NITRIFICATION TREATMENT OF SWINE WASTEWATER WITH ACCLIMATED NITRIFYING SLUDGE IMMOBILIZED IN POLYMER PELLETS

M. B. Vanotti, P. G. Hunt

ABSTRACT. *Nitrification of ammonia (NH_4^+) is a critical component for improved systems of animal wastewater treatment. One of the most effective processes uses nitrifying microorganisms encapsulated in polymer resins. It is used in Japan in municipal wastewater treatment plants for higher nitrification rates, shorter hydraulic retention times (HRT), and lower aeration treatment cost. We evaluated whether this technology could be adapted for treatment of higher-strength lagoon swine wastewaters containing ~230 mg NH_4-N L^{-1} and 195 mg BOD_5 L^{-1}. A culture of acclimated lagoon nitrifying sludge (ALNS) was prepared from a nitrifying biofilm developed in an overland flow soil using fill-and-draw cultivation. The ALNS was successfully immobilized in 3- to 5-mm polyvinyl alcohol (PVA) polymer pellets by a PVA-freezing method. Swine wastewater was treated in aerated, suspended bioreactors with a 15% (w/v) pellet concentration using batch and continuous flow treatment. Alkalinity was supplemented with inorganic carbon to maintain the liquid pH within an optimum range (7.7-8.4). In batch treatment, only 14 h were needed for nitrification of NH_4^+. Ammonia was nitrified readily, decreasing at a rate of 16.1 mg NH_4-N L^{-1} h^{-1}. In contrast, it took 10 d for a control (no-pellets) aerated reactor to start nitrification; furthermore, 70% of the N was lost by air stripping. Without alkalinity supplements, the pH of the liquid fell to 6.0-6.2, and NH_4^+ oxidation stopped. In continuous flow treatment, nitrification efficiencies of 95% were obtained with NH_4^+ loading rates of 418 mg-N L-reactor^{-1} d^{-1} (2.73 g-N g-pellet^{-1} d^{-1}) and an HRT of 12 h. The rate of nitrification obtained with HRT of 4 h was 567 mg-N L^{-1} d^{-1}. In all cases, the NH_4-N removed was entirely recovered in oxidized N forms. Nitrification rates obtained in this work were comparable to rates obtained with municipal systems. This indicates that immobilized ALNS pellets were not greatly affected by high NH_4^+ or BOD concentration of swine wastewater. Thus, immobilized pellet technology can be adapted for fast and efficient removal of NH_4^+ contained in anaerobic swine lagoons using acclimated microorganisms.*

Keywords. *Swine lagoons, Ammonia removal, Nitrification, Immobilized nitrifiers, Animal wastewater treatment, Nitrifying pellets.*

During recent decades, animal production methods in the U.S. have changed from small, individual operations to large, confined, commercial enterprises. This change has caused many problems for the swine industry including emission of ammonia (NH_3) from lagoons. Liquid swine manure is usually treated and stored in large (0.25 to 5 ha) anaerobic lagoons before land application. It may be anticipated that 50 to 80% of the nitrogen (N) entering animal lagoons will escape to the atmosphere through NH_3 volatilization (Miner and Hazen, 1977; Barrington and Moreno, 1995; Braum et al., 1997). Recent estimates of NH_3 emissions from swine lagoons in North Carolina indicate that about 30 000 kg of N per day may volatilize from a total of 2000 ha of lagoons (Crouse et al., 1997). Its subsequent deposition across the landscape may be the largest form of nitrogen non-point source pollution in the region.

Biological removal of N through the process of nitrification and denitrification is regarded as the most efficient and economically feasible method available for removal of N from wastewaters (Focht and Chang, 1975; Tchobanoglous and Burton, 1991; Furukawa et al., 1994). The effectiveness of the biological nitrogen removal process depends on the ability of nitrifying organisms to oxidize ammonium ions (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-). Subsequent reduction to molecular N, denitrification, may be essential as well, but not considered in the present study. This step is rapid with available carbonaceous substrate and an anaerobic environment, conditions which are typically found in farm settings in constructed wetlands (Rice et al., 1998) or liquid manure storage units (Bernet et al., 1996). The reaction rate of nitrification is extremely low compared to that of denitrification, so that nitrification will be normally a rate limiting step in biological nitrogen removal process.

The basic problem related to nitrification in wastewaters with a high content of organic carbon is the low growth rate of the nitrifying bacteria; the generation time of these microorganisms is about 15 h. Compared to heterotrophic microorganisms, which have generation times of 20 to 40 min, the nitrifiers compete poorly for limited oxygen and nutrients and tend to be overgrown or washed out of reactors (Figueroa and Silverstein, 1992; Wijffels et al.,

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The authors are **Matias B. Vanotti**, Soil Scientist, and **Patrick G. Hunt**, ***ASAE Member***, Soil Scientist, USDA-ARS, Coastal Plains Soil, Water, and Plant Research Center, Florence, S.C. **Corresponding author:** Matias B. Vanotti, USDA-ARS, 2611 West Lucas St., Florence, SC 29501-1242, phone: 843.669.5203, ext. 108, fax: 843.669.6970, e-mail: <vanotti@florence.ars. usda.gov>.

1993). The nitrification of lagoon swine wastewater is an especially difficult process because of the very low numbers of *Nitrosomonas* and *Nitrobacter* usually found after anaerobic treatment (Blouin et al., 1990). Even when the oxygen supply is plentiful, an adaptation period is needed to reach a minimum bacteria concentration before effective nitrification. Recycling surplus activated sludge in an aerobic reactor or long hydraulic retention time (HRT) is required to retain slow growing autotrophic nitrifiers. Unfortunately, in the absence of enriched nitrifying populations, aerobic treatment of lagoons can potentially add to problems by stripping ammonia into the atmosphere, particularly if uncontrolled or excessive flow rates of air are used (Burton, 1992).

The efficiency of the nitrification process can be increased by increasing the nitrifiers' retention time independent from the wastewater retention time (Wijffels et al., 1993). In most cases this is done by immobilization of nitrifiers. One advantage of this technology is that increased wastewater flow is possible with minimal washout of immobilized bacteria. Immobilization has been widely used in wastewater treatment applications by taking advantage of spontaneous attachment of cells to the surface of inert support materials. Applications of attached growth for treatment of swine wastewater have been developed by Ciaccolini et al. (1984) and St.-Arnaud et al. (1991) who reported higher nitrification rates compared to systems where microorganisms were in suspension.

Advances in biotechnology using immobilization technology have shown that higher nitrification efficiencies are possible through the entrapment of cells in polymer gels, a common technique in drug manufacturing and food processing. The successful application for nitrification treatment of municipal wastewater has been demonstrated using both natural polymers such as calcium alginate (Lewandowski et al., 1987) and synthetic polymers such as polyethylene glycol, PEG (Tanaka et al., 1991), or polyvinyl alcohol, PVA (Furukawa et al., 1994). Pellets made of synthetic polymers are superior to natural polymers in terms of strength and durability; their estimated life span is about 10 years. These characteristics are very important in long-term biotreatment operation. For this reason, synthetic polymer pellets are preferred for pilot- and plant-scale purposes. There are currently several full-scale municipal wastewater treatment plants using this technology in Japan (Takeshima et al., 1993).The nitrifiers are entrapped in 3- to 5-mm polymer pellets permeable to NH_4^+, oxygen, and carbon dioxide needed by these microorganisms, resulting in a fast and efficient removal of N. Tanaka et al. (1991) reported nitrification rates three times higher than those of the conventional activated sludge process. Previous work with nitrifying pellets has been done exclusively in municipal-type systems where typical NH_4^+ concentrations are about 30 mg N L^{-1} and BOD_5 < 90 mg L^{-1}. There are no reports whether the high performance of these pellets can be maintained with animal wastewater containing 10 times more N and high concentrations of carbonaceous materials.

Our objectives were to determine the feasibility of using immobilized nitrifying pellets for fast removal of NH_4^+ contained in lagoon swine wastewater. In this article, we report on procedures used for preparation of immobilized ALNS pellets and the evaluation of the nitrifying activity of the pellets through batch and continuous nitrification experiments of swine wastewater.

Materials and Methods

Preparation of Nitrifying Culture

A culture of acclimated lagoon nitrifying sludge (ALNS) was prepared with seed biofilm sludge obtained from the surface soil of an overland flow field plot located in Duplin County, North Carolina. The overland flow provided nitrification treatment for a wastewater effluent from an anaerobic swine lagoon (Vanotti et al., 1998). At the time of sample collection, it had been operational for 1.5 yr receiving daily loading rates of 60 to 100 kg N ha^{-1}. The biofilm sludge sample contained large amounts of intermixed plant materials that were separated by rinsing the sample with tap water over an 0.8-mm opening sieve. The fine organic particles collected in the filtrate, comprising the nitrifying sludge, were concentrated by sedimentation in a Imhoff settling vessel and washed with water to remove residual dissolved organic materials. The settled sludge was then transferred into 12-L aeration tanks and diluted to a level of 100 mg MLSS L^{-1} (dry weight basis) with a synthetic inorganic salts medium (table 1). The ALNS cultures were prepared in the aeration tanks using a fill-and-draw cultivation method, where the aeration was stopped once each day, the suspensions allowed to settle for 30 min, the supernatant withdrawn and replaced with fresh inorganic salts medium, and aeration resumed. Temperature and dissolved oxygen (DO) in the culture tanks were maintained at 35°C and 6.5 mg L^{-1}, respectively. The alkalinity concentration of the inorganic salts medium (table 1) was 1125 mg $CaCO_3$ L^{-1} and supplied approximately half the alkalinity needs for complete nitrification of 300 mg NH_4-N L^{-1}. In order to maintain the pH at 8.5, the mixed liquor was supplemented with carbonates. During the first 5 d of cultivation, the pH of the mixed liquor was adjusted to 8.5 by addition of 1 *N* K_2CO_3 two to five times a day. Thereafter, a pH 8.5 $CO_3^=/HCO_3^-$ buffer consisting of 31 mg Na_2CO_3 L^{-1} and 1660 mg $NaHCO_3$ L^{-1} (alkalinity = 1017 mg $CaCO_3$ L^{-1}) was added daily after 8 h of aeration. The nitrifying cells were harvested by sedimentation after 20 d of incubation; the cultivation procedure yielded 0.968 g-MLSS of ALNS L^{-1} (0.755 g-MLVSS L^{-1}).

Immobilization Technique

The ALNS was encapsulated in polymer gels according to the PVA-freezing method of Hashimoto et al. (1986). The ALNS was concentrated to 58 g L^{-1} by sedimentation

Table 1. Composition of inorganic medium used for preparation of ALNS* cultures

Chemical Name	Final Concentration
NH_4-N as $(NH_4)_2SO_4$	300 mg L^{-1}
K_2HPO_4	100 mg L^{-1}
$NaHCO_3$	1225 mg L^{-1}
Na_2CO_3	359 mg L^{-1}
$MgSO_4 \cdot 7H_2O$	60 mg L^{-1}
$FeSO_4 \cdot 7H_2O$	8 mg L^{-1}
$CaCl_2 \cdot 2H_2O$	8 mg L^{-1}
Trace elements solution†	0.1 mL L^{-1}

* ALNS = Acclimated lagoon nitrifying bacteria.
† Tanaka et al., 1981.

and washed free of the medium with distilled water by repeated (three times) resuspension and settling. The solution of polymer for the preparation of cryogels was obtained as follows: 200 g of PVA (HC; 100% saponification, 2000 polymerization, Kuraray Co., Tokyo, Japan) was dispersed in 1 L of distilled water and left to swell at room temperature for 1 h; then the suspension was heated on a boiling water bath under stirring to complete PVA dissolution (30 min). One unit of concentrated ALNS was mixed on a weight basis with one unit of 20% (w/v) PVA warm aqueous solution at 45°C. The mixture was then poured into a plastic tray to make a sheet approximately 3 mm thick, cooled for 2 h at 4°C and frozen for 16 h at –4°C. After fast thawing over a Teflon-coated aluminum tablet, immobilized ALNS-pelletized cubes of 3 to 5 mm were prepared using a sharp knife. The immobilized pellets were washed with the inorganic salts medium (table 1) under aeration until foaming by unpolymerized PVA stopped. Pellets were produced at a rate of 766 g (wet) or 740 mL pellets/1000 g of ALNS-PVA initial mixture and contained 37.9 mg ALNS g-pellet^{-1} (wet). Recovery of immobilized ALNS was performed during 2 d in an incubator at 35°C using the inorganic salts medium under a loading rate of 2.0 mg NH_4-N g-pellet^{-1} d^{-1}. After this recovery period, the nitrifying pellets were tested with swine lagoon wastewater in batch and continuous experiments.

Liquid Swine Manure

The wastewater used was a lagoon effluent from a swine operation near Kenansville, in Duplin County, North Carolina. This was a 4100-m^3 single-stage anaerobic lagoon providing treatment and storage of flushed swine manure from 2,600 pigs. The lagoon liquid contained, on the average, 233 mg NH_4-N L^{-1}, 250 mg Total Kjeldahl N L^{-1}, and 0 mg L^{-1} NO_2^- and NO_3^-. Other characteristics were 320 mg total suspended solids L^{-1}, 1690 mg total solids L^{-1}, 195 mg BOD_5 L^{-1}, 159 mg carbonaceous-BOD_5 L^{-1}, 814 mg COD L^{-1}, 1357 mg alkalinity L^{-1}, and a pH of 8.3.

Reactor Configuration

A diagram of the experimental apparatus used in the nitrification experiments is shown in figure 1. The basic reactor consisted of a 1.2 L aeration tank and was modeled after the Pegasus process (Tada et al., 1991; Takeshima et al., 1993). Pellets were added at 183.6 g (wet) per reactor, equivalent to a 15.3% (w/v) pellet concentration. A 1-mm wedge-wire screen was placed before the outlet port to separate the pellets and free cells, and retain the pellets inside the reactor. Air was supplied from the bottom of the tank at a flow rate of 0.4 L min^{-1} to ensure full fluidization of immobilized pellets. The diffuser consisted of an aquarium porous stone that provided fine bubble aeration. The resulting dissolved oxygen (DO) concentration in the mixed-liquor was > 3 mg L^{-1} (range 3.1 to 4.2 mg L^{-1}), which is consistent with DO level recommendations for non-limiting nitrification (Focht and Chang, 1975; Sharma and Ahlert, 1977; Tchobanoglous and Burton, 1991). Process temperature was controlled at 30°C using a heat regulator and a circulated water bath that accommodated up to six reactors. A wastewater temperature of 30°C was selected because previous work on nitrification and nitrogen removal (Focht and Chang, 1975; Sharma and Ahlert, 1977) indicate that the overall optimum temperature for the nitrification process is in the range of 28 to 36°C . A variable flow peristaltic pump was used in the continuous flow experiments to feed the lagoon wastewater to the nitrification tank. The NH_4-N loading rate was adjusted by varying the flow rates from 1029 to 7200 mL d^{-1}. These flow rates provided hydraulic retention time (HRT) treatment in the range of 28 to 4 h, respectively. Each flow rate was maintained for 1 week; water quality samples were collected daily at days three through seven from both the influent and the effluent liquid. The batch experiments were conducted under the same conditions described above using 1.2 L reactors without an influent line. All the experiments were duplicated.

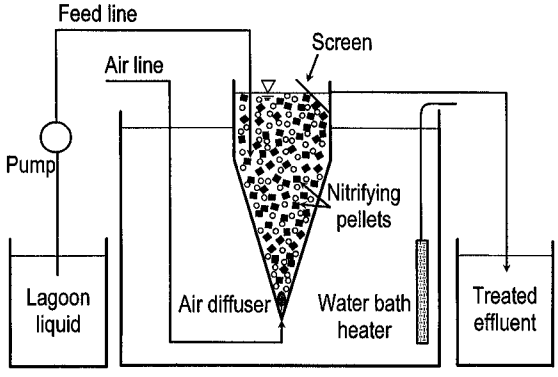

Figure 1–Schematic diagram of reactor used for continuous treatment of swine wastewater with nitrifying pellets.

Analytical Methods

Wastewater analyses were done according to Standard Methods (APHA, 1992). The NO_3–N, NO_2–N, and NH_4-N concentrations were determined by automated analyses (Technicon Instruments Corp., 1977; 1978) of filtered samples. Wastewater samples were collected in 3.5 mL vials, stored at 4°C immediately after sampling, and analyzed for nutrients within 24 h. Changes in N forms during storage using this procedure were < 1%. Alkalinity was determined by acid titration to the bromocresol green endpoint (pH = 4.5) and expressed as mg $CaCO_3$ L^{-1}. This pH coincided with the inflection point of the titration curve. Process pH was monitored throughout the experiments using temperature-compensated pH probes. The concentrations of un-ionized ammonia [free ammonia, NH_3 (FA)] and un-ionized nitrous acid [free nitrous acid, HNO_2 (FNA)] were calculated using the equations given by Anthonisen et al. (1976) based on temperature (°C), pH, and total NH_4-N or NO_2-N concentration (mg L^{-1}) values:

$$\text{FA as } NH_3 \text{ (mg L}^{-1}) = \left(\frac{17}{14}\right) \times \left(\frac{NH_4\text{-N} \times 10^{pH}}{\frac{K_b}{K_w} + 10^{pH}}\right) \quad (1)$$

$$\text{FNA as } HNO_2 \text{ (mg L}^{-1}) = \left(\frac{46}{14}\right) \times \left(\frac{NO_2\text{-N}}{K_a \times 10^{pH}}\right) \quad (2)$$

where K_b, K_w, and K_a are ionization constants for NH_3, H_2O, and HNO_2, respectively. Both the ratio (K_b/K_w) and K_a may be related to temperature: (K_b/K_w) = exp [6344/(273 + T)] and Ka = exp [– 2300/(273 + T)]. Samples used for mixed liquor suspended solids (MLSS) and volatile suspended solids (MLVSS) determinations were collected in 50 mL vials while continuously mixing the cultures with a bench stirrer. The residue retained on a glass-fiber filter was dried to 105°C for suspended solids determination and ignited to 500°C for volatile suspended solids (APHA, 1992). The nitrifying activity of the cultures, expressed as mg N oxidized g-MLSS^{-1} h^{-1}, was calculated from the rate of increase of (NO_3^- + NO_2^-)-N concentration during the first 4 h of aeration of a fresh inorganic salts medium. Weight of pellets is reported in a wet stage determined after draining off liquids with a 1.2-mm mesh sieve. Corresponding pellet volume was determined by water displacement using a weighed pellet sample and a graduated cylinder.

Results and Discussion

Preparation of Nitrifying Polymer Pellets

The ALNS exhibited high nitrification activity and good settling characteristics. The nitrification activity of ALNS, obtained before immobilization using the inorganic medium (300 mg NH_4-N L^{-1}), was 15.4 mg-N g-MLSS^{-1} h^{-1}. This rate is similar to values from 13.1 to 17.5 mg-N g-MLSS^{-1} h^{-1} reported by Furukawa et al. (1993) with marine nitrifiers but higher than typical values of 0.2-6 mg N g-MLSS^{-1} h^{-1} reported for fresh water activated sludge-type systems (Wild et al., 1971; Loehr et al., 1973; Sharma and Ahlert, 1977). The composition of the inorganic medium used to grow the ALNS (table 1) was selected to prevent nitrification inhibition during lagoon liquid testing by high NH_3. For example, the initial concentration of NH_3 in these batch cultures (at 35°C and a pH of 8.5, eq. 1) was 96.2 mg L^{-1}, or about 2.5 times higher than levels in the aerated lagoon liquid tested.

The nitrifying sludge also showed extremely good settling properties as it was concentrated to 58.0 g-MLSS L^{-1} using simple sedimentation procedures. A high concentration of nitrifying sludge in the polymerization mixture is desirable in order to increase the number of microorganisms contained in the pellet and the nitrification potential of the pellets, but there are practical limits. For example, centrifugation at 3,000 rpm for 10 min further increased concentration to 96.6 g L^{-1}. However, its handling during preparation of the PVA-ALNS mixture was difficult due to loss of fluidity. Therefore, the concentration of cells by sedimentation was preferred. The mixture of PVA-HC aqueous solution and concentrated ALNS, with a final PVA concentration of 9.91%, was successfully immobilized using the PVA-freezing method. The immobilized pellets had an elastic, rubber-like appearance, and exhibited excellent gel strength that did not deteriorate even after long-term treatment. This was evaluated in one of the original reactors used in the continuous flow experiments that was kept running uninterrupted for 2.5 years receiving swine lagoon wastewater at loading rates of 400 to 600 g NH_4-N L-reactor^{-1} d^{-1}. Periodic examination showed no noticeable changes in their original shape and a total pellet loss from the reactor of only 5.4 g (< 3% by weight). In fact, after this 2.5-year period of continuous reaction, the immobilized ALNS pellets exhibited two times greater nitrification rate in an experiment with high NH_4^+ concentration wastewater (Vanotti et al., 1999).

Nitrification of Swine Wastewater Using Batch Treatment

The nitrifying pellets showed excellent capability for fast and efficient removal of NH_4-N from lagoon wastewater. Data in figure 2 show nitrification performance in a batch run in which the lagoon liquid was supplemented with 1 g L^{-1} of a carbonate mixture composed of 98.15% $NaHCO_3$ and 1.85% Na_2CO_3. This supplement provided an extra 600 mg L^{-1} of alkalinity and kept the process pH within a narrow range of 7.7 to 8.3 for optimum nitrification treatment. The NH_4^+ in the lagoon liquid was completely removed in only 14 h of aeration treatment. It was transformed into oxidized N forms without losses of NH_3 by volatilization; full conversion of NO_2^- to NO_3^- was completed in approximately 24 h. Ammonia was nitrified readily, decreasing linearly (r = 0.998) at a rate of 16.1 mg NH_4-N L^{-1} h^{-1}. Corresponding (NO_3^- + NO_2^-)-N production was also linear with time (r = 0.998) and averaged 15.9 mg N L^{-1} h^{-1} . These rates are comparable to values of 7.7 to 11.6 mg NH_4-N L^{-1} h^{-1} reported by Tanaka et al. (1991) using nitrifying pellets applied to municipal wastewater containing 20 to 25 mg NH_4-N L^{-1}. Our results are significant because they show that nitrification efficiency of nitrifying pellets was not affected with animal wastewater containing ~10 times more NH_4^+ than municipal systems as well as higher BOD (> 100 mg L^{-1}). The initial concentration of ALNS contained in the PVA gel was 37.9 mg g-pellet^{-1}. At the loading rate of 153 g-pellet L^{-1} of reactor volume (15.3%), the concentration of immobilized ALNS in the reactor was 5800 g-MLSS L^{-1} (4500 g-MLVSS L^{-1}). Wild et al. (1971) and Srinath et al. (1976) reported that the time

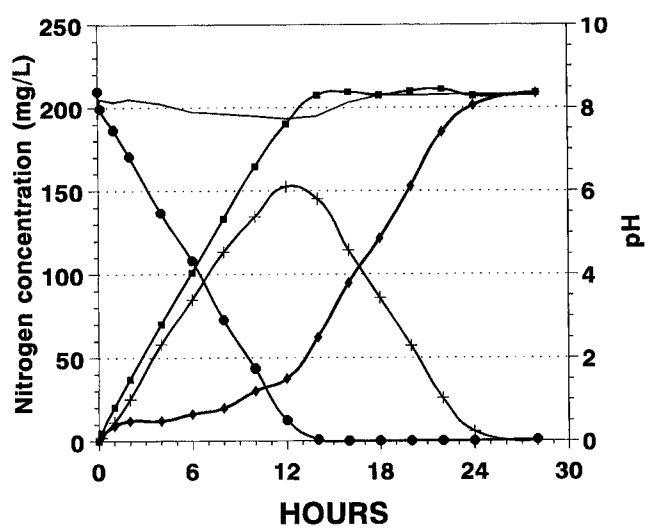

Figure 2–Nitrification of lagoon wastewater with immobilized nitrifiers in batch treatment using a $CO_3^=/HCO_3^-$ buffer for optimum NH_4^+ removal performance; (●) NH_4^+, (+) NO_2^-, (◆) NO_3^-, (■) NO_2^- plus NO_3^-, and (–) pH. Each symbol is the mean of two replications; largest SD are 4.8, 4.7, and 6.2 mg N L^{-1} for NH_4^+, NO_2^-, and NO_3^-, respectively, and 0.16 for pH.

required for a given degree of ammonia removal was inversely proportional to the concentration of nitrifiers present for a range of MLVSS from 60 to 6000 mg L^{-1}. Although we are uncertain of the actual number of nitrifiers that remained functional after the polymerization process, their numbers should have been considerable based on both the high nitrification efficiency observed and reports of other workers. These increased numbers are very important for nitrification of lagoon wastewater. For instance, Ballester et al. (1992) and Blouin et al. (1990) have shown the need to inoculate with nitrifying microorganisms for rapid nitrification of anaerobically digested liquid swine manure because of the extremely low concentration of nitrifiers contained in these wastes. Using batch treatment with a 10% (v/v) inoculum of enriched nitrifying cultures, Ballester et al. (1992) reported a nitrification efficacy of 6 to 10 mg NH_4-N oxidized L^{-1} h^{-1}, which was obtained after an initial lag phase of 4 to 6 d of zero nitrification. Without inoculation, the initial lag phase was extended to 17 d, and the nitrification rates obtained thereafter were much lower (~1.7 mg N L^{-1} h^{-1}). Similarly, Blouin et al. (1990) showed higher nitrification efficacy (12.0 mg NH_4-N oxidized L^{-1} h^{-1}) for swine waste inoculated (10% v/v) with enriched nitrifying populations in high (10^6-10^7 MPN/mL) concentration; compared to rates of about 0.9 mg NH_4-N oxidized L^{-1} h^{-1} with addition of an inoculum consisting of activated sludge from a municipal plant, or no nitrification at all (49 d) in an aerated control that was not inoculated. Thus, the rapid NH_4^+ oxidation of 16 mg N L^{-1} h^{-1} obtained with immobilized pellets (fig. 2) indicates a high concentration of active nitrifying microorganisms in the pellets since comparable nitrification rates for anaerobically digested swine waste have been obtained only after massive inoculation of nitrifying bacteria. One of the advantages of using immobilized technology applied to nitrification of swine wastewater is that problems of maintaining enriched nitrifying populations often reported for systems where cells are in suspension (St.-Arnaud et al., 1991) are minimized due to the gel entrapment conditions.

In addition to poor nitrification, aerobic treatment of lagoons can potentially add to environmental problems by stripping out ammonia in the absence of enriched nitrifying populations. This is illustrated in figure 3, showing the nitrogen transformations in a control batch treatment without addition of nitrifying pellets or alkalinity supplements. Nitrification did not start until day 10, and by that time a significant amount of NH_4^+ was already lost. This loss was likely due to NH_3 volatilization and air stripping and not from simultaneous nitrification/denitrification or microbial biomass uptake because: (1) process pH did not decrease (as it would be expected with active nitrification of swine wastewater without alkalinity supplements); and (2) total Kjeldhal N values were not maintained (as expected with microbial growth and uptake). Nitrification was completed at day 17 with 30% of the initial NH_4^+ oxidized (only NO_2^- was formed) and the remaining 70% unaccounted, presumably lost through NH_3 stripping. The patterns of nitrification and N transformations shown in figure 3 are typical of results obtained by other authors such as Ballester et al. (1992), Blouin et al., (1990), and Sievers (1995) with aeration treatment of anaerobically digested swine wastewater

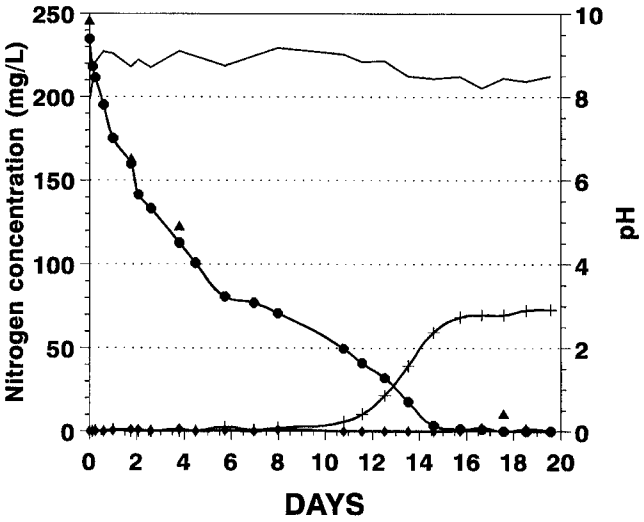

Figure 3–Nitrification of lagoon wastewater in a control batch treatment receiving only aeration, without immobilized nitrifiers or pH correction; (●) NH_4^+, (+) NO_2^-, (◆) NO_3^-, (▲) TKN, and (–) pH. Each symbol is the mean of two replications, largest SD are 28.2, 21.1, 1.3, and 31.9 mg N L^{-1} for NH_4^+, NO_2^-, NO_3^-, and TKN, respectively, and 0.14 for pH.

without addition of nitrifiers and illustrate the need to increase the concentration of nitrifying microorganisms for the rapid nitrification of these wastes.

Impact of pH Control

Control of pH is also an important consideration for optimum nitrification of swine wastewater. During nitrification, there is a release of hydrogen ions, at a rate of 2 mol for each mole of NH_4^+ oxidized, that decreases the pH to an extent related to the buffering capacity of the system. The alkalinity concentration in the lagoon wastewater (1357 mg $CaCO_3$ L^{-1}) was lower than 1670 mg L^{-1} estimated for complete oxidation of 233 mg NH_4-N L^{-1}, considering an alkalinity consumption ratio of 7.14 mg-$CaCO_3$ per mg NH_4-N oxidized (Sherrard, 1976). Figure 4 shows a typical pattern of acidification of lagoon liquid by nitrification activity of the pellets. Results of this and other batch runs (not shown) conducted on the same wastewater without initial alkalinity supplementation showed that NH_4^+ oxidation was always halted at about 12 h when the pH of the mixed liquor reached a value of 6.0 to 6.2, leaving at that point approximately 20% of the N untreated (~50 mg N L^{-1}).

One mechanism by which pH affects the rate of nitrification has been proposed by Anthonisen et al. (1976). They proposed that nitrifying bacteria are inhibited by the un-ionized rather than the total ion concentration of ammonia and nitrite. Their studies showed that NH_4^+ oxidation by *Nitrosomonas* sp. is usually inhibited at concentrations of un-ionized nitrous acid (HNO_2) of 0.2 to 2.8 mg L^{-1} and concentration of un-ionized ammonia (NH_3) of 10 to 150 mg L^{-1}, while NO_2^- oxidation by *Nitrobacter* sp. is inhibited with NH_3 concentrations in the range of 0.1 to 1.0 mg L^{-1}. Our data fit well within these inhibitory values. Figure 4 shows that oxidation of NO_2-N was inhibited during the first 8.5 h and during the pH adjustment period. Corresponding free NH_3 concentrations shown in figure 5 indicate that NO_2^- oxidation was

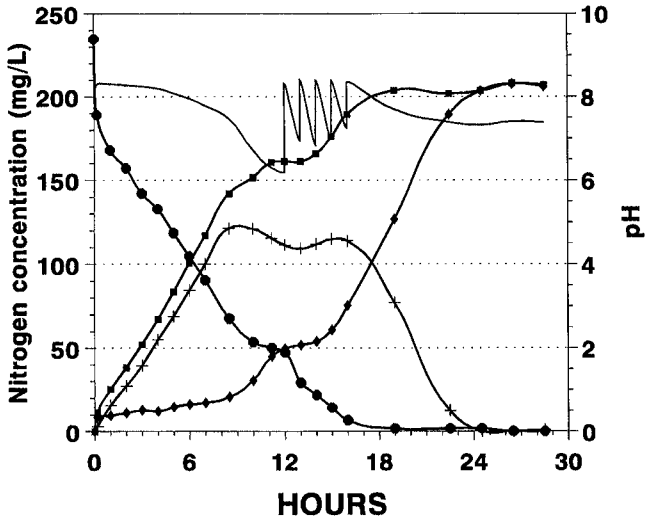

Figure 4–Nitrification of lagoon wastewater with immobilized nitrifiers in batch treatment. The pH was adjusted to 8.4 at 12-15 h with 1 *N* NaOH providing an extra 390 mg L^{-1} of alkalinity to finish NH_4^+ oxidation; (●) NH_4^+, (+) NO_2^-, (◆) NO_3^-, (■) NO_2^- plus NO_3^-, and (–) pH. Each symbol is the mean of two replications, largest SD are 9.8, 13.7, and 13.3 for NH_4^+, NO_2^-, and NO_3^-, respectively, and 0.27 for pH.

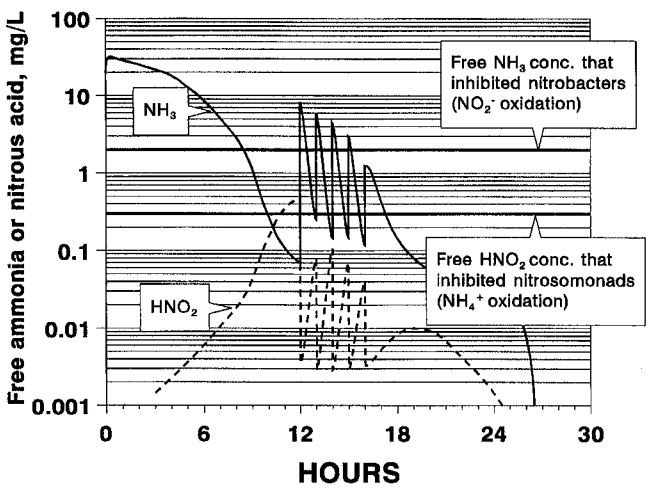

Figure 5–Changes in un-ionized ammonia and nitrous acid during inhibited nitrification of lagoon wastewater. Values were determined using equations 1 and 2 with the pH and total NH_4^+ and NO_2^- concentration shown in figure 4.

inhibited at NH_3 concentration levels between 2.0 and 31.2 mg L^{-1}. As the NO_2-N accumulated and the pH decreased during progression of nitrification (fig. 4), the free nitrous acid increased to a value of 0.3 mg L^{-1} (fig. 5), which inhibited NH_4^+ oxidation. This inhibition was relieved with pH corrections at 12 to 16 h, and NH_4^+ oxidation was rapidly completed. These data indicate that NH_4^+ oxidation using immobilized ALNS pellets is not affected by free ammonia levels of swine lagoon wastewater and that inhibition caused by high HNO_2 concentration during the progress of nitrification can be easily corrected through pH control. Results from such a system are shown in figure 2 where the pH was kept buffered within a narrow, optimum range through inorganic carbon addition. It maintained HNO_2 concentrations lower than 0.02 mg L^{-1} even when total nitrite-N accumulated to excessively high levels (153 mg L^{-1}). Our data also show that NO_2^- oxidizing microorganisms in the pellets were more sensitive to free NH_3 levels in the lagoon liquid, and this process was inhibited when NH_3 exceeded 2.0 mg L^{-1}. An aerated lagoon liquid with a total NH_4-N concentration of 230 mg L^{-1} and a pH of 8.3 contains 38.7 mg NH_3 L^{-1} at 30°C (eq. 1). Thus, during batch treatment, initially high NH_3 levels in the waste will extend the total process time needed to complete nitrification to NO_3^- (figs. 2 and 4). This limitation, however, may not be consequential if the goal in lagoon wastewater treatment is to remove N through a nitrification/denitrification cycle. For example, Focht and Chang (1975) suggested that NH_4^+ oxidation to NO_2^- and then reduction to N_2 represents an improvement over complete oxidation to NO_3^- and subsequent reduction. This is because the overall process results in less energy expenditure and time and since toxicity of NH_3 to *Nitrobacter* does not have to be considered, the loading rates of high NH_4-N concentration can be increased.

Nitrification of Swine Wastewater Using Continuous Flow Treatment

Ammonia removal potential of ALNS pellets was also evaluated under continuous flow with increasing NH_4^+ loading rate treatments. Ammonia loading rates were gradually increased during a two-month period from 194 to a maximum of 1287 mg-NH_4-N L-reactor^{-1} d^{-1} (corresponding from 1.27 to 8.40 mg-NH_4-N g-pellet^{-1} d^{-1}, respectively). Loading rates were adjusted by changing the influent flow rate in order to provide a range of hydraulic retention time (HRT) from 24 to 4 h (table 2). Alkalinity was supplemented in all the treatment runs with 1 g L^{-1} of a carbonate mixture added to the influent wastewater and consisted of 98.15% $NaHCO_3$ and 1.85% Na_2CO_3.

Tables 2 and 3 present the treatment results of these continuous experiments. The NH_4^+-N removed was entirely recovered in NO_3^- and NO_2^- forms. Nitrification efficiencies of more than 90% were successfully obtained, even at relatively short hydraulic retention time of 12 h and NH_4^+ loading rate of 418 mg N L-reactor^{-1} d^{-1}. The pellet-to-reactor volume ratio was 15.3% (w/v); the corresponding NH_4^+ loading rate for unit mass of pellet (wet) was 2.73 mg N g-pellet^{-1} d^{-1}. Nitrification performance of pellets obtained with swine wastewater is comparable with performance in municipal wastewater applications. Furukawa et al. (1994) reported > 80% nitrification efficiencies for treatment of NH_4^+ polluted landfill leachate (containing 35 mg NH_4^+-N L^{-1}) by using PVA immobilized nitrifiers with an NH_4-N loading rate of 2.9 mg N g-pellet^{-1} d^{-1}. High nitrification performance (> 90% efficiency at NH_4-N loading of 1.7 to 1.9 mg N g-pellet^{-1} d^{-1}) has been also reported by Tanaka et al. (1991) with PEG pellets applied to municipal wastewater containing 20 to 30 mg N L^{-1} d^{-1}. Our results indicate that the nitrifying potential of immobilized ALNS was not affected by certain wastewater quality characteristics in animal lagoon liquid such as high NH_4^+ and BOD strength that were thought to be limiting factors. Consequently, our results verified for the first time that the nitrification treatment of animal wastewater by immobilized pellets is feasible.

Table 2. Treatment of lagoon swine wastewater with nitrifying pellets under continuous flow

Run (no.)	HRT* (h)	Q† ($L\ d^{-1}$)	NH_4^+ Loading Rate‡ (mg N L-reactor^{-1} d^{-1})	Influent NH_4^+ Conc.§	Effluent Quality: NH_4^+	NO_3^-	NO_2^-	NO_3^- plus NO_2^-	pH
				mg N L^{-1}					
1	28	1.03	194	226.7 (2.7)‖	3.2 (0.8)	227.3 (4.6)	5.5 (1.9)	232.7 (2.7)	7.70 (0.06)
2	24	1.20	227	226.6 (5.2)	3.5 (0.1)	231.8 (3.4)	8.4 (0.9)	240.2 (3.8)	7.75 (0.07)
3	20	1.44	260	216.7 (2.6)	5.3 (0.3)	218.9 (4.3)	13.9 (4.3)	232.8 (1.4)	7.83 (0.07)
4	16	1.80	326	217.4 (7.6)	9.8 (0.8)	198.3 (0.1)	20.0 (1.1)	218.3 (1.3)	7.90 (0.04)
5	12	2.40	418	209.0 (3.7)	27.5 (1.9)	168.9 (6.5)	29.8 (6.9)	198.7 (0.4)	8.07 (0.03)
6	8	3.60	634	212.3 (2.9)	78.2 (3.8)	92.9 (10.1)	46.0 (7.1)	138.9 (3.0)	8.16 (0.03)
7	6	4.80	884	221.1 (1.4)	96.6 (0.4)	69.7 (1.3)	55.1 (0.5)	124.7 (0.8)	8.26 (0.04)
8	4	7.20	1284	214.6 (1.0)	114.0 (3.4)	42.2 (3.1)	52.3 (1.5)	94.5 (1.8)	8.39 (0.09)

* Hydraulic retention time.
† Q, Influent flow rate.
‡ NH_4^+ loading rate = Q $(NH_4^+)_i$ /V; where $(NH_4\text{-}N)_i$ = Influent NH_4-N conc., and V = reactor volume (1.2 L).
§ Lagoon influent contained 250 mg L^{-1} of TKN and 0 mg L^{-1} of NO_3^- and NO_2^-.
‖ Means of two replicate runs with SD in parentheses.

Table 3. Nitrification efficiency of nitrifying pellets using continuous treatment

Run (no.)	HRT* (h)	NH_4^+ Loading Rate	NH_4^+ Removal Rate	NO_3^- + NO_2^- Production Rate	NH_4^+ Removal Efficiency (%)	Nitrification Efficiency (%)
		mg N L-reactor^{-1}d^{-1}				
1	28	194	193	199	99	100
2	24	227	223	240	98	100
3	20	260	254	279	98	100
4	16	326	311	327	96	100
5	12	418	363	397	87	95
6	8	637	402	417	63	65
7	6	884	498	499	56	56
8	4	1287	604	567	47	44

* Hydraulic retention time.

The metal screen placed inside the reactor prevented attachment of heterotrophic bacteria and the formation of a deleterious biofilm on the surface of the pellets due to a cleaning action during fluidization and separation of free cells in the effluent. This mechanism may explain the excellent nitrification efficiency obtained with high BOD_5 test values of 195 mg L^{-1} in the influent. The average BOD_5 concentration in the effluent obtained at optimum nitrification performance (HRT = 12 h) was 24 mg L^{-1}.

Nitrification rates obtained with continuous flow were comparable to those obtained with batch treatment. For example, the rate of $(NO_3^- + NO_2^-)$-N production was 397 mg N L-reactor^{-1} d^{-1} in the continuous experiment at optimum HRT of 12 h, which is similar to the 382 mg N L-reactor^{-1} d^{-1} (15.9 mg N L^{-1} h^{-1}) obtained in batch treatment (fig. 2). However, high NO_2^- concentrations were not detected in the continuous experiments using optimum HRT, and NO_3^- was the predominant form in the effluent indicating that NO_2^- oxidizing bacteria in the pellet were not inhibited and operated simultaneously with NH_4^+ oxidizers (table 2). This difference is explained by the much lower NH_4^+ concentration maintained in the reactor; therefore, lower "free" NH_3 levels in the system to cause inhibition of NO_2^- oxidation. For example, using the effluent NH_4^+ concentration and pH data in table 2, the calculated NH_3 levels in the reactor at HRT of 16 and 12 h were 0.7 and 2.6 mg L^{-1}, respectively, which contrast with initial conditions (38.7 mg NH_3 L^{-1}) estimated for the batch system that suppressed activity of NO_2^- oxidizing bacteria. Thus, continuous treatment is desirable for rapid, complete nitrification of high NH_4^+ strength animal wastewater.

By increasing the NH_4-N loading rate to 634 mg N L-reactor^{-1} d^{-1} (4.16 mg N g-pellet^{-1} d^{-1}; 8 h HRT), the nitrification efficiency was decreased to 65% (table 3). Nitrification efficiencies decreased further to 44% at the highest rate of 1287 mg N L-reactor^{-1} d^{-1} (8.40 mg N g-pellet^{-1} d^{-1}; 4 h HRT). The total NH_4-N removed, however, increased with loading rates. At the HRT of 4 h, the NH_4^+ removal rate was 604 mg N L-reactor^{-1} d^{-1}, which is 66% higher than the removal rate obtained at HRT of 12 h providing optimum nitrification efficiency. Higher efficiencies may be useful for systems designed to meet stream discharge requirements. Such complete treatment is unlikely to be necessary for most farms, and a retrofit nitrification unit operated at shorter retention times may be appropriate because it will remove higher amounts of NH_4^+ ammonia from a lagoon. This strategy has the potential advantage of reducing the total cost of aeration per unit of nitrate-N produced.

Conclusions

The use of large populations of nitrifying bacteria entrapped in polymer resins offers the potential for NH_4^+ removal rates that are much faster than those occurring in conventional waste treatment systems based on activated sludge. Although this technology has been successfully used for treating municipal wastewaters that have lower concentrations of nitrogen and organic carbon compared to liquid animal manure, there was a concern that concentrated animal waste may be harmful to the immobilized bacteria, specifically because of high free NH_3 and BOD. Our approach was to use immobilized pellet technology with nitrifying sludge acclimated to lagoon swine wastewater (ALNS). The ALNS was obtained from a nitrifying biofilm that developed in an overland flow system used to treat swine wastewater and exhibited high nitrification activity (15.4 mg N g-MLSS^{-1} h^{-1}) in a batch culture with free NH_3 levels of 96 mg L^{-1}. This result show that ALNS was very tolerant of high NH_3

concentrations since toxicity threshold for NH_4^+ oxidizers has been reported as 10 to 150 mg L^{-1}.

The ALNS was successfully immobilized in polymer pellets using a PVA-freezing method. Our results show that immobilized ALNS can rapidly convert NH_4^+ from lagoon wastewater to NO_3-N and at rates that are comparable to those obtained in municipal applications. Optimum performance was obtained when the process pH was controlled with alkali supplements. This was important to prevent inhibition of Nitrosomonas by HNO_2. Addition of 400 to 600 mg alkalinity L^{-1} was needed for complete NH_4^+ oxidation in the lagoon sample. In batch treatment, only 14 h were needed for complete oxidation of NH_4^+ (~210-230 mg N L^{-1}) to NO_3^- and NO_2^- using immobilized ALNS pellets. The rate of nitrification was extremely low in a control without the pellets, where most of the NH_4^+ was lost through air stripping. This demonstrated the problems with using aeration for nitrification of lagoon liquid or other animal wastes having low concentration of nitrifiers. In continuous flow, nitrification efficiencies > 95% were obtained with HRT ≥ 12 h and NH_4^+ loading rates ≤ 418 mg N L^{-1} d^{-1}. Although the nitrification efficiency decreased with higher loading, the NH_4^+ removal rates increased; the highest rate, 604 mg N L^{-1} d^{-1}, was obtained with HRT of 4 h. In all cases, all of the NH_4-N removed was entirely recovered in oxidized N forms. Immobilization is an attractive way to maintain high cell concentration and prevent washout of the slow-growing autotrophic nitrifiers under high flow rate conditions such as those used in this study. Rates of NH_4^+ oxidation were similar in both continuous flow and batch treatment; but high NH_3 levels in batch mode affected NO_2^- oxidation to NO_3^-. However, complete conversion to NO_3^- may not be a critical factor if the overall goal is to remove surplus NH_4^+ from a farm through nitrification/denitrification processes.

The results of this study indicate that immobilized pellets are a useful technology for the nitrification treatment of swine wastewater. Although this study shows the magnitude of the capacity for NH_4^+ removal from animal lagoons by encapsulated ALNS, pilot-scale testing would be required to evaluate the most efficient operating strategy for a full-scale nitrification reactor.

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References

Anthonisen, A. C., R. C. Loher, T. B. Prakasam, and E. G. Srinath. 1976. Inhibition of nitrification by ammonia and nitrous acid. *Journal WPCF* 48(5): 835-852.

American Public Health Association. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th Ed. Washington, D.C.: APHA, AWWA, WEF.

Ballester, M., C. Bizeau, and R. Moletta. 1992. Study of nitrification of swine waste after methanization. Selection and comparative study of two microbial nitrifying ecosystems. *Environ. Technol.* 13: 837-845.

Barrington, S. F., and R. Moreno. 1995. Swine manure nitrogen conservation using Sphagnum moss. *J. Environ. Quality* 24(4): 603-607.

Bernet, N., N. Delgenes, and R. Moletta. 1996. Denitrification by anaerobic sludge in piggery wastewater. *Environ. Technol.* 17: 293-300.

Blouin, M., J.-G. Bisaillon, R. Beaudet, and M. Ishaque. 1990. Nitrification of swine waste. *Canadian J. Microbiol.* 36: 273-278.

Braum, S. M., J. F. Moncrief, and S. C. Gupta. 1997. Nitrogen losses from a liquid dairy manure management system. In *Agron. Abstracts.* Madison, Wis.: ASA.

Burton, C. H. 1992. A review of the strategies in the aerobic treatment of pig slurry: Purpose, theory and method. *J. Agric. Eng. Res.* 53: 249-272.

Ciaccolini, I., G. Cosmai, A. Micheli, P. Vitali, and M. Corrado. 1984. Tests of nitrification of effluents from anaerobic digestion of swine wastes, with recovery of fertilizers for agricultural use. *Acqua-Aria* 2: 145-154.

Crouse, D. A., S. C. Hodges, W. P. Robarge, R. L. Mikkelsen, and J. L. Havlin. 1997. Nitrogen budget for a swine production facility in North Carolina. In *Agron. Abstracts.* Madison, Wis.: ASA.

Figueroa, L. A., and J. Silverstein. 1992. The effect of particulate organic matter on biofilm nitrification. *Water Environ. Res.* 64(5): 728-733.

Focht, D. D., and A. C. Chang. 1975. Nitrification and denitrification processes related to waste water treatment. *Adv. in Appl. Microbiol.* 19: 153-186.

Furukawa, K., A. Ike, and M. Fujita. 1993. Preparation of marine nitrifying sludge. *J. Ferment. Bioeng.* 76(2): 134-139.

Furukawa, K., S. Ryu, M. Fujita, and I. Fukunaga. 1994. Nitrogen pollution of leachate at a sea-based solid waste disposal site and its nitrification treatment by immobilized acclimated nitrifying sludge. *J. Ferment. Bioeng.* 77(4): 413-418.

Hashimoto, S., K. Furukawa, and H. Hama. 1986. Studies on the immobilization of activated sludge by polyvinylalcohol. In *Proc. Environ. & Sani. Eng. Research* 22: 195-203.

Lewandowski, Z., R. Bakke, and W. G. Characklis. 1987. Nitrification and autotrophic denitrification in calcium alginate beads. *Wat. Sci. Tech.* 19: 175-182.

Loehr, R. C., T. B. S. Prakasam, E. G. Srinath, and Y. D. Yoo. 1973. Development and demonstration of nutrient removal from animal wastes. Environmental Protection Technology Series, Report EPA-R2-73-095. Washington, D.C.: EPA.

Miner, J. R., and T. E. Hazen. 1977. Transportation and application of organic wastes to land. In *Soils for Management of Organic Wastes and Waste Waters*, 379-425, eds. L. F. Elliot, and F. J. Stevenson. Madison, Wis.: ASA/CSSA/SSSA.

Rice, M., A. Szogi, S. Broome, F. Humenik, and P. Hunt. 1998. Constructed wetland systems for swine wastewater treatment. In *Proc. Animal Production Systems and the Environment*, 501-505. Des Moines, Iowa: Iowa State University.

Sharma, B., and R. C. Ahlert. 1977. Nitrification and nitrogen removal. *Water Research* 11: 897-925.

Sherrard, J. H. 1976. Destruction of alkalinity in aerobic biological wastewater treatment. *J. Water Pollution Control Fed.* 48(7): 1834-1839.

Sievers, D. M. 1995. Reduced ammonia levels in anaerobic lagoon effluent by low rate aeration. In *Proc. 7th Int. Symp. on Agricultural and Food Processing Wastes*, ed. C. C. Ross, 514-520. St. Joseph, Mich.: ASAE.

Srinath, E., R. C. Loehr, M. Asce, and T. B. S. Prakasam. 1976. Nitrifying organism concentration and activity. *J. Environ. Eng.* 102(April): 449-463.

St-Arnaud, S., J.-G. Bisaillon, and R. Beaudet. 1991. Microbiological aspects of ammonia oxidation of swine waste. *Canadian J. Microbiol.* 37: 918-923.

Tada, M., S. Harada, S. Koike, Y. Kawaguchi, T. Mizuguchi, H. Emori, N. Mori, T. Yamadera, M. Hotta, H. Nakamura, T.

Sumino, T. Kimata, and Y. Fujii. 1991. System for treating waste water by nitrification. U.S. Patent No. 5,061,368.

Takeshima, T., K. Motegi, H. Emori, and H. Nakamura. 1993. Pegasus: An innovative high-rate BOD and nitrogen removal process for municipal wastewater. In *Proc. 66th Annual Water Environment Federation Conf.*, 173-181. Anaheim, Calif.: WEF.

Tanaka, H., S. Uzman, and I. Dunn. 1981. Kinetics of nitrification using fluidized bed reactor with attached growth. *Biotechnol. Bioeng*. 23: 1686-1702.

Tanaka, K., M. Tada, T. Kimata, S. Harada, Y. Fujii, T. Mizuguchi, N. Mori, and H. Emori. 1991. Development of new nitrogen removal system using nitrifying bacteria immobilized in synthetic resin pellets. *Water Sci. & Technol.* 23: 681-690.

Tchobanoglous, G., and F. L. Burton. 1991. *Wastewater Engineering: Treatment, Disposal, and Reuse*. Boston, Mass.: Irwin/McGraw-Hill.

Technicon Instruments Corp. 1977. Individual/simultaneous determination of nitrogen and/or phosphorus in BD acid digests (dialyzer). Industrial method 337-74W/B. Tarrytown, N.Y.: Technicon Instruments Corp.

_____. 1978. Nitrate and nitrite in water and wastewater. Industrial method 100-70W/B. Tarrytown, N.Y.: Technicon Instruments Corp.

Vanotti, M. B., A. A. Szogi, P. G. Hunt, F. J. Humenik, and J. M. Rice. 1998. Nitrification options for swine wastewater treatment. In *Proc. Animal Production Systems and the Environment*, 795-800. Des Moines, Iowa: Iowa State University.

Vanotti, M. B., M. Nakaoka, P. G. Hunt, A. Ellison, and S. Odamura. 1999. Treatment of high-ammonia animal wastewater with nitrifying pellets. ASAE Paper No. 99-4092. St. Joseph, Mich.: ASAE.

Wijffels, R. H., E. J. T. M. Leenen, and J. Tramper. 1993. Possibilities of nitrification with immobilized cells in wastewater treatment: Model or practical system? *Wat. Sci. Tech.* 27(5-6): 233-240.

Wild, H. E., C. N. Sawyer, and T. C. McMahon. 1971. Factors affecting nitrification kinetics. *J. Water Pollut. Control Fed.* 43(9): 1845-1854.