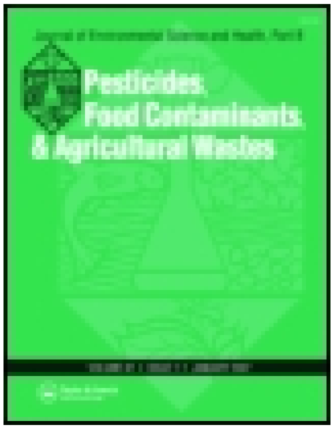

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A novel use of anaerobically digested liquid swine manure to potentially control soybean cyst nematode

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Experiments were carried out in two steps to determine the effect of anaerobically digested swine manure on soybean cyst nematode (SCN) egg control. In the first step, liquid swine manure underwent anaerobic digestion to search for the best digestion time for both volatile fatty acids (VFA) and ammonium nitrogen (NH_4^+) enrichment. The results showed that about 17 and 28 days of incubation were needed, respectively, to reach the maximal levels of VFA and NH_4^+ in the manure. In the second step, raw, VFA-enriched, and NH_4^+-enriched manure were applied separately, at four different rates (25, 50, 100, and 200 mL/pot), to soil pots inoculated with nematode eggs in a greenhouse environment. Soil samples were collected 35 and 61 days after inoculation to determine the effect of such treated manure on SCN egg productivity. The data indicated that the SCN egg counts were inversely related to the manure application rates in a linear manner with correlation coefficients of 0.998, 0.967, and 0.900 for raw, NH_4^+-enriched, and VFA-enriched manure for the 35-day samples. While no such relationships were found for the 61-day samples, implying that none of the treatments were still effective 61 days after application. At the four application rates, the VFA-enriched manure performed best in reducing SCN egg counts (by 18.1, 19.5, 34.3, and 18.6%) as compared to the raw manure treatment. In contrast, the NH_4^+-enriched manure achieved mostly negative reductions. To achieve the best control of SCN egg growth, the VFA-enriched manure should be used and applied to soybean fields every 35 days.

Keywords: swine manure; soybean cyst nematode egg control; anaerobic digestion.

Introduction

Currently, rigorous efforts have been put forth to research alternative ways of using swine manure to recuperate its values besides fertilizer, with emphasis primarily on energy recovery through anaerobic digestion and fermentation. However, the beneficial use of swine manure should not be only limited to the energy sector and needs to be diversified to better promote waste recycling and reuse. A promising area that has largely been untouched is the possibility of converting liquid swine manure into a pesticide, such as nematicide. Soybean cyst nematode (SCN) is a known problem, with few effective controlling techniques available, which affects soybean yields and causes many root diseases that interfere with nutrient uptake by the crop (iron deficiency is one example), leading to reduced soybean productivity. Swine manure is traditionally applied to corn field but there appears a growing trend of disposing of liquid swine manure to soybean production fields. Available information shows that about one-third of Iowa livestock producers are actually applying swine manure to soybean fields [1] and some producers in Minnesota are doing the same, too. The most exciting news with this practice rests with preliminary observations by a number of researchers that swine manure may have potential of reducing SCN infection of soybean roots. [1−3] Since swine manure contains high levels of mineral nutrients along with organic acids and ammonia toxic to SCN, applying swine manure to soybean fields may not only control SCN but also provide the needed nutrients for soybean growth at the same time, thereby improving soybean health, minimizing SCN damage, and increasing soybean productivity and profitability. Following that line, research is needed to develop either chemical or biological processes in order to engineer swine manure into a product with SCN control capability.

Volatile fatty acids (VFAs) and ammonium (NH_4^+) are the two major constituents in swine manure, which are reported to be biocidal to pathogens by a number of previous workers. [4,5] Limited lab-scale experimental data showed that a soil containing high level of VFAs would usually

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be suppressive to plant-pathogens. [2,3] A field experiment conducted by the Iowa State University revealed that soybean yields were increased in fields treated with swine manure as compared to those without due potentially to the impact of VFA and NH_4^+. [1] These findings are encouraging and could all point to a postulate that swine manure, after certain treatments, may posses the capability of controlling parasitic losses of soybean yields caused by SCN.

It is well established that VFAs are produced during anaerobic decomposition of liquid animal manure.[6] Undigested fresh swine manure normally contains a large amount of proteins that can be converted to VFAs and ammonia via anaerobic digestion, with VFAs being precursors for methane production. It is therefore possible to achieve high VFAs concentrations in swine manure by controlling the digestion process. Although there are a few reports in literature that have documented the observation of VFA increase during the digestion process, [7–9] more detailed information is definitely required to develop strategies for better using this process to maximize the production of VFAs in swine manure.

The objectives of this study were to first investigate the best incubation times for both VFA and NH_4^+ enrichment in swine manure undergoing anaerobic digestion. Such prepared manure with elevated levels of VFA and NH_4^+, together with raw manure, was then separately applied to SCN-egg inoculated soils contained in pots that were placed in a greenhouse to evaluate the feasibility and effectiveness of the partially digested manure for SCN control. Based on the information obtained, suggestions for practical use of this method were also made.

Materials and methods

Experiment for VFA and NH_4^+ enrichment through anaerobic digestion of swine manure

At this stage, experiments were carried out focusing on the enrichment of volatile fatty acids (VFAs) and ammonium (NH_4^+) in swine manure using anaerobic digestion. Raw manure was collected from a finishing barn, equipped with a pull-plug manure handling system, at the University of Minnesota Southern Research and Outreach Center at Waseca. The collected manure (around 4–5% total solids level with the initial levels of VFA, NH_4^+, and pH being 6030 ± 864 mg/L, 1524 ± 0.0 mg/L, and 7.39 ± 0.02, respectively) was then placed in lab-scale anaerobic digesters fabricated from clear acrylic columns (in triplicate for both VFA and NH_4^+ determination), each measuring 15 cm in diameter and 91 cm in height with a working volume of 15 L. The digesters, after being loaded with manure, were maintained airtight throughout the experiment to create a strictly anaerobic environment inside the vessels. A liquid sampling port was located about 20 cm from the bottom on the sidewall of each digester and samples of 50 mL each were collected every other day from all columns during the entire experimental period. During sampling, pressurized nitrogen gas was passed through an air diffuser placed at the bottom of the digester for about 5 minutes to agitate the manure in the column so a well-mixed sample could be taken. The length of experiment for either VFA or NH_4^+ was determined when the concentration of these two constituents in the manure under digestion had reached and passed their respective maximal values with a downhill pattern clearly demonstrated. Ammonia (NH_4^+-N) in the liquid samples was determined using the standard methods.[10] The VFA measurement was based on esterification of the carboxylic acids present in the sample followed by colorimetric determination of the esters produced by the ferric hydroxamate reaction.[11] The manure pH was measured by a Digi-Sense digital pH/Temperature/mV/ORP meter (Model No. 5938-10, Coleparmer Company, Illinois, USA). All tests were run under room temperature.

Experimental design for studying the effect of digested manure on soybean cyst nematode control

Manure preparation

As stated early, three manure types were used for the greenhouse experiments, i.e., raw, VFA-enriched, and NH_4^+-enriched. Based on results from the previous stage, a big tank (4.5 m^3) was used as an anaerobic digester to digest the freshly collected manure from the pit to boost both VFA and NH_4^+ levels. The withdrawal of enriched manure began when the incubation times had elapsed for VFA and NH_4^+ to reach their respective maximal values, which were determined with reference to the benchmarks obtained from the Stage 1 experiments. Raw manure treatment used manure collected directly from the pit without going through the digestion process. All collected manure was stored in a freezer at -20°C, if not used immediately after collection.

Preparation of soybean cyst nematode eggs

The SCN eggs were obtained via several steps in a greenhouse environment. First, the soybean cyst nematodes were cultured in pots planted with susceptible soybeans for one and a half months. Cysts were then collected from the soybean roots by hand-decanting, sieving (850-μm-pore for the top sieve, 250-μm-pore for the bottom sieve), and centrifugation in a 63 % (w/v) sucrose solution at 1,500*g* for 5 minutes. The next step was to skim off the collected cysts from the top of the tube onto a 250-μm-pore sieve, and crushed them mechanically to release the SCN eggs. [12] Finally, the eggs were captured using nested sieves (75-μm-pore on top of a 25-μm-pore sieve) and cleaned using centrifugation in a 38% (w/v) sucrose solution to remove most of the remaining debris. The collected eggs were maintained in tap water until use.

Experimental setup

Natural field soil (loamy type) without SCN was collected from an experimental field of University of Minnesota Southern Research and Outreach Center at Waseca, MN. The soil was screened and well mixed prior to use. Each 16-cm (diameter) clay pot received an amount of 1,500 g (0.8 liter) of soil that was uniformly inoculated with SCN eggs at 6,000 eggs/100 cc soil by thorough mixing. Four different application rates (25, 50, 100, and 200 mL/pot, equivalent to 23.4, 46.8, 93.6, and 187.2 m^3/hectare) were used for the three treatments (raw, VFA-enriched, and ammonium-enriched manure), with each pot receiving the same amount of liquid of 200 mL. For those rates with a liquid amount less than 200 mL, water was used to balance the difference. A treatment with water was also included as control. These application rates were established with reference to the commonly used range of manure application in the field by soybean farmers (28.0–140.1 m^3/hectare). [13] In the field study conducted by the Iowa State University, the application rate of manure was reported to be 56 m^3/hectare.[1] The manured pots were then planted with SCN-susceptible soybean cultivar "Sturdy". A randomized block design with three treatments and four replicates was used for the experiment. All the pots were placed on the bench in the greenhouse and watered daily to maintain soil moisture. Two sets of pots, each having four replicates, were established for two sampling times to determine the SCN egg population density, with one set sampled 35 days, and the other 61 days, after planting.

Soybean cyst nematode eggs density determination

On day 35 and 61 after the experiment started, SCN females were dislodged from roots by rubbing and then the roots were cut into fine pieces and mixed thoroughly with soil. A sub-sample of 100 cc (185 g) of soil from each pot was taken to determine SCN egg population density. Similar procedures used in egg preparation described early were followed to collect SCN eggs with the exception that an elutriator was used here instead of hand-decanting. The collected eggs were refrigerated in water using 50-ml tubes until counted and the counting was performed using an inverted microscope.

Statistical analysis

Where applicable, statistical paired-t tests were employed for all the comparisons between treatment means at a significant level of $\alpha = 0.05$.

Results and discussion

Changes of volatile fatty acids, ammonium, and pH in the manure during the digestion process

Figure 1 shows the changes in volatile fatty acids (VFAs) and pH over the digestion course. The VFA level increased

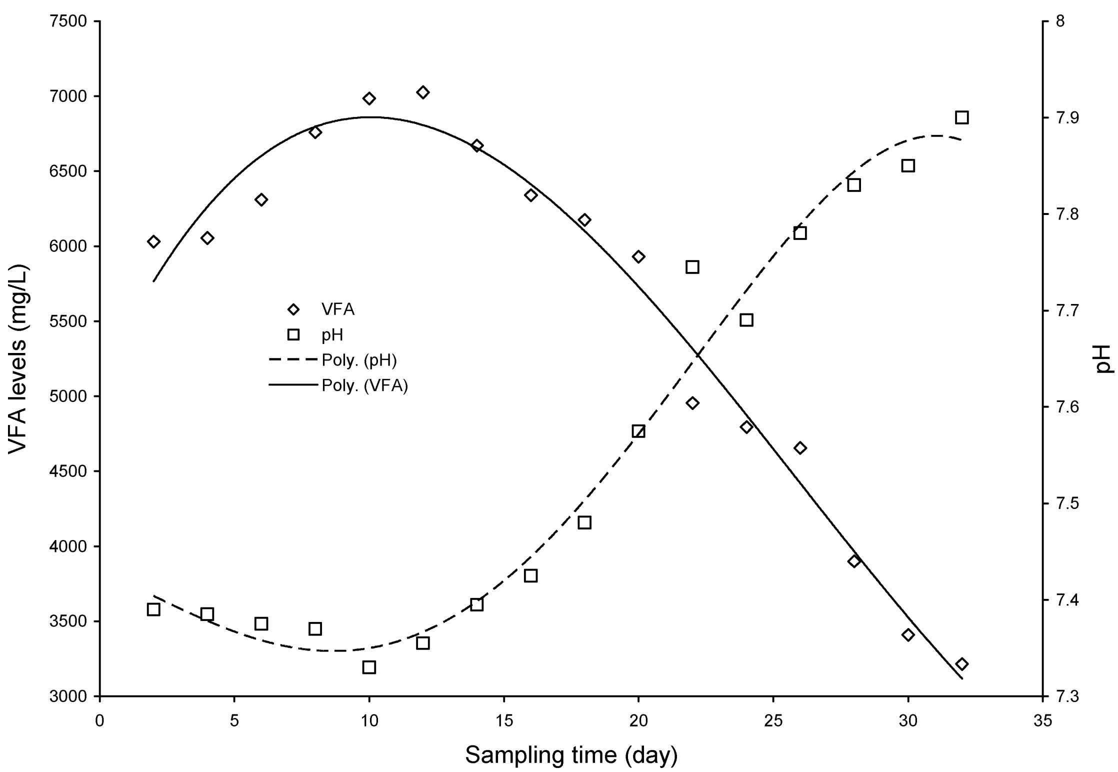


Fig. 1. Variations in pH and VFA concentrations in the manure under digestion over the sampling period.

shortly after the digestion started, with its peak reached on around day 10 or 11 before heading down. The total increase in VFA level in the manure under digestion was about 17% (from 6000 to 7000 mg/L) that appeared to be achieved in the first ten or so days of the treatment. This observation is in agreement with the results presented by previous researchers who have reported that VFAs are the major metabolic products of anaerobes in the early stage of anaerobic digestion. Spoelstra described a laboratory experiment in which a mixture of freshly voided feces and urine was anaerobically incubated and about 43% of the crude protein in the mixture was found to be degraded to VFAs and carbon dioxide. [7] Rainville and Morin reported, based on their study, an increase in VFA level by about 22% in the anaerobically stored swine slurry happened in the first 7 to 10 days.[8] Ndegwa et al. observed a remarkable 77% increase in VFA level in the anaerobically treated liquid swine manure on around day 20.[9] All this information, together with the results from this study, clearly demonstrates that liquid swine manure can be enriched with VFA through anaerobic digestion. However, it also has to be recognized that the extent of VFA enrichment differs from one study to another, depending probably upon many factors such as the age of manure used, the age of pigs, feed types, and manure handling practices. For instance, in this study, since a pull-plug system was used for the finish building, which discharged manure every two weeks, the manure in the shallow pit could be as old as up to two weeks before being placed in the anaerobic digester. Considering the fact that fresh manure is produced on a continuous basis, it may be reasonable to assume that one week is a good approximation of the storage time for the bulk manure in the pit. Along that line, it may be inferred that the optimal incubation time to reach the highest VFAs level in the manure through anaerobic digestion could be around 17–18 days. If such treated manure is not used shortly after 17 days of digestion, the VFA level in the manure will decrease, as reflected in Figure 1, due to the continued anaerobic degradation of the VFAs produced into methane.[14] The result also suggests that liquid swine manure anaerobically stored longer than 4 weeks should not be used in applications where high VFA content is sought.

Another interesting phenomenon observed in Figure 1 is the inverse relationship between VFA and pH in the manure during the digestion process. Production of VFA by the anaerobes appeared to lower the manure pH at the beginning of the digestion process; however, as the process progressed, the manure pH rebounded as the VFA concentration declined. When the data of VFA and pH are plotted against one another (Fig. 2), a good linear relationship is manifested with the correlation coefficient of 0.963, implying that the VFA concentration may be a critical factor in

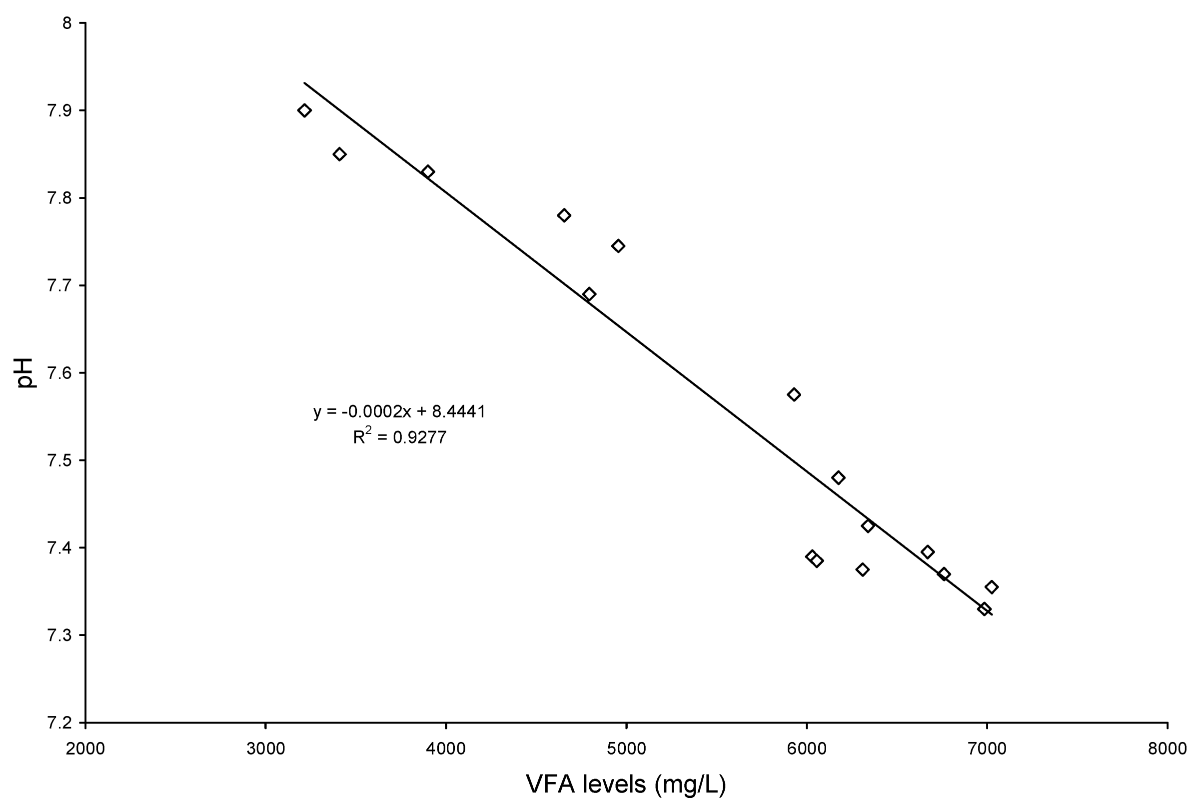


Fig. 2. The linear correlation between manure pH and VFA in the digested liquid swine manure.

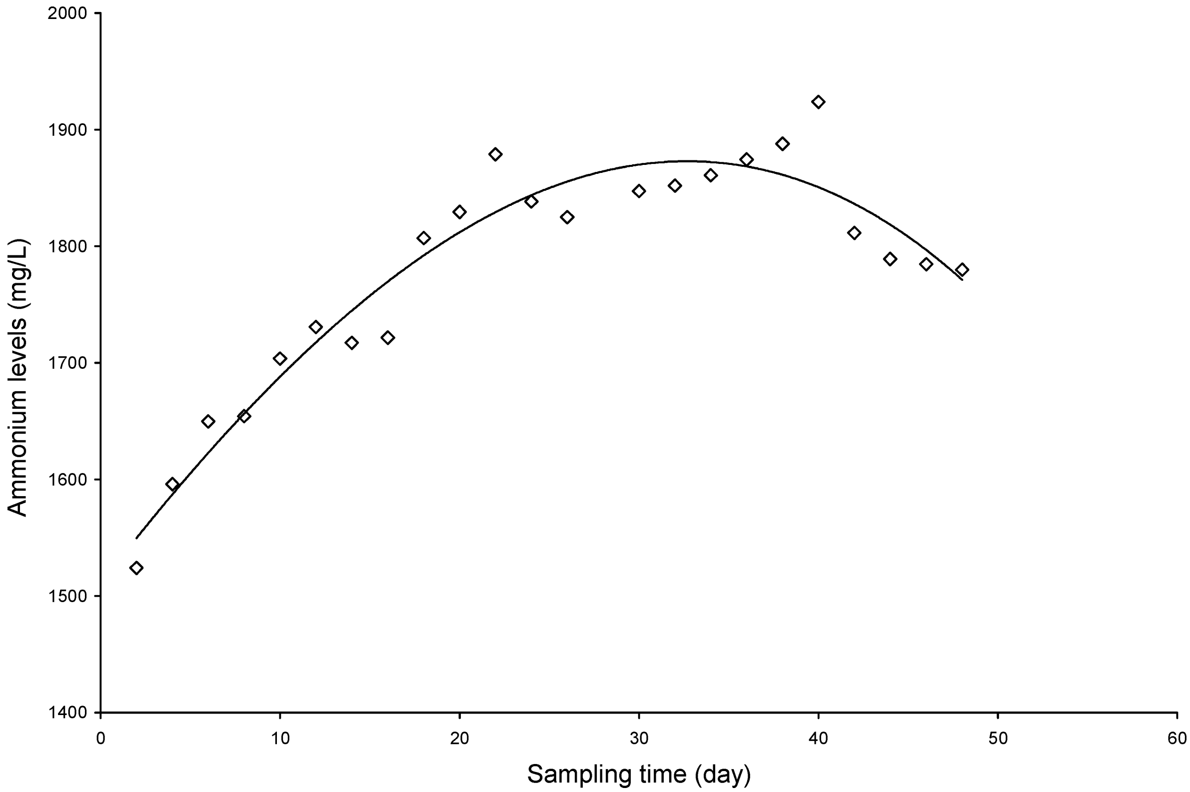


Fig. 3. Variation in NH_4^+-N in the manure under digestion over the sampling period.

influencing manure pH in anaerobic digestion. A linear correlation at this significance level ($R = 0.963$) between these two parameters may provide a way of estimating the VFA level in manure under anaerobic digestion by simply measuring the liquid pH, which is usually relatively convenient to perform. This recommendation, nonetheless, is open to further investigation to verify its veracity. The linear equation to determine VFA by pH is presented below.

$$\mathrm{VFA(mg/L)} = -(\mathrm{pH} - 8.4441) \times 5000 \qquad (1)$$

The change in ammonium nitrogen (NH_4^+) during the digestion process is illustrated in Figure 3, based on which a few comments can be made. First, the concentration curve featured a slightly different trend from that for VFAs in that it had a relatively flat top, a plateau, with a maximal increase in NH_4^+ by about 23% taking place on approximately day 21. Similarly, when the pit storage period is included, which is 7 days, it comes to the requirement of at least 28 days for NH_4^+ to reach its maximal plateau. Second, it can also be seen in Figure 3 that, to guarantee the highest NH_4^+ concentration in application, it is necessary to use the manure that has been under anaerobic digestion between 28 and 46 days (the plateau region). The nearly unchanging NH_4^+ concentration during this time frame may actually increase the flexibility in application by offering a big window (18 days) for manure utility without losing its effectiveness. Finally, comparing Figure 1 and 3 reveals that NH_4^+ does not increase in step with VFA because the latter can reach its maximum much faster than the former (17 days vs. over 28 days). Therefore, purely from the viewpoint of turnaround time, processing manure for high VFA concentration should thus be preferred.

Effect of manure application rate on soybean cyst nematode egg counts

The SCN egg counts from soil samples taken and assayed 35 days after application of manure at different rates are presented in Figure 4. General speaking, the SCN egg counts from soils treated with either raw or VFA- or NH_4^+-enriched manure decreased as the manure doses increased. Among all the treatments, the VFA-enriched manure demonstrated the lowest egg counts at all application rates, while the NH_4^+-enriched manure showed the least effect on the reduction of the SCN eggs for all dosages tested, which was even worse than the treatments with raw manure (the 'raw manure' here could be digested for 7 days if considering the pit storage time) and water (indicated by an 'x' in Fig. 4). Percentage wise, the VFA-enriched manure reduced the SCN egg counts by 18.1, 19.5, 34.3, and 18.6%, respectively, as compared to raw manure at application rates of 25, 50, 100, and 200 mL/pot. In contrast, for the NH_4^+ enriched manure, these percentages turned out to be 5.4, −14.0, −19.2, and −40.4%. As such, it may be concluded that the NH_4^+ enrichment treatment will not improve the manure's

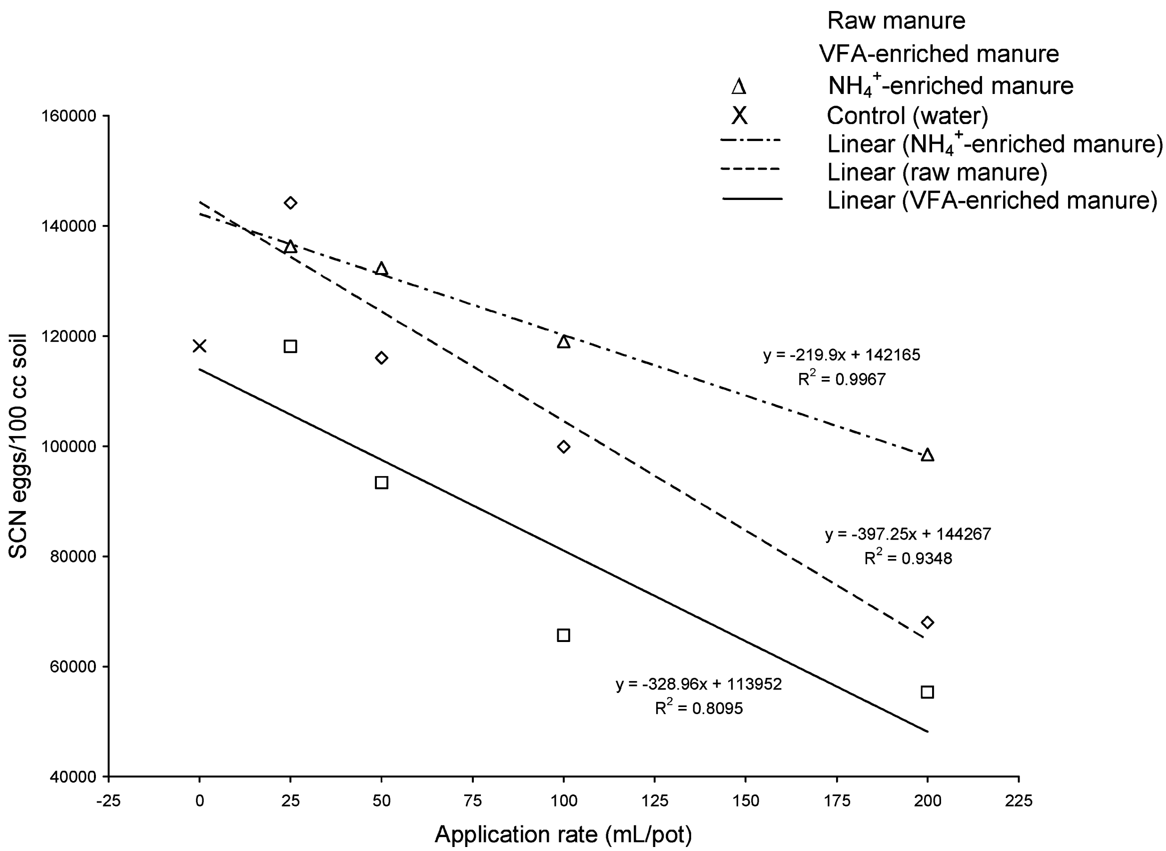


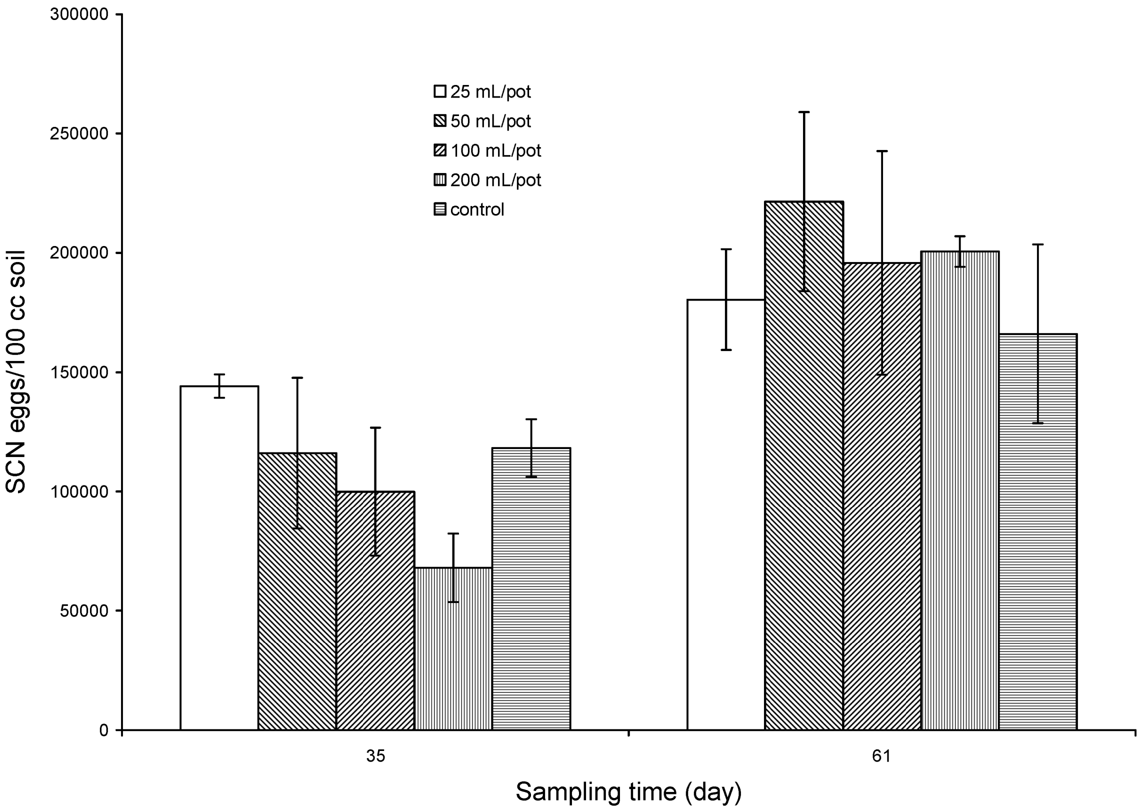
Fig. 4. Profiles of SCN egg counts per 100 cc soil in relation to the application rates of raw manure, VFA-enriched manure, NH_4^+-enriched manure, and the control.

capability of controlling the development of SCN eggs in the soil.

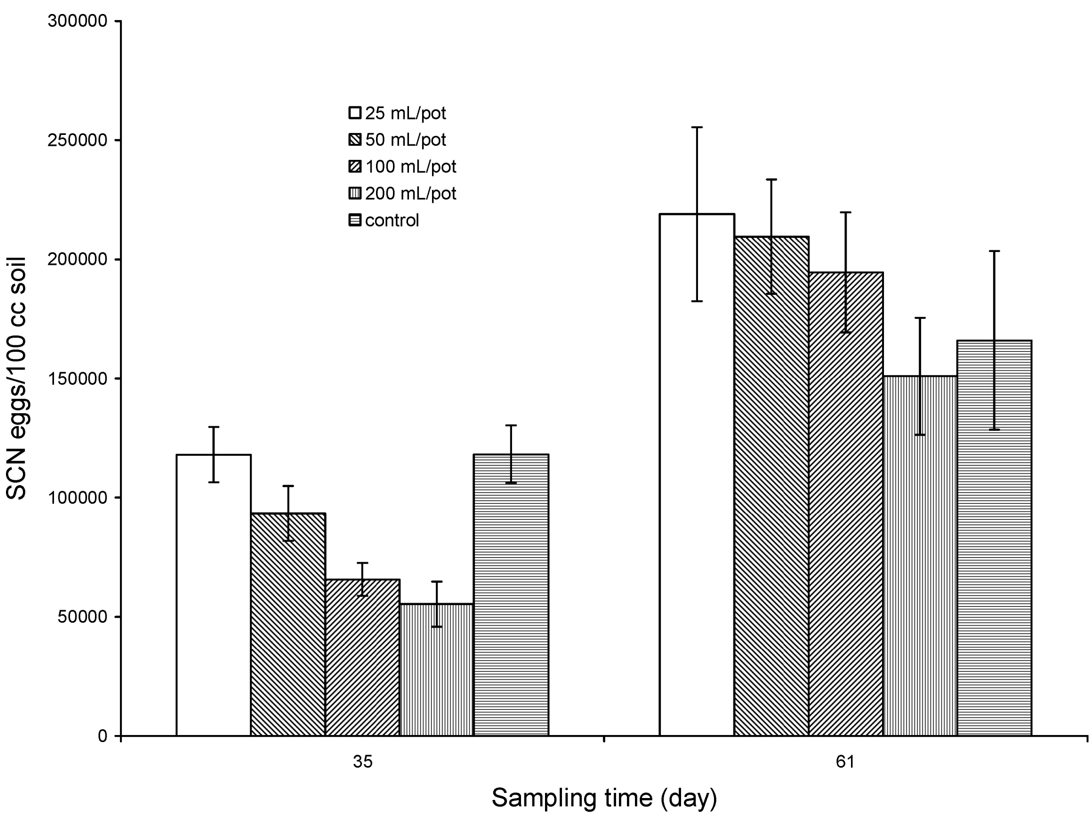
It can also be seen that the treatment effects feature linear relationships between application rates and egg counts per 100 cc soil for raw, NH_4^+-enriched, and VFA-enriched manure, with correlation coefficients of 0.998, 0.967, and 0.900 for, respectively. Another scenario worth of mentioning is the reduction rate of SCN eggs in response to the manure application rate increase (egg counts/mL manure). The three egg reduction rates could be obtained directly from the linear equations presented in Figure 4 (the slopes), i.e., 397.3, 219.9, and 329.0 egg count/mL manure for raw, NH_4^+-enriched, and VFA-enriched manure, suggesting that for every mL increase in the applied manure, the raw manure treatment could result in the highest SCN egg killing. Obviously, this discussion is put forward solely on the grounds that linear regression is the best goodness-of-fit for the data of all three treatments. In fact, if the trend for the VFA-enriched manure treatment is closely examined, the best curve to fit the data is a quadratic equation with a correlation coefficient being 0.998. This argument is readily supported by the four data points shown on Figure 4 in that the last point (200 mL/pot) virtually falls out of a straight line badly. If only the first three data points were used in calculation, a significant increase for egg reduction rate could be achieved, which was 700 egg count/mL manure, rather than 329.0 egg count/mL manure obtained above. Same argument apparently cannot be said to the other two manure treatments. One last point that needs to be emphasized is that, based on the distribution of data points for the VFA-enriched manure, it doesn't appear to provide sufficient ground for doubling the manure application rate (from 100 mL/pot to 200 mL/pot) for the sole purpose of controlling the proliferation of SCN eggs because the benefit of doing so seems to be relatively marginal (only gains about 15.6% more reduction).

The effective duration of manure treatment on soybean cyst nematode egg development

To determine the effective duration of manure treatment since application, the data of SCN egg counts from soil samples collected on day 35 and 61, including means and standard deviations, are presented together in Figure 5. For raw manure (Fig. 5a), the controlling effect on SCN egg generation appeared to be totally lost in two months, regardless of the manure application rate. Due to large variations observed for the 61-day samples, there was hardly any statistically significant difference in terms of egg count among all the treatments according to the paired *t* test results.

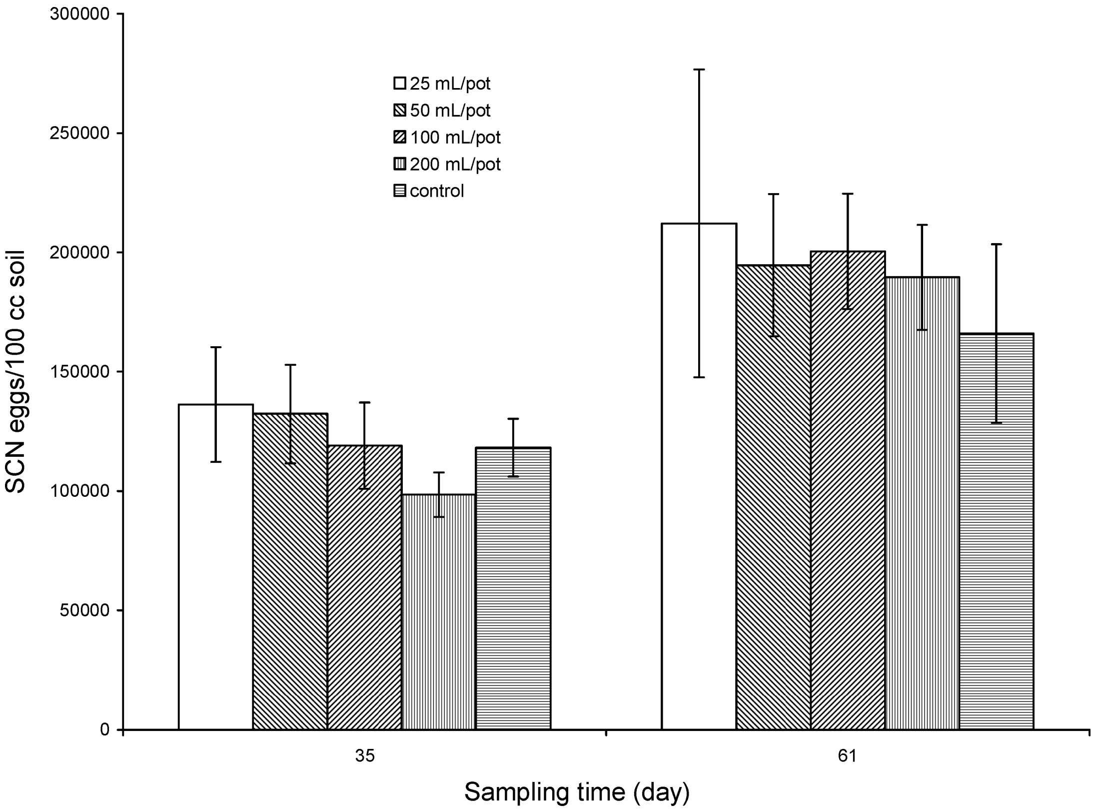


(a)



(b)

Fig. 5. Means and standard deviations of SCN egg counts per 100 cc soil collected on day 35 and 61 for all the treatments. (a) raw manure; (b) VFA-enriched manure; (c) NH_4^+-enriched manure. *(Continued)*



(c)

Fig. 5. (Continued)

However, this was not the case for the 35-day samples where statistically significant differences were found between application rates of 200 mL/pot and 25 mL/pot, and the control treatment as well. Since no data were collected between day 35 and 61, it is not possible to determine the time when the loss of effectiveness started, and the pattern how this loss evolved. Further effort in this area is certainly encouraged.

For the VFA-enriched manure treatment, the data on day 61 show that there are still significant differences in terms of egg count among different treatments (Fig. 5b). The 200 mL/pot treatment reduced more eggs than the 25 and 50 mL/pot treatments. This significance in difference became disappeared when compared with the 100 mL/pot treatment because of the large variations observed in data, although a drop in egg count by 50,000 was revealed (200,000 vs. 150,000). In contrast, the 35-day treatments produced a clear distinction in egg reduction between the bottom three application rates (25, 50, and 100 mL/pot), while there was no significant difference between the 100 and 200 mL/pot rates. And the 25 mL/pot rate was primarily the same as the control treatment in terms of the SCN egg count.

Analogous to raw manure, the NH_4^+-enriched manure also performed poorly on day 61 at all application rates in controlling SCN egg development. Despite large variations in data, the means of egg count for all the treatments didn't appear to be differing from one another by large margins (212,000, 195,000, 200,000, and 190,000 for application rates of 25, 50, 100, and 200 mL/pot, respectively). For the 35-day data, none of the manure treatments were able to significantly reduce the SCN egg count, as opposed to the control treatment.

Figure 5 also depicted a number of points of interest. First, regardless of application rates and manure types, the SCN egg counts on day 61 were significantly higher than those on day 35, indicating that the capability of the anaerobically digested manure to enrich either VFA or NH_4^+ aimed at controlling SCN egg production was forfeited somewhere between day 35 and 61 and the exact time when that happened couldn't be determined from the available information obtained in this study. Second, there was no significant difference in SCN egg count for soils treated with raw, VFA-enriched, and NH_4^+-enriched manure 61 days after the treatments. This conclusion is predicated upon statistical *t* tests by grouping data within treatments and then comparing them between treatments, as a result of which the means and standard deviations for all the manure treatments are 199,500 ± 16,900, 193,500 ± 30,000 and 199,200 ± 9,800 for raw, VFA-enriched, and NH_4^+-enriched manure, respectively. Third, the variation in data seemed to be much larger for the 61-day samples than for the 35-day samples, evidenced by the longer error bars shown in Figure 5.

One explanation for this observation could be that when the inhibitory effect of the processed manure on egg production goes away over time, many other factors, such as the distribution of nutrients and water, may locally govern the SCN egg production process that potentially leads to different growth rates for different pots and larger standard deviations when the data from different pots are employed to run the statistical analysis. Finally, from the perspective of practical use of the anaerobically digested manure to control SCN egg growth, it is recommended that (1) the VFA-enriched manure should be used and (2) application should be repeated every 35 days to ensure the best results, according to the data from this study.

Conclusion

Anaerobic digestion can be used to enrich both VFA and NH_4^+ in swine manure and the optimal incubation times to reach the highest levels for these two constituents are 17-18 days for VFA (about 17% increase) and 28 days for NH_4^+ (about 23% increase), respectively. For the VFA-enriched manure, its utility has to take place immediately after incubation for maximal strength, while for the NH_4^+-enriched manure, application can happen anywhere between 28 and 46 days during incubation without losing its maximal strength.

A linear relationship was observed between the manure VFA and pH with the correlation coefficient of 0.963 (VFA (mg/L) $= -$ (pH $-$ 8.4441) $\times$ 5000). With this equation, the VFA level in manure under anaerobic digestion may be estimated by simply measuring the pH in the liquid. More research is needed to verify this observation before a valid recommendation can be made.

For soil samples collected 35 days after receiving the manure treatments (raw, VFA-enriched, and NH_4^+-enriched manure), the SCN egg counts in general decreased as the manure doses increased, with the VFA-enriched treatment demonstrating the lowest egg counts at all application rates. The NH_4^+-enriched manure showed the least effect on the reduction of SCN eggs for all the rates tested, which was even worse than raw manure and water treatments. Compared to raw manure at the four application rates studied (25, 50, 100, 200 mL/pot), the VFA-enriched manure reduced SCN egg counts by 18.1, 19.5, 34.3, and 18.6%, while the NH_4^+-enriched manure achieved mostly negative reductions.

The SCN egg counts were found to be linearly related to manure application rates with correlation coefficients of 0.998, 0.967, and 0.900 for raw, NH_4^+-enriched, and VFA-enriched manure, respectively. The benefit of using the VFA-enriched manure to control egg growth increased with the dose increases from 25 to 100 mL/pot, while a further increase in dosage to 200 mL/pot only brought about 15.6% more reduction.

The controlling effect between different application rates on SCN egg population appeared to be totally lost in two months for all manure treatments except the VFA-enriched one at a rate of 200 mL/pot. Regardless of application rates and manure types, the SCN egg counts in soils sampled on day 61 were significantly higher than those sampled on day 35 and the edge in effectiveness that the VFA-enriched manure had gained over other treatments was no longer existing. Therefore, it can be concluded that for the best treatment result, the VFA-enriched manure should be applied to soybean fields every 35 days in order to effectively suppress SCN egg production.

Acknowledgments

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