Microbiological Aspects of Aerobic Thermophilic Treatment of Swine Waste

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A thermophilic strain (D2) identified as a Bacillus sp. was isolated from an aerobic digester of swine waste after several months of operation at 55°C. Aerobic thermophilic batch treatment of swine waste inoculated with strain D2 was studied in a 4-liter fixed-bed reactor. Stabilization of the waste was achieved in less than 30 h when the original chemical oxygen demand (COD) was between 15 and 20 g/liter or in less than 48 h when the COD was around 35 g/liter. When the COD was higher than 30 g/liter, the pH of the waste reached 9.2 to 9.5 during the treatment, and periodic adjustment of the pH to 8.5 was necessary to maintain the activity of the biofilm. In this reactor, ammonical nitrogen was completely eliminated by desorption in less than 72 h of incubation. The different packing materials used resulted in similar rates of degradation of organic matter. The thermophilic treatment was also efficient in the 75-liter digester, and stabilization was achieved in approximately 50 h. A bank of 22 thermophilic bacterial strains originating from different environments and adapted to the thermophilic treatment of swine waste was established. This thermophilic treatment allows, in one step, rapid stabilization of the waste, elimination of the bad smell, and complete elimination of ammonia nitrogen by stripping.

Serious environmental problems are associated with pig production. Land spreading has been the main method of manure disposal and has resulted in direct and indirect pollution of adjacent watercourses. Swine waste has large amounts of organic matter, ammonia nitrogen, malodorous compounds (24, 25), and potential viral, bacterial, and parasitic pathogens for humans and animals (3, 17, 18). Aerobic biological treatment offers a rapid and effective means of degrading the substrate in an inoffensive manner. The treatment is theoretically carried out in three steps: oxidation of organic matter, nitrification, and, finally, denitrification in order to remove ammonia nitrogen (10). However, the last two steps are complex and difficult to realize with this waste (16). Swine manure contains a high proportion of suspended solids that resist biological degradation. The rate of biodegradation is enhanced by the removal of these solids (14).

The microbiological aspects of mesophilic aerobic treatment of swine waste have been studied (4, 5, 7, 19, 20). An Acinetobacter sp. is the dominant microorganism, and its prevalence is correlated with the periods of rapid reduction in chemical oxygen demand (COD) (7, 20).

Aerobic thermophilic treatment has been applied to sewage sludge and animal waste (12, 13, 22, 23). Significant advantages of aerobic thermophilic digestion have been thought to include increased rates of oxidation, resulting in smaller digester volume requirements; destruction of most pathogenic bacteria, viruses, and parasites; weed seed destruction, and increased ease of liquid-solid separation (15).

The present work was carried out to study some microbiological aspects of aerobic thermophilic treatment of swine waste in order to increase its efficiency. The laboratory process developed allowed, in one step, rapid stabilization of the waste, elimination of the bad smell, and complete elimination of ammonia nitrogen by stripping.

MATERIALS AND METHODS

Swine waste. Samples of swine waste were collected under the slatted floor of a fattening house. The farm had approximately 1,000 pigs of different ages. The animals were fed with commercial rations. Feed and water were provided ad libitum. The piggeries were routinely operated without any special alterations for this study. The waste samples were stored at 4°C until required. Before being used, the waste was centrifuged at 5,000 × g for 30 min at 4°C or filtered through a 30-mesh screen to remove coarse, suspended solids. The centrifuged or filtered waste was used throughout this study, and it is referred to hereafter as simply the waste.

Analysis. COD, ammonia, and Kjeldahl nitrogen were determined by the standard methods for the examination of water and wastewater (1).

CFU of the swine waste microflora per milliliter were determined by inoculating 0.1 ml of the appropriate decimal dilutions of the culture on Columbia blood agar and incubating it at 37 or 55°C for 48 h. The biofilm in fixed-bed reactors was evaluated on the packing material collected from the top, middle, and bottom of each digester. Each packing material was placed in 4.5 ml of sterile phosphate-buffered saline and mixed vigorously on a Vortex mixer for 30 s, and the number of CFU per milliliter was determined. The average of three samples of the packing material was calculated and expressed as CFU per centimeter2.

Digestors. Cultures of swine waste were carried out in a bench-top chemostat (Bioflo model C30; New Brunswick Scientific Co., Inc., Edison, N.J.) containing 500 to 600 ml of waste. The agitation was at 400 rpm, and the airflow was 500 ml/min. The temperature of the digestor was controlled at 55°C.

The fixed-bed reactor used was a 4-liter Plexiglas (Rohm & Haas Co., Philadelphia, Pa.) beaker incubated in a water bath maintained at 55°C (Fig. 1). The reactor contained 2 liters of swine waste and packing material for a total volume of 3 liters. The aeration was carried out with seven fritted-glass gas dispersion tubes. The airflow rate was generally 3

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different strains was carried out on solid brain heart infusion medium adjusted to pH 7.5, 8.0, 8.5, 9.0, or 9.5 with 0.3 M NaOH. The inoculated plates were incubated at 55°C and observed for the presence of growth after 48 h. The maximal growth temperatures of the strains were determined on Columbia blood agar. The temperatures studied were 55, 60, 65, and 70°C. The inoculum came from a 20-h culture at 55°C, and the growth was determined after 24 h of incubation. Proteolytic activities were estimated by the presence of hydrolysis after 24 h of growth of the strain at 55°C on solid medium composed of 0.8% (wt/vol) nutrient broth (Difco Laboratories, Detroit, Mich.), 2.0% (wt/vol) milk powder (Steinberg, Montreal, Canada), and 1.5% (wt/vol) agar. Lipase activity with Tween 80 as a substrate and amylase activity on starch were evaluated on solid medium at pH 7.5 and 8.5 by the methods of Smibert and Krieg (21). The plates were made in two series, one series of plates was adjusted at pH 7.5, and the other was adjusted at pH 8.5 with 0.3 M NaOH. The plates were observed after 24 h of growth at 55°C. The identification of some strains was carried out by standard conventional methods at the Québec Laboratory of Public Health, Sainte-Anne-de-Bellevue, Québec.

Biological treatment. Swine waste in the fixed-bed reactor (4 liters) was inoculated with a standardized culture of Bacillus sp. strain D2 or other purified bacterial strains. These strains were precultured by incubating them for 18 h at 55°C on Columbia blood agar medium, and the waste was inoculated to obtain 10^5 to 10^6 CFU/ml. After stabilization of the waste, 75% of the treated waste was replaced with fresh waste. This procedure was repeated for 3 to 4 weeks. The treated waste was then replaced by fresh waste, and chemical and biological analysis were carried out at different time intervals to assess the efficiency of the treatment. Except for experiment 1, the pH was adjusted periodically to 8.5. The airflow rate was 3 liters/min for experiments 1 to 6 and 9; 4 liters/min for experiments 7, 8, and 10; and 5 liters/min for experiment 11.

The 75-liter digester containing 50 liters of swine waste was inoculated with 2 liters of waste of an active preculture of the 4-liter fixed-bed reactor inoculated with Bacillus sp. strain D2 and operated for several months. This preculture contained an estimated 2 × 10^9 CFU/ml of thermophilic bacteria per ml. After stabilization, about 75% of the waste was kept as an inoculum for the next batch. The digester was in operation for several weeks, and the efficiency of the treatment was evaluated.

RESULTS

Waste inoculated with Bacillus sp. strain D2 in a fixed-bed reactor. Bacillus sp. strain D2 was isolated from swine waste in an aerobic thermophilic digester after several months of operation. This strain was inoculated to swine waste in the 4-liter fixed-bed reactor. The digester was operated for several weeks in order to obtain the formation of the biofilm, which was estimated to contain around 10^9 CFU/cm².

The efficiency of the treatment was assessed by the reduction of COD. As the waste is variable during the year, wastes with different COD contents were used. Waste with a COD between 30 and 35 g/liters was stabilized in less than 48 h (Fig. 2A). When the pH was not controlled, values between 9.1 and 9.5 were observed after 40 h, and the biofilm was assessed to contain less than 10^8 CFU/cm². In experiments 2 and 3, the pH was adjusted periodically to 8.5 in order to favor the formation of the biofilm. In these experiments, the biofilms contained 2 × 10^9 and 1 × 10^8 CFU/cm².

![Diagram](http://aem.asm.org/DownloadedFrom/aem.asm.org)

**FIG. 1.** Reactor (4 liters) with packing material. A, Pump; B, water bath; C, gas dispersion tubes; D, packing material.
respectively, and the stabilization was achieved more rapidly.

Waste with a COD between 20 and 30 g/liters was also stabilized after approximately 48 h (Fig. 2B). The biofilm, in experiments 4 to 8, was estimated to contain $5 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$, $6 \times 10^6$, and $6 \times 10^6$ CFU/cm², respectively. Waste with a low COD (15 to 17 g/liter) was also investigated. The stabilization was achieved in less than 30 h (Fig. 2C) with a biofilm estimated to contain $9 \times 10^5$ CFU/cm². The efficiency of the treatment was not related to the airflow rate used.

The ammoniacal nitrogen content was also determined in these assays. Generally, the ammoniacal nitrogen was completely eliminated by stripping in less than 72 h (Fig. 3).

The efficiency of the treatment was determined with some other packing materials: washed stones, gravel, and polybutylene tubes. The results of these assays are presented in Fig. 4. Although the wastes used had different CODs, the rates of degradation were the same. The stabilization of the waste was dependent upon initial COD and was generally achieved in less than 50 h.

The washed stones were too friable as a packing material and the assays were performed for only 5 weeks. The gravel permitted the formation of a biofilm containing $9 \times 10^5$ CFU/cm². However, the efficiency of the treatment was not good, with a decrease of COD to only 54% in 55 h (results not shown). It was thought that this material was too compact and did not allow a good agitation of the waste. A pump was used to mix the waste, which was taken at the top of the reactor and pumped to the bottom at a flow rate of 12 liters/h. The efficiency of the treatment was better, and the COD was decreased to 62% in 48 h. However, the increase in pH was slower and the stripping of ammonia was less. On polybutylene tubes, the biofilm was estimated to contain $8.7 \times 10^6$ CFU/cm². This material did not interfere with the agitation of the waste and can be manipulated easily.

Selection of new bacterial strains. In an attempt to increase the efficiency and stability of the treatment, 22 thermophilic bacterial strains originating from different sources were isolated after incubation of the samples for 2 weeks in swine waste with thermophilic treatment (Table 1). These strains

![FIG. 2. Reduction of COD of different swine wastes as a function of incubation time in the 4-liter reactor.](image1)

![FIG. 3. Reduction of ammoniacal nitrogen as a function of incubation time. The numbers refer to the assays shown in Fig. 2.](image2)

![FIG. 4. Comparison of the effects different packing materials on the reduction of COD as a function of the time of incubation in the 4-liter reactor. A, Polybutylene tubes; B, gravel; C, washed stones; D, glass rashig rings.](image3)
were gram-variable bacilli of different sizes. The presence of spores was observed for 17 strains; 12 were mobile; 21 could grow at 60°C, 9 could grow at 65°C, and none of the strains tested grew at 70°C. All strains except five could grow at pH 9.5. Only 1 strain showed protease activity, 12 had amylase activity, and 9 had lipase activity. Identification was carried out for six strains: T4, T5, T6, and S10 belonged to the genus Bacillus, and S8 and S13 are Bacillus licheniformis strains.

**Waste inoculated with strains T4, T5, T6, and D2.** Strains T4, T5, and T6 were chosen because they can grow at 65°C and at pH 9.5. These strains and Bacillus sp. strain D2 were inoculated to waste in the 4-liter fixed-bed digester, and the treatment was continued for several weeks. The efficiency of this treatment was compared with that obtained with the waste inoculated only with strain D2. The treatments were carried out at a constant temperature (55°C), and the biofilms were estimated to contain 2.6 × 10^7 and 3.1 × 10^8 CFU/cm², respectively. Under these conditions, both treatments showed similar efficiencies (results not shown).

**Treatment in the 75-liter digester.** The 75-liter digester was packed with polyethylene tubes, and the waste was inoculated with Bacillus sp. strain D2. Three different assays were performed, and the stabilization of the waste was achieved in approximately 30 h (Fig. 5). Table 2 shows some parameters measured during one of these treatments. The pH was 9.5 at the end of treatment, and 98.4% of ammoniacal nitrogen was eliminated by stripping. Total thermophilic bacterial count increased to 4 × 10^8 CFU/ml after 19 h and decreased to 8 × 10^6 CFU/ml at 73 h. The formation of biofilm on the packing materials was very slow during our assays. After 2 months of operation, the biofilm was assessed as having 10^7 CFU/cm², and a 72% reduction in COD was observed after 40 h.

**DISCUSSION**

Although the bacterial population of swine waste is abundant and includes some thermophilic organisms, it has already been reported that inoculation of fresh waste with previously aerated waste had a beneficial effect for its stabilization (12, 19). In agreement with Blouin et al. (4) and Graczyk and Kolaczkowski (12), the stabilization of the waste was reached after a period of incubation time proportional to the initial concentration of organic matter. The stabilization is generally achieved in less than 2 days. The reduction of COD is generally between 70 and 80%, which is in accord with the results of Garraway (11). A nonbiodegradable fraction is always present and is proportional to the concentration of the input waste (4, 8).

The biofilm on the packing materials was mainly com-

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**TABLE 1. Characterization of thermophilic strains isolated**

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<th>Amylase at pH:</th>
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<th>Mobility</th>
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* O, Oval; R, round; T, Terminal; D, deformation; ND, No deformation; C, Center; ST, subterminal.

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**FIG. 5.** Reduction of COD as a function of the time of incubation in the 75-liter reactor packed with polyethylene tubes. The numbers I, II, and III refer to three different assays.
posed of strain D2 and was generally stable. However, an important decrease in the number of CFU per milliliter was observed in the assays of waste with a COD higher than 30 g/liter. Under these conditions, the pH during the treatment reached 9.5. A periodic adjustment of the pH to 8.5 was necessary to maintain the viability of the biofilm. When the COD of the waste was lower than 30 g/liter, the control of the pH was not necessary.

In aerobic mesophilic treatment carried out with or without pH control, the ammonia is not eliminated (7, 16), and further steps of nitrification and denitrification of the treated slurry are necessary to achieve this goal. Because of the high concentration of ammonia in swine waste, these steps are complex and increase the cost of the treatment. However, as shown in this work, the conditions of pH and temperature obtained in the thermophilic treatment allowed the complete loss of ammonia by desorption. In order to reduce the cost of the treatment, ammonia could be easily recovered from the exit air of the digestor by precipitation as ammonium salt in a sulfuric acid solution and used as the nitrogen component in a chemical fertilizer.

The efficiency of the treatment was reduced when the temperature was higher than 60°C (result not shown). In an attempt to increase the efficiency and stability of the treatment, we have isolated new thermophilic microorganisms from the environment and have adapted them to swine waste. Some strains have been identified as belonging to the genus Bacillus; the other strains isolated were not fully identified, but they have similar morphologies and characteristics. Sonnleitner and Fiechter (23) reported that 95% of the thermophilic microorganisms isolated from an aerobic sewage sludge pilot plant are Bacillus stearothermophilus strains. This group of microorganisms is very heterogeneous and includes acidophilic and alkalophilic bacteria which can grow between pH 5 and 9.5 and at temperatures up to 70°C.

The thermophilic microorganisms that we have isolated can rapidly degrade the organic matter of the waste, and some strains can grow at extreme pH (9.5) and temperature (65°C). However, only a few strains showed enzyme activity. This could be due to the low sensitivity of the methods used. Proteases are excreted at relatively low levels by most thermophilic bacteria compared with mesophilic organisms (6). The spores found for the majority of the strains isolated could be important in maintaining the active thermophilic population when the conditions of the digestor are unfavorable, e.g., when there is a break in the aeration system or when the treatment is stopped for a time.

Our results suggest that there is no advantage to inoculating the waste with strains D2, T4, T5, and T6 compared with using only strain D2. However, in a practical manner, the use of several strains could be important in preserving the stability and efficiency of the treatment. By using a mixture of strains, waste with different compositions could be treated and extreme conditions of pH and temperature could frequently be tolerated.

The assays in the 75-liter digestor have confirmed the results obtained in the 4-liter fixed-bed digestor. Rapid stabilization of the waste and elimination of the ammoniacal nitrogen by stripping was achieved. The air exit system of the reactor with a pipe passing through an incineration chamber did not favor the rapid stripping of ammonia. A direct air exit to the exterior would probably have reduced the time necessary to completely eliminate the ammonia during the treatment.

The presence of packing materials could be important for obtaining a better oxygenation of the waste. The slow ascension of the air bubbles through the packing materials in the digestor favors better oxygen transfer to the waste. Doyle et al. (7) have observed a higher efficiency of their mesophilic treatment when the reactor was filled with packing materials. They have attributed it to the presence of a biofilm on the supports and to the increase of the total biomass in the reactor.

The greater efficiency of aerobic thermophilic treatment of swine waste compared with that at mesophilic temperatures has already been shown, and 55°C was regarded as the optimum temperature; raising the temperature from 55 to 65°C had a slightly detrimental effect (8, 12). The heat released from aerobic treatment of animal wastes and wastewater sludge has been studied by some investigators (2, 9, 15). Microbiological metabolism in aerobic treatment of swine waste can produce enough heat to reach thermophilic temperatures.

The aerobic thermophilic treatment carried out in this work was by batch culture. Further study with continuous cultures could improve the treatment; it could maintain a relatively constant physical and chemical environment and improve the efficiency of the active microorganisms.

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LITERATURE CITED