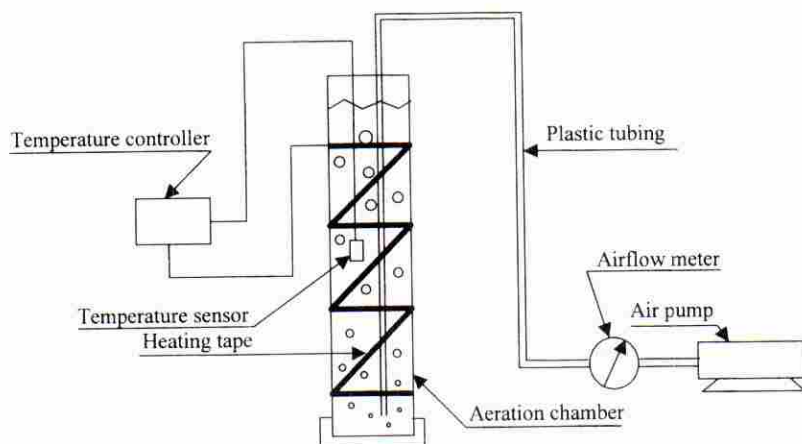


Figure 1. Schematic of the aeration apparatus design.

temperatures of 5 °C, 10 °C, 15 °C, 20 °C, and 25 °C, respectively, with a fixed airflow rate of approximately 0.129 L/min/L manure throughout the experiment. This airflow rate was determined in several preliminary trials to ensure that the ORP in manure fell into a range between -120 mV and 200 mV, considered as low level aeration. The experiment was carried out in winter to allow establishment of the desired temperatures using heating tapes.

Fresh pig slurry from a finishing building was used to run the test and each reactor was loaded once with no addition of manure during the test. For sampling, the manure in each reactor was thoroughly stirred using a motorized paddle-stirrer (Tline Laboratory Stirrer, Model 102, Talboys Engineering) and a sample was drawn from approximately the mid-depth of each reactor for laboratory analyses. Manure sampling was performed every two days starting from day 1 for the entire test period of 21 days.

Sample Analyses

All manure samples were analyzed for pH, ORP, VFAs, and total anaerobic and aerobic bacterial counts. The pH for all the samples was determined immediately after sampling using a pH meter (Orion model 720A, Orion Research), while ORP was determined directly from each reactor at each sampling time using an oxidation-reduction potential meter (DIGI-SENSE, model 5938-52, Cole-Parmer Instrument). The total anaerobic and aerobic bacterial counts were determined using Oxyrase plates (Catalog No. O-BRU-BA, Oxyrase, Mansfield, Ohio 44901). Manure samples were first diluted to 10^{-5} , based on previous trials, and droplets of 20 μL of the diluted solutions were spotted onto the Oxyrase plates. When the droplets were absorbed into the nutrient medium, the plates were either closed with special lids that came with the plates for anaerobic growth, or left open for aerobic incubation, as instructed by the manufacturer. Both aerobic and anaerobic plates were moved into an incubator with temperature set at 37 °C. After incubating for five days, the number of bacterial colonies on the nutrient media plates

were counted by eye and the concentration of bacteria from the manure was calculated based on the dilution level used.

For VFA determinations, a well-mixed sample was diluted and then centrifuged at 3000 g for 30 minutes. The centrifuged samples were then filtered using GF/A Whatman filter papers. The VFAs in the filtrates were determined using an esterification method (12). This method is based on esterification of the carboxylic acids present in the sample followed by colorimetric determination of the esters produced by the ferric hydroxamate reaction. All volatile fatty acids are reported as their equivalent mg/L acetic acid (13). Whenever analysis was not done immediately after sampling, the samples were stored at -20°C and only thawed and allowed to reach room temperature prior to analyses.

Statistical Paired *t* test and *F* test were used to compare results using a significance level at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Temperature Effect on the Oxidation–Reduction Potential

Figure 2 illustrates the variations of oxidation-reduction potential (ORP) over the experimental period for the five different temperatures. It can be seen that all ORPs increased during the first day of aeration; however, four out of five ORPs drastically decreased in the following day except for the one at 5°C , which decreased slowly over a span of several days and became somewhat steady after day 9. The ORPs for temperatures from 10 to 25°C , after dropping from the initial peak values, remained relatively steady for the rest of test period after day 3. This phenomenon differs pig slurry from clean water in response to aeration process due to the presence of a consortium of microbes including aerobes. When aeration started, the aerobic bacteria were not activated immediately, thus,

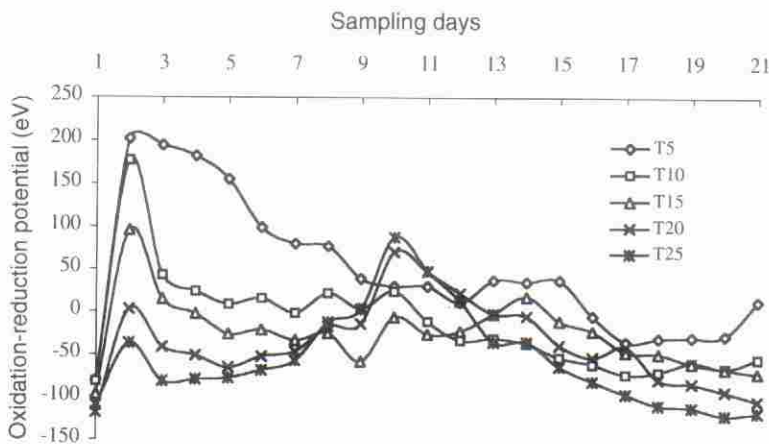


Figure 2. Variations of oxidation-reduction potential for temperatures from 5°C to 25°C during the test period.

an initial steady rise of ORP was observed due to the accumulation of dissolved oxygen in the liquid. In about one day, a rapid decline in ORP occurred as a result of the increased respiration rate by the activated aerobes that outstripped the supply of oxygen in the liquid because of a constant aeration rate. Similar results were also reported by Hissett et al. (9).

The sharp decline of ORPs after one-day operation may reveal another facet of aeration technique in treating pig slurry. Since aerobes are the major working force in this process, keeping up a critical aerobic population is the key to the success of aeration treatment. In order to do so, it is essential to maintain a necessary level of oxygen in the treated liquid to support aerobic activities. Obviously, the data from this study do not appear to provide evidence that maintaining a relatively stable oxygen concentration in the liquid can be accomplished by aerating pig slurry at a constant rate (Figure 2). It can therefore be inferred that, due to the complexity of the slurry environment of biological nature, more detailed research is needed to apply the above findings to the practical design of a better aeration system.

Also, it is shown in Figure 2 that the increase in ORPs was inversely related to temperature, i.e., the higher the temperature, the lower was the increase in ORPs. This observation is consistent with the well-established theory governing gas-liquid surface transfer, which states that the solubility of a gas in a liquid is inversely proportional to the liquid temperature. This also implies that more energy is needed at higher temperatures than at lower temperatures to aerate pig slurry to reach the same oxygen concentration in the liquid.

In theory, the oxidation-reduction potential reflects the oxidative/reductive environment in the liquid under treatment. Charpentier et al. (14) proposed a guideline in describing the oxidative/reduction status of a liquid based on ORP levels. When the ORP in the liquid reaches 100 mV and above, the liquid environment is said to be aerobic with detectable dissolved oxygen present and the aerobic respiration prevails. When the ORP in the liquid drops to -300 mV and below, anaerobic environment will dominate and the fermentation process will come into play. Any ORPs falling in between these two limits will be indicative of an anoxic environment. According to this rule, only three ORPs at temperatures of 5 °C, 10 °C, and 15 °C either reached or exceeded 100 mV after one-day aeration. While only one ORP (at 5 °C) maintained in the aerobic zone through day 6 of aeration and all ORPs were in the anoxic zone thereafter, regardless of temperature. It appears that temperature effect on aeration efficiency becomes insignificant for extended aeration under a constant aeration rate. However, even when all the ORPs are in the anoxic zone, the ORP gradient still is in opposition to the temperature gradient, as seen in Figure 2 for the latter part of the aeration process, clearly indicating the temperature dependence of ORPs. This observation is not in agreement with the report by Cumby (5), in which aerator performance was found to be consistently better at raised temperatures. Obviously, such a claim not only violates the temperature related gas-liquid transfer law, but also was evidenced to be incorrect by the data from this study.

Temperature Effect on Bacterial Counts

The means and standard deviations of total aerobic and anaerobic bacterial counts in the entire test period are presented in Table 1. The measurements on day 1 prior to aeration were based on raw samples in bulk volume without duplication. It is interesting

Table 1. Total Aerobic and Anaerobic Bacterial Counts at Different Temperatures ($\times 10^5$)[†]

Sampling Days	Bacterial Type	Temperature (°C)				
		5	10	15	20	25
1	Aerobes	73	73	73	73	73
	Anaerobes	63	63	63	63	63
3	Aerobes	23 ± 5.6 ^{a,c,x,y}	28 ± 2.3 ^{a,x}	38 ± 6.7 ^{b,x,z}	20 ± 3.4 ^{a,c,x}	5 ± 2.4 ^{d,x}
	Anaerobes	28 ± 4.6 ^{a,x}	30 ± 6.3 ^{a,x,z,t}	50 ± 4.2 ^{b,x,u}	25 ± 4.1 ^{a,x,y}	13 ± 3.1 ^{c,y,u}
5	Aerobes	23 ± 4.1 ^{a,x,y}	48 ± 2.1 ^{b,y}	25 ± 3.7 ^{a,y,r}	28 ± 4.1 ^{a,x,y,u}	73 ± 8.9 ^{c,z}
	Anaerobes	14 ± 4.9 ^{a,y}	35 ± 5.1 ^{b,z}	35 ± 6.2 ^{b,y,z}	22 ± 5.6 ^{c,x,y}	3 ± 2.2 ^{d,x}
7	Aerobes	110 ± 11.6 ^{a,z}	50 ± 9.8 ^{b,y,w}	60 ± 10.1 ^{b,u,s,t}	33 ± 5.6 ^{c,y,z,w}	16 ± 7.8 ^{d,y,u}
	Anaerobes	58 ± 7.4 ^{a,u,w}	98 ± 9.6 ^{b,u}	65 ± 9.8 ^{a,u,s,t}	23 ± 4.9 ^{c,x,y}	38 ± 5.1 ^{d,y}
9	Aerobes	78 ± 8.8 ^{a,v}	88 ± 9.5 ^{a,u}	50 ± 6.7 ^{b,x,z,s}	43 ± 7.1 ^{b,z,w,t}	10 ± 5.4 ^{c,x,y}
	Anaerobes	30 ± 5.6 ^{a,x}	33 ± 7.1 ^{a,x,z}	53 ± 8.6 ^{b,x,z,s}	43 ± 8.2 ^{a,b,z,w,t}	44 ± 4.3 ^{b,x,y}
13	Aerobes	80 ± 8.1 ^{a,c,v}	70 ± 4.8 ^{a,v}	90 ± 7.3 ^{c,v}	50 ± 6.7 ^{d,t}	15 ± 3.7 ^{c,y,u}
	Anaerobes	45 ± 7.1 ^{a,u}	50 ± 4.2 ^{a,y,w}	100 ± 12.4 ^{b,v}	48 ± 3.7 ^{a,t}	43 ± 2.9 ^{a,y,u}
15	Aerobes	63 ± 5.4 ^{a,w}	60 ± 5.6 ^{a,w}	83 ± 6.4 ^{b,v,w}	40 ± 7.1 ^{c,z,v,w,t}	18 ± 4.2 ^{d,y}
	Anaerobes	88 ± 7.4 ^{a,v}	33 ± 3.4 ^{b,x,z}	73 ± 5.3 ^{c,u,w}	30 ± 4.2 ^{b,v,u,w}	53 ± 2.9 ^{d,y,u}
17	Aerobes	40 ± 7.8 ^{a,u}	45 ± 8.2 ^{a,y,z}	50 ± 7.9 ^{a,x,z,s}	28 ± 8.4 ^{b,x,y,w}	7 ± 4.2 ^{c,x,y}
	Anaerobes	53 ± 5.7 ^{a,u}	30 ± 6.5 ^{b,x,t}	73 ± 8.9 ^{c,w,t}	18 ± 6.2 ^{d,x}	40 ± 5.3 ^{b,x,y}
19	Aerobes	18 ± 5.3 ^{a,x}	40 ± 8.7 ^{b,y,z}	55 ± 7.9 ^{b,s}	28 ± 4.8 ^{a,y,u,v,w}	4 ± 2.7 ^{c,x}
	Anaerobes	12 ± 7.6 ^{a,c,y}	20 ± 4.7 ^{a,t}	20 ± 6.5 ^{a,t}	25 ± 8.6 ^{a,x,y,u,v}	36 ± 5.8 ^{d,x,u}
21	Aerobes	25 ± 4.8 ^{a,x}	68 ± 8.3 ^{b,v,w}	30 ± 4.3 ^{a,y,r}	48 ± 5.2 ^{c,t}	3 ± 2.1 ^{d,x}
	Anaerobes	5 ± 4.3 ^{a,t}	10 ± 3.7 ^{a,s}	25 ± 5.1 ^{b,y,r}	33 ± 5.3 ^{b,y,z,u,v,w}	23 ± 2.5 ^{b,x}

[†]Same letters (a, b, c, d, e) indicate no statistical difference between columns at a significance level of $\alpha = 0.05$. Same letters (x, y, z, u, v, w, s, t) indicate no statistical difference between rows at a significance level of $\alpha = 0.05$.

to note that when aeration was initiated, both aerobic and anaerobic bacterial counts dropped drastically as opposed to the original counts. The reason why the aerobic counts showed the same trend as the anaerobic counts is not clear and may connote a lag phase that normally exists before the aerobes adapt themselves to the changed environment.

According to Table 1, the aerobic counts started to increase significantly on day 7 for columns with temperature maintained at 5 °C and 15 °C (from 23×10^5 and 25×10^5 to 110×10^5 and 60×10^5 , respectively), and on day 5 for the column with temperature of 10 °C (from 28×10^5 to 48×10^5). After the increase, the counts fluctuated between the range of 40×10^5 and 80×10^5 for the 5 °C column, 48×10^5 and 88×10^5 for the 10 °C column, and 50×10^5 and 90×10^5 for the 15 °C column, indicating an active aerobic flora present in the liquid manure due to aeration. The aerobic growth appeared to die down on day 19 and 21 for the 5 °C and 15 °C treatments and was statistically at the same level at the end of the test. While in the 10 °C column, this decrease in aerobic counts was not observed. In spite of this difference, it may still be inferred that there appears no difference in terms of temperature effect (5 °C, 10 °C and 15 °C) on the aerobic growth, based on the growth pattern of aerobes under the three temperature regimes.

In contrast, a completely different scenario was seen for aerobic growth for columns with temperatures at 20 °C and 25 °C. Although there was a significant increase

in aerobic counts on day 7 for the 20 °C column, the overall level in the entire test period was much lower than those in the lower temperature columns. The situation was even worse for the 25 °C column in which all the aerobic counts were significantly lower than in the other four columns except for the reading on day 5. It is commonly assumed that aerobic activities would be enhanced at the raised temperatures. Obviously, raising temperature alone does not meet this postulate based on the data presented in Table 1. As a matter of fact, increasing temperature played a role of inhibiting aerobic growth under the test environment in this study.

The decrease in aerobic counts could be caused by the diminishing supply of oxygen in the liquid associated with the temperature increase. Comparing the aerobic counts with the anaerobic counts in Table 1 may prove this hypothesis. At low temperatures (≤ 15 °C), the aerobic counts are generally significantly higher than anaerobic counts, implying that aerobes may dominate in this temperature range. However, when the temperature goes above 15 °C, the number of aerobes declines sharply while the number of anaerobes steadily increases to become dominant. When temperature reaches 25 °C, more than 75% of the initial aerobic cells are probably destroyed. This switch in position indicates a change in manure environment, i.e., from aerobic to anaerobic, which can be seen in Figure 2. The average oxidation–reduction potential (ORP) decreases drastically from about +40 mV to –60 mV, accompanied by an increase in temperature from 5 °C to 25 °C. It is generally accepted that with increasing temperatures, the solubility of oxygen in liquids decreases. Therefore, to bring about increase in biodegradation, more oxygen is required which, in turn, means a higher energy demand (13). Since low temperatures generally mean low biodegradation rates but high solubility of oxygen in the liquid, the data from this study strongly suggest that there should be an optimal point at which a cost-effective combination of temperature and aeration rate can be found. Unfortunately, little information in this aspect is available in the literature.

Manure pH and VFA Changes Over the Temperature Range

The means and standard deviations for manure pH and VFA changes over the temperature range are presented in Table 2. It is seen from Table 2 that when the temperature increased from 10 °C to 15 °C, the manure pH decreased significantly from

Table 2. Manure pH and VFA Changes Over the Temperature Range[†]

Temperature (°C)	Mean pH	Standard Deviation	Mean VFAs (mg/L)	Standard Deviation
5	8.35 ^a	0.18	8.56 ^c	0.59
10	8.15 ^a	0.09	9.72 ^c	0.49
15	7.68 ^b	0.25	11.33 ^d	0.74
20	7.54 ^b	0.40	11.16 ^d	0.63
25	7.28 ^b	0.44	12.24 ^d	0.64

[†]Different letters mean statistical differences between data at a significance level of $\alpha = 0.05$.

8.15 to 7.68. What is interesting here is that accompanied with the pH decrease is the simultaneous increase in VFA level in the manure. This increase in VFA levels may reveal the transition of manure environment from aerobic to anaerobic. Since VFAs are typical intermediate and end products of anaerobes (15), the enhanced production of VFAs in the test manure signals the enhanced anaerobic activity that is obviously favorably supported by the forming anaerobic environment. Another more general conclusion that may be drawn from the data in Table 2 is that the manure pH may be used as an indicator of the aerobic and/or anaerobic environment in the manure. For an aerobic environment to prevail, the manure pH should be maintained at least above 7.68.

The Correlation of ORP with the Total Aerobic and Anaerobic Counts

The influence of ORP on aerobic bacterial growth is shown in Figure 3. It can be seen that the aerobic bacterial counts are affected by the ORP in the liquid. When ORP reached about -125 mV, the aerobic counts almost approached zero, revealing the lower limit of ORP for aerobes to grow. A relatively good, quadratic relationship is observed for aerobes with ORP (correlation coefficient: $R = 0.8374$). Charpentier et al. (14) reported that when ORP was below 0 mV, no dissolved oxygen (O_2) was present. And the potential oxygen source was nitrates (NO_3^-) for ORPs between 0 mV and -300 mV. Theoretically, nitrates can still be used by some facultative aerobes as the electron acceptor to continue their metabolic activity as long as the ORP is above -300 mV. However, the data from this study indicates that the aerobic metabolism could be slashed even when the ORP is well above -300 mV (-125 mV here). This observation may

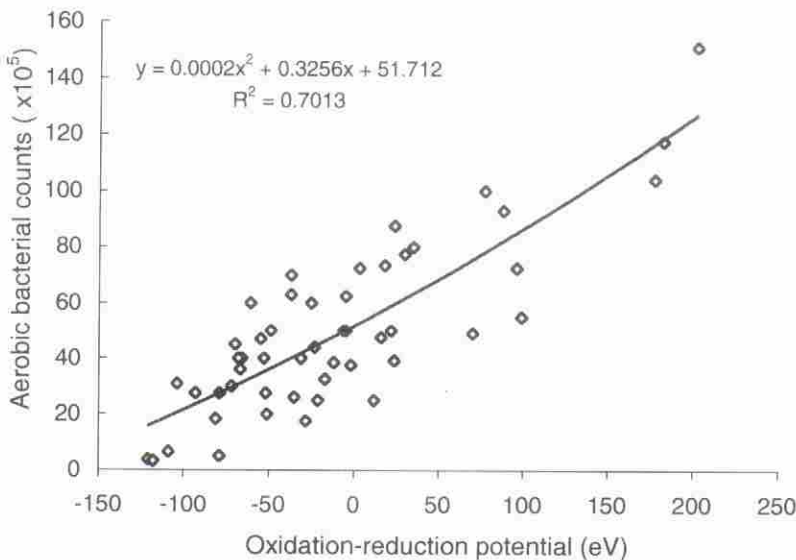


Figure 3. Changes in aerobic bacterial counts vs. the oxidation-reduction potential.

imply that there are not enough nitrates in the manure. The cause of this possible nitrate deficiency was explained by Evans et al. (1986) in which it was stated that, at low aeration level with ORP around -50 mV, nitrification was inhibited and the concentration of ammonia and organic nitrogen in most cases remained similar to that in the fresh manure. Therefore, it may be inferred that when ORP falls into the negative regime, its role in assisting aerobes to grow will become critical. Without adequate ORP in the liquid, aerobic metabolism may not be able to proceed properly. For this reason, Evans et al. (16) suggested that an ORP level of -50 mV in the manure be required to maintain the minimal aerobic activity.

As compared to aerobes, the relationship for anaerobes with ORP is not obvious (Figure 4). Apparently, increasing ORP from about -125 mV to $+200$ mV has a greater influence on the aerobic growth than on the anaerobic inhibition, if Figures 3 and 4 are examined. However, the effect of ORP on anaerobic growth is still phenomenal in that the anaerobic bacterial counts are reduced when ORP surpasses $+35$ mV. Although past researchers suggested that a minimal ORP of -50 mV be maintained for aerobic respiration (16), large quantities of anaerobes would coexist in the ORP ranging from -50 to $+35$ mV, as shown in Figure 4. In this coexisting ORP regime, these anaerobes can be active in their metabolic activities. Therefore, from the standpoint of odor control, this functioning anaerobic consortium may still be capable of producing quantities of odorous compounds. A similar study conducted by Williams et al. (17) showed that odor offensiveness could be controlled if the ORP was maintained at above $+13$ mV for manure with temperature ranging from 28 °C to 35 °C. Another study by Burton et al. (18) showed that if the ORP was controlled at $+133$ mV for manure at temperature of 22 °C, the odor intensity was measured as between very faint and faint. Although the temperatures used in these two studies differed slightly, their results could be considered

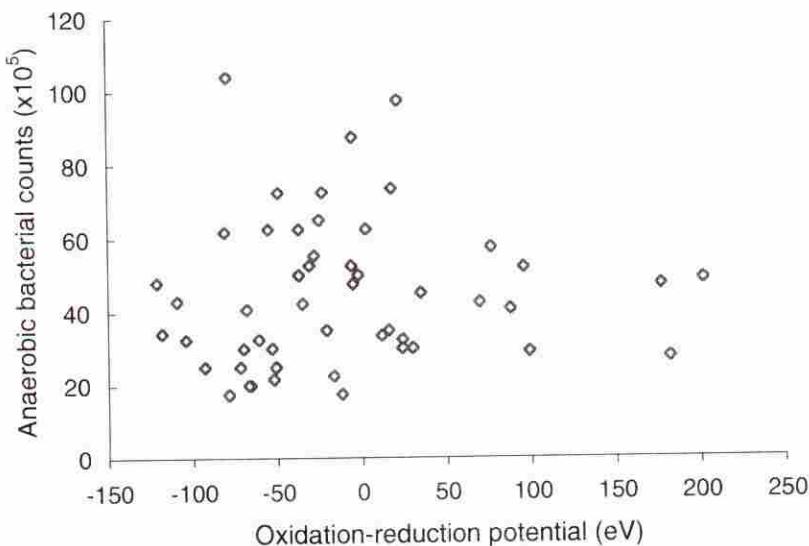


Figure 4. Changes in anaerobic bacterial counts vs. the oxidation-reduction potential.

consistent with the finding of minimal ORP from this study in which a minimal ORP of +35 mV is suggested to be maintained in the liquid in order to reduce the anaerobic activity, thereby reducing the potential of odor generation. Since odor offensiveness is not measured in the current study, the above suggestion needs to be verified.

CONCLUSIONS

The data clearly indicate that with the current experiment design (at an airflow rate of 0.129 L/min/L manure), the manure temperature should be kept under 15 °C to maintain a dominant level of aerobic bacterial groups. When the temperature increases from 15 °C to 25 °C, about 75% of the aerobic bacteria in the manure will be destroyed. Also, the average oxidation–reduction potential will decline from around +40 mV to –60 mV due to the increase in temperature from 5 °C to 25 °C, resulting in an environmental transition from aerobic to anaerobic.

The data also suggested that for odor control, the manure ORP should be maintained at 35 mV or higher to effectively assist the aerobic growth and, at the same time, hinder the anaerobic activity. However, this postulate needs further verification due to lack of odor offensiveness data in this study.

A quadratic relationship between the aerobic bacterial counts and the ORP is obtained with a correlation coefficient of 0.8374. This relationship illustrates the importance and significance of maintaining a certain level of ORP in the liquid manure to assist aerobic growth. Although such relationship is not observed between the anaerobic bacterial counts and the ORP, the data still imply that keeping the manure ORP in the positive regime would certainly help prevent anaerobic zones from forming.

According to the data, manure pH may be used as an indicator of the environment (high pH, aerobic; low pH, anaerobic). It thus follows that intentionally increasing manure pH to some extent may enhance aerobic growth but hinder anaerobic growth. This area obviously needs more research. Also, it appears that the temperature-induced increase in pH will become negligible when the temperature goes beyond 15 °C.

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