

**NITRIFICATION TREATMENT OF SWINE WASTEWATER  
BY ENCAPSULATED NITRIFIERS**

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**Summary:**

The use of nitrifying bacteria encapsulated in polymer resin pellets is a new technology in municipal wastewater treatment plants for higher nitrification rates and more efficient reactors. We evaluated if the technology has potential application for biological removal of ammonia nitrogen from swine lagoons. Nitrification efficiencies of 95% were obtained in bench experiments with ammonia loading rates of 418 mg N/L/d and hydraulic residence time (HRT) of 12 h. A prototype plant was set up in a swine operation in Duplin Co., NC. At the initial 48 h HRT, nitrification activity increased from 21 to 200 g N/m<sup>3</sup>/d in 30 d. Nitrification activity further increased to 319 and 433 g N/m<sup>3</sup>/d at HRT's of 32 and 24 h, respectively.

**Keywords:**

Swine wastewater, Ammonia removal, Nitrification treatment, Lagoon, Immobilized Nitrifiers, Constructed Wetlands.

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## INTRODUCTION

During recent decades, animal production methods in the U.S.A. have undergone dramatic changes. The predominant trend has seen animal production changing from small, individual operations into large, confined, commercial enterprises. Typical facilities use flush or pit-recharge systems to remove manure from the confinement houses. The flushed waste is mostly treated and stored in large (0.25- to 5-ha) anaerobic lagoons and later applied to cropland. Anaerobic lagoons are designed to perform a significant reduction in the organic content of the flushed waste. Although the anaerobic digestion process can reduce 80% or more of the organic matter from these high-strength wastewaters, these open lagoon systems are not without significant adverse environmental impact. Specifically, organic N is converted to free ammonia ( $\text{NH}_3$ ) with much of it volatilized from the lagoon's surface. For storage periods of 180 days typical of the Southeast, it may be anticipated that more than 50% of the nitrogen (N) entering the lagoon will escape to the atmosphere (Miner and Hazen, 1977; Muck and Steenhuis, 1982; Barrington and Moreno, 1995). Recent estimates of  $\text{NH}_3$  emissions from swine lagoons in North Carolina indicate that about 30 Mg of  $\text{NH}_3$  per day volatilizes from a total of 2,000 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. It is estimated that airborne pollution now accounts for about one-third of the 2,300 Mg of N that enter the Neuse River basin of the eastern U.S.A. each year (Hans Paerl, pers. comm.). These and other considerations, such as the potential for contaminated ground and surface waters, fish kills, and unexpected ecological shifts, provide ample reason for a greatly increased interest in finding alternative methods of N management for confined animal production that are functional and affordable.

## NITRIFICATION-DENITRIFICATION TREATMENT

A possible solution is to remove ammonia from animal lagoon wastewater through on-farm biological nitrification-denitrification systems. The effectiveness of such biological nitrogen removal processes depends on the ability of nitrifying organisms to oxidize  $\text{NH}_3$  to nitrate ( $\text{NO}_3\text{-N}$ ). Once in a nitrate form, the transformation into  $\text{N}_2$  (or denitrification process) needs two conditions: a source of carbon and an anaerobic environment. These conditions are typically found in wetlands or liquid manure storage units. Using lagoon swine wastewater with a nitrification pretreatment, Rice and coworkers (1998) increased more than five times the N removal potential of constructed wetlands. Bernet and coworkers (1996) found that denitrification can also be carried out in the same tank used for anaerobic digestion of swine wastewater. Their results indicate that design of a practical process combining anaerobic digestion and denitrification coupled to a nitrifying reactor needs consideration of the carbon (C) to  $\text{NO}_3\text{-N}$  ratio in order to obtain complete  $\text{NO}_3\text{-N}$  reduction to molecular N.

The basic problem related to nitrification of animal wastewaters is the low growth rate of the nitrifying bacteria; the generation time of these microorganisms is about 15 hours. Compared to heterotrophic microorganisms, which have generation times of 20-40 minutes, the nitrifiers compete poorly for limited oxygen and nutrients and tend to be overgrown or washed out (Figueroa and Silverstein, 1992; Wijffels et al., 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., 1989). Even when the oxygen supply is plentiful, an adaptation period is needed to reach a minimum bacteria concentration before effective nitrification (Vanotti et al., 1998). 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).

To overcome low nitrification rates in swine wastewater, we evaluated a new technology that uses nitrifying bacteria encapsulated in polymer pellets. The technology has been successfully applied to municipal wastewater treatment providing higher nitrification rates, shorter hydraulic residence times (HRT), and smaller reactors. This is an attractive approach to biological ammonia removal as applied to animal systems because the capacity of the reactor can be increased by increasing the nitrifiers' retention time independent from the wastewater retention time.

## **ENCAPSULATED NITRIFIERS**

Advances in biotechnology using immobilization technology have shown that conditions can be modified to enhance the activity of specific microorganisms performing a desirable chemical process. The immobilization of microorganisms in polymer resins is a widely applied technique in drug manufacturing and food processing. The application for municipal wastewater treatment has been recently developed and tested in Japan (Tanaka et al., 1991; Takeshima et al., 1993), and there are currently several full-scale municipal wastewater treatment plants using this technology. This was the result of a 10-year comprehensive research project intended to solve wastewater treatment problems using biotechnology. Through the immobilization process, the nitrifying microorganisms are provided with a very suitable environment to perform at maximum effectiveness. The nitrifiers are entrapped in 3- to 5-mm pellets made of polymers that are permeable to  $\text{NH}_3$ , oxygen and carbon dioxide needed by these microorganisms, resulting in a fast and efficient removal of  $\text{NH}_3$ . Typical materials are polyethylene glycol (PEG) and polyvinyl alcohol (PVA); these pellets are functional for more than 10 years. Wastewater is treated in a nitrification tank equipped with a wedge-wire screen to retain the pellets and a whole-floor aeration system to ensure high oxygen transfer and appropriate fluidization. Pellet volume is usually 7 to 15% of the total reactor volume. Nitrification rate with this technology can be three times higher than those of the conventional activated sludge process (Tanaka et al., 1991). This is important when assessing the application of nitrification technologies for animal systems because construction and aeration cost can be limiting factors.

## **BENCH EXPERIMENTS**

Laboratory experiments were conducted to elucidate conditions that optimize nitrification of lagoon wastewater. An active culture of acclimated swine wastewater nitrifying bacteria (ANB) was prepared from seed sludge obtained from an overland flow treatment field used for nitrification of anaerobic lagoon wastewater effluent. The ammonia strength of the inorganic salts medium (pH 8.5) was increased gradually to 300 mg/L to overcome inhibitory effects caused by

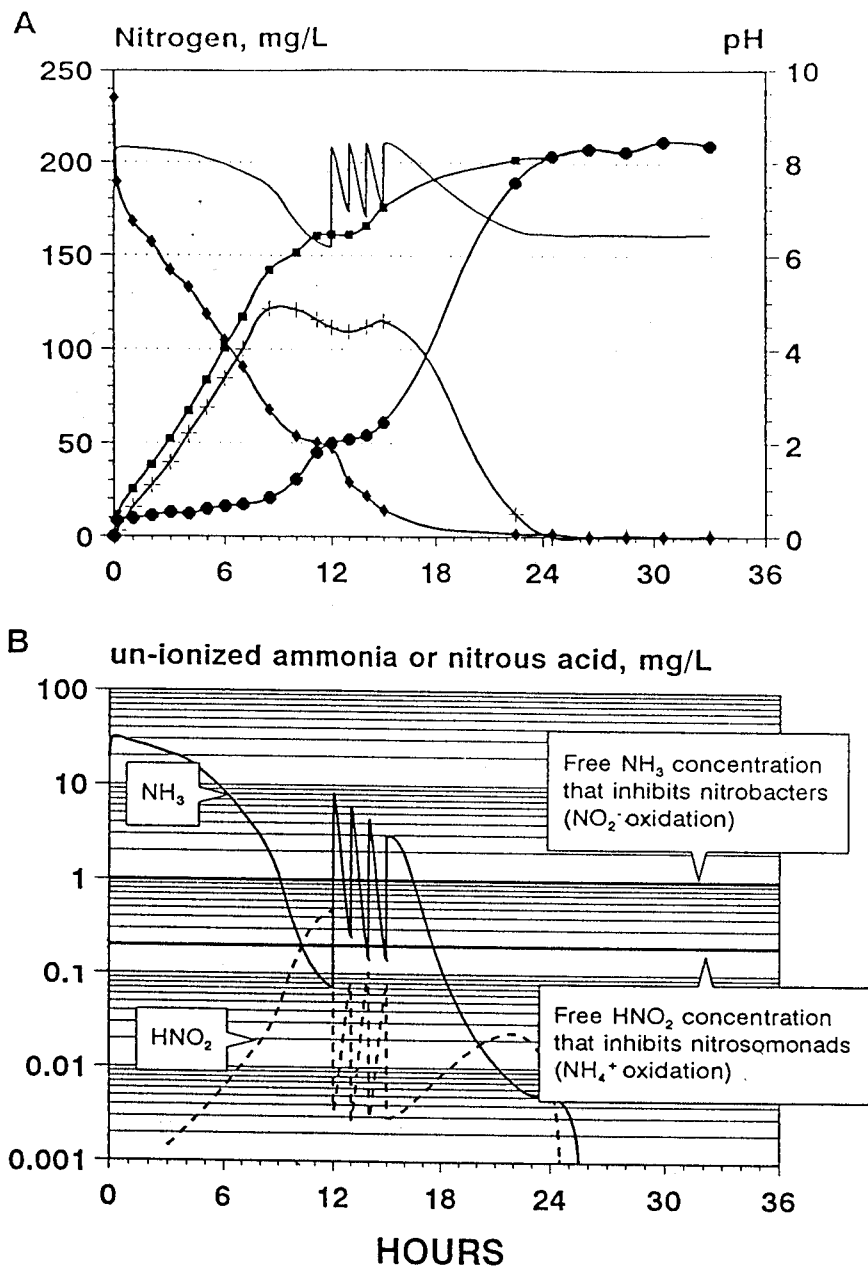
high levels of free ammonia in swine wastewater, similar to procedures used for acclimation of marine nitrifiers (Furukawa et al., 1993). After 10 days of incubation at 35°C, the cultivation procedure yielded 1 g-MLSS/L with a nitrification activity of 7.06 mg NH<sub>4</sub>-N/g-MLSS/h. Acclimated nitrifying cells were harvested by sedimentation, mixed with polyvinyl alcohol gel, and the mixture was polymerized using a freezing method (Vanotti and Hunt, 1998). Pelletized cubes of 3-5 mm containing ANB were prepared using a sharp blade. Pellets were produced at a rate of 766-g (wet) or 875-ml pellets per 1000 g of ANB-PVA initial mixture and contained 37.9 mg ANB/g-pellet (wet).

The bench reactors consisted of conic aeration tanks with air supplied from the bottom of the tanks to ensure full fluidization of nitrifying pellets. Average dissolved oxygen concentration was 7.7-mg O<sub>2</sub>/L. Pellets were added at 15% (w/v) [17% (v/v)] pellet to tank volume ratio. Temperature was controlled using a circulated water bath and heat regulator. All experiments were conducted at 30°C. The wastewater used was a lagoon effluent from a swine operation in Duplin County, North Carolina. It contained 233-mg NH<sub>4</sub>-N/L, 250-mg TKN/L, and 0 nitrate and nitrite. Other characteristics were 200-mg TSS/L, 150-mg BOD<sub>5</sub>/L, 1357-mg alkalinity/L, and a pH of 8.3.

### **Nitrification of Swine Wastewater using Batch Treatment**

Data in Fig. 1 identify inhibitory boundary conditions of NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> oxidation of swine wastewater by immobilized nitrifiers. During NH<sub>4</sub><sup>+</sup> oxidation, there is a release of hydrogen ions that decreases the pH to an extent related to the buffering capacity of the system. The alkalinity concentration of the wastewater (1357-mg CaCO<sub>3</sub>/L) was lower than the 1670 mg/L needed for complete oxidation of 233-mg NH<sub>4</sub>-N/L (assuming 7.14-mg CaCO<sub>3</sub>/mg NH<sub>4</sub>-N). As the NO<sub>2</sub>-N accumulated and the pH decreased during progression of nitrification, the free un-ionized nitrous acid (HNO<sub>2</sub>) increased to a value (0.2 mg/L) that inhibited NH<sub>4</sub><sup>+</sup> oxidation. Addition of NaOH pulses at 12-15 h relieved this inhibition, and NH<sub>4</sub><sup>+</sup> oxidation was completed. On the other hand, oxidation of NO<sub>2</sub>-N was inhibited during the first 9 h and during the pH adjustment period when un-ionized (free) NH<sub>3</sub> levels were higher than 1 mg/L. These values are consistent with the benchmark nitrification work of Anthonisen et al. (1976). Their studies showed boundary concentrations of 0.2 to 2.8 mg/L for free nitrous acid inhibiting NH<sub>4</sub><sup>+</sup> oxidation, and 0.1 to 1.0 mg/L for free ammonia affecting the oxidation of NO<sub>2</sub>-N.

Inhibition of NH<sub>4</sub><sup>+</sup> oxidation by free nitrous acid can be easily relieved with pH control. Such a system is shown in Fig. 2; swine wastewater was supplemented with a pH 8.5 CO<sub>3</sub><sup>=</sup>/HCO<sub>3</sub><sup>-</sup> buffer in order to add an extra 600 mg/L of alkalinity and meet H<sup>+</sup> demands of NH<sub>4</sub><sup>+</sup> oxidation. Under these conditions, NH<sub>4</sub><sup>+</sup> was completely oxidized in 14 h. But oxidation of NO<sub>2</sub>-N was still inhibited by high initial free NH<sub>3</sub> in the lagoon wastewater; therefore, a total of 24 h was needed for complete nitrification to NO<sub>3</sub>-N. This limitation is, however, an opportunity for bioengineering research through development of Nitrobacter strains adapted to higher levels of free NH<sub>3</sub>, such as those acclimated to nitrify under harsh saline environments with free NH<sub>3</sub> concentration of 10 to 20 ppm (Furukawa et al., 1993).



**Figure 1.** Nitrification of lagoon wastewater with immobilized nitrifiers, batch treatment. **A:** Nitrogen transformations during inhibited nitrification; ( $\blacklozenge$ )  $\text{NH}_4^+$ , ( $+$ )  $\text{NO}_2^-$ , ( $\bullet$ )  $\text{NO}_3^-$ , ( $\blacksquare$ )  $\text{NO}_2^-$  plus  $\text{NO}_3^-$ , and ( $-$ ) pH. **B:** Inhibitory boundary conditions of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation.

In the absence of enriched nitrifying populations, aerobic treatment of lagoons can potentially add to problems by stripping out ammonia. This problem is illustrated in Fig. 3, showing the nitrogen transformations in a control treatment that was conducted parallel to the experiment shown in Fig. 1. Nitrification of lagoon wastewater started at 10 d and 69% of  $\text{NH}_4\text{-N}$  was lost by ammonia volatilization. These results are not surprising because of the low number of nitrifying microorganisms usually found after anaerobic stabilization (Blouin et al., 1989).

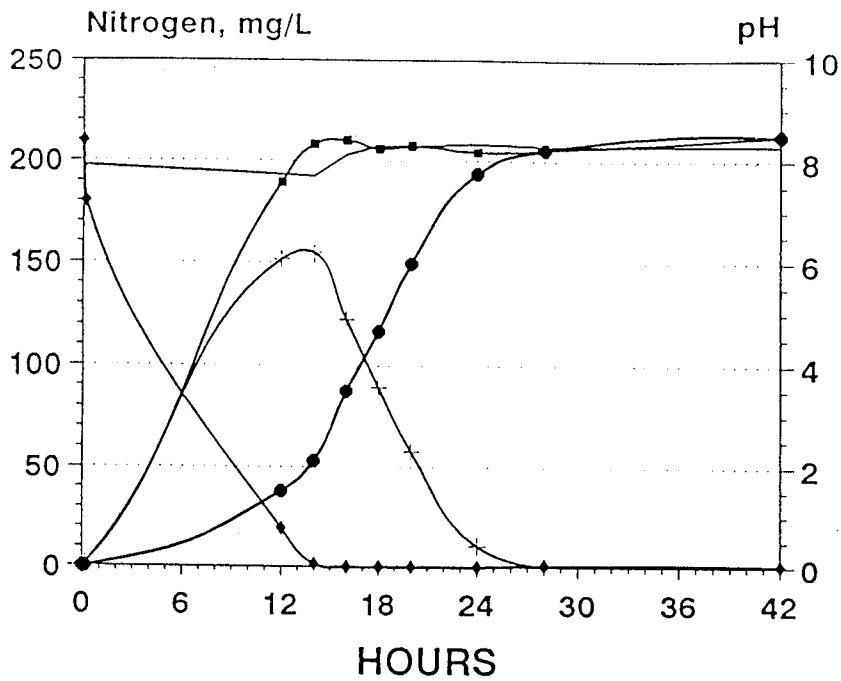


Figure 2. Nitrification of lagoon wastewater with immobilized nitrifiers in batch treatment using a  $\text{CO}_3^{2-}/\text{HCO}_3^-$  buffer for optimum process; ( $\blacklozenge$ )  $\text{NH}_4^+$ , (+)  $\text{NO}_2^-$ , ( $\bullet$ )  $\text{NO}_3^-$ , ( $\blacksquare$ )  $\text{NO}_2^-$  plus  $\text{NO}_3^-$ , and (-) pH.

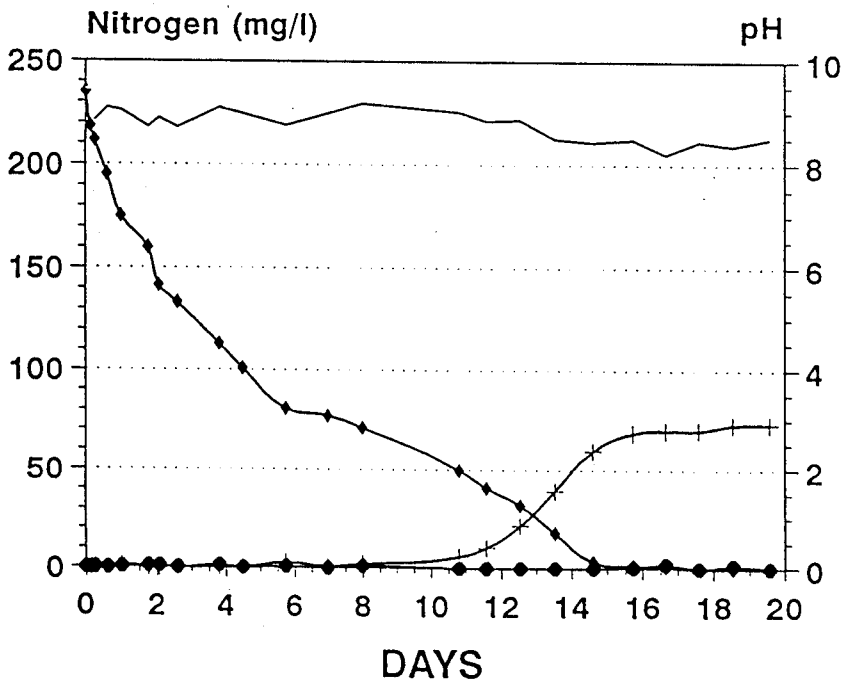


Figure 3. Nitrification of lagoon wastewater in a control batch treatment receiving only aeration, without immobilized nitrifiers or pH correction; ( $\blacklozenge$ )  $\text{NH}_4^+$ , (+)  $\text{NO}_2^-$ , ( $\bullet$ )  $\text{NO}_3^-$ , and (-) pH.

## Nitrification of Swine Wastewater using Continuous Flow Treatment

Ammonia removal potential of ANB pellets was also evaluated under continuous flow treatment. Ammonia loading rates were gradually increased from 227 to a maximum of 1287-g  $\text{NH}_4\text{-N}/\text{m}^3$  of aeration tank per day (corresponding from 1.48- to 8.40-mg  $\text{NH}_4\text{-N}/\text{g-pellet/d}$ , respectively). Loading rates were changed by decreasing the hydraulic residence time (HRT) from 24 h to 4 h. Alkalinity requirements were corrected by adding an extra 600-mg alkalinity/L to the influent swine wastewater using a pH 8.5  $\text{CO}_3^{2-}/\text{HCO}_3^-$  buffer. Pellets were retained inside the reactor with a 1-mm wedge-wire screen placed at the outflow. Other experimental conditions were similar to those described for the batch experiments.

**Table 1.** Treatment of lagoon wastewater with encapsulated nitrifiers under continuous flow.

HRT†	Ammonia Loading Rate	Ammonia Removal Rate‡	Nitrate + Nitrite Production Rate§	Nitrification Efficiency¶
hours	-----g N/m <sup>3</sup> reactor volume/day-----			%
24	227	223	240	100
20	260	254	279	100
16	326	311	327	100
12	418	363	397	95
8	637	402	417	65
6	884	498	499	56
4	1287	604	567	44

† Hydraulic residence time

‡ Ammonia removal rate = flow\*( $\text{NH}_4\text{-N}$  conc. inflow -  $\text{NH}_4\text{-N}$  conc. outflow)

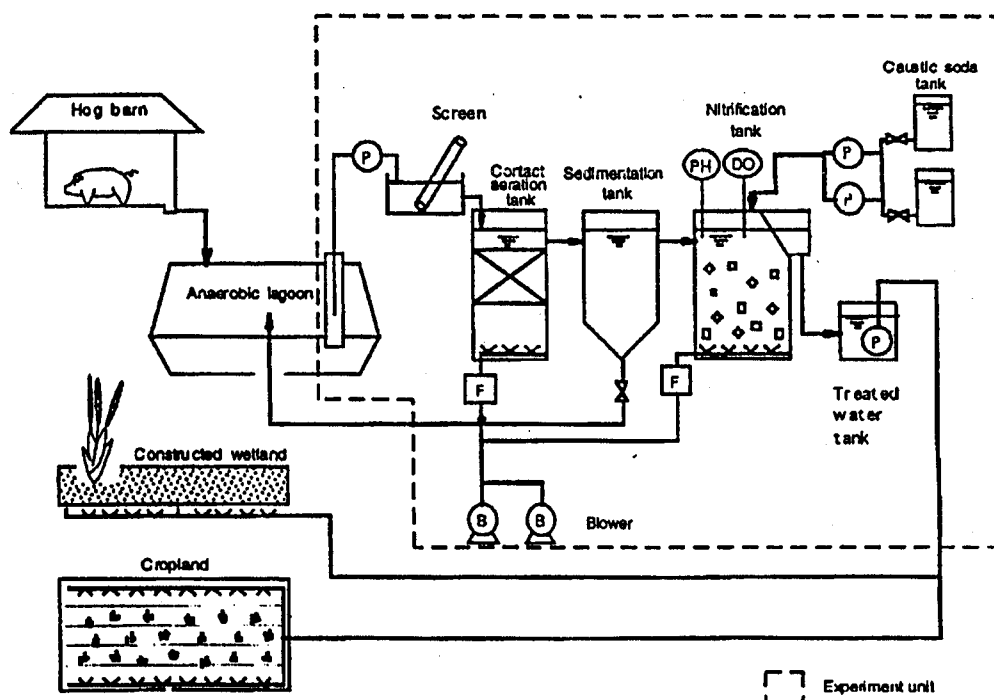
§  $\text{NO}_x\text{-N}$  production rate = flow\*( $\text{NO}_3\text{-N}$  +  $\text{NO}_2\text{-N}$  conc. outflow); Inflow  $\text{NO}_x$  concentration=0

¶ Nitrification efficiency = ( $\text{NO}_x\text{-N}$  conc. outflow/ $\text{NH}_4\text{-N}$  conc. inflow)\*100

Nitrification efficiencies of more than 90% were obtained with ammonia loading rates lower than 2.73-mg N/g-pellet/d and HRT higher than 12 h. Nitrification efficiencies decreased to 44% at the highest rate of 8.40-mg N/g-pellet/d (HRT = 4 h). All the ammonia-N removed was converted into nitrate and nitrite forms. Nitrate was predominant at HRT higher than 12 h, while equal amounts of nitrate and nitrite were produced at the highest load. Although higher loading rates resulted in lower nitrification treatment efficiencies, the total amount of  $\text{NO}_x\text{-N}$  produced was higher, with the maximum ammonia removal rate obtained with HRT of 4 h. Higher efficiencies may be useful for total systems designed to meet stream discharge requirements. However, if the objective is to remove large amounts of ammonia from the lagoon, then a retrofit nitrification unit operated at shorter retention times would be recommended. This strategy has the advantage of reducing the total cost of aeration per unit of nitrate-N produced.

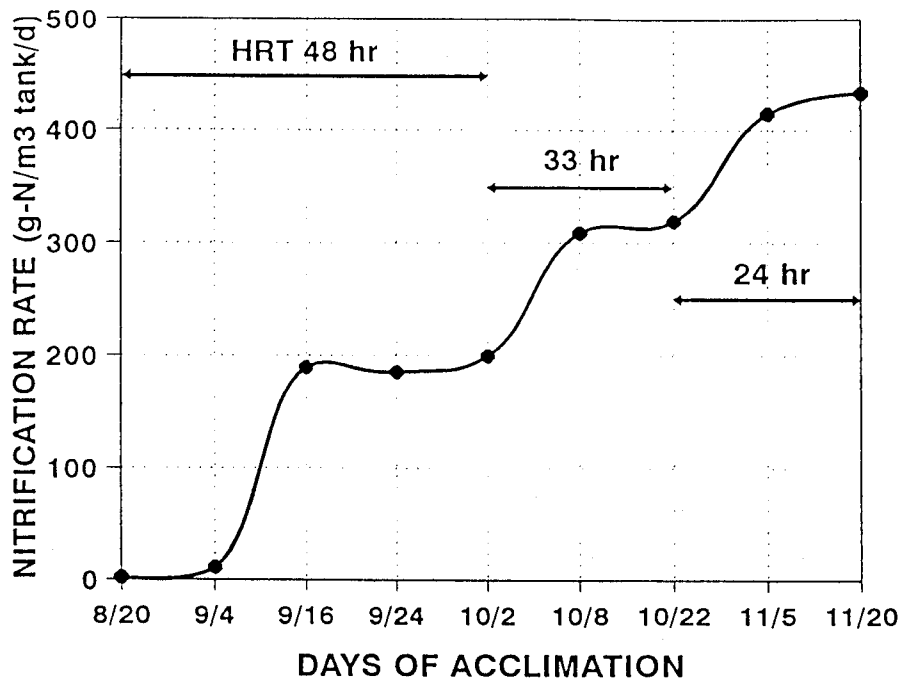
## PILOT EXPERIMENT

Results of bench scale research were used to design and construct a pilot plant for biological ammonia removal from swine lagoons. The unit was set up in a swine operation in Duplin Co., North Carolina. Flow diagram of the pilot plant is shown in Fig. 4. It consisted of a 0.34 m<sup>3</sup> contact aeration tank made of PVC crossflow media used to lower influent BOD, followed by a 0.18 m<sup>3</sup> sedimentation tank, and a 1.3 m<sup>3</sup> aerated fluidized tank for nitrification. A 1-mm wedge-wire screen was installed at the outflow of the nitrification tank to separate the pellets and activated sludge and retain the pellets inside the nitrification tank. Polyethylene glycol (PEG) pellet cubes (3- to 4-mm size) were added in the nitrification tank at 10% of the effective tank volume. The PEG pellets were produced in Japan by the Hitachi Plant Engineering Company using a sheet polymerization technique (Aoki et al., 1989), and contained 2% activated municipal sludge. The pellets were transported to the field site immediately after production for acclimation to swine wastewater. Air was provided to the bottom of both aeration tanks with a compressor and fine air diffusers (8.6% efficiency). Air flow rate of 50 L/min was applied to the contact aeration tank and 80 L/min to the nitrification tank. This ensured appropriate fluidization of the pellets and maintained DO concentration of the mixed liquor at more than 3 mg/L. The unit was completed with a pH controller and chemical tank used to keep the process pH in the nitrification tank above 7.8 through NaOH injections.



**Figure 4.** Prototype plant set up in a swine operation in Duplin Co., North Carolina for nitrification treatment of swine wastewater.





**Figure 5.** Field acclimation of PEG pellets to lagoon swine wastewater with decreasing hydraulic residence times (HRT).

The difference between the PVA pellets used in the bench experiments and the PEG pellets used in the pilot study is that the PVA pellets were prepared using an active culture of swine wastewater nitrifying bacteria (ANB), which were fully functional after only two days of recovery treatment following immobilization (Vanotti and Hunt, 1998). Instead, the PEG pellets were produced with activated sludge from a municipal wastewater treatment plant. Therefore, we conducted a procedure to adapt the nitrifiers for swine wastewater treatment. The pellets were successfully acclimated to lagoon effluent during a 3-month period in which the ammonia loading rate was increased by decreasing the hydraulic residence time (HRT). At the initial 48 h HRT, nitrification activity of pellets increased from 21 to 200 g N/m<sup>3</sup> tank/d (0.02 to 2.0 g N/L-pellet/d) in about 30 d (Fig. 5). Nitrification activity further increased to 319 and 433 g N/m<sup>3</sup> tank/d (3.2 to 4.3 g N/L-pellet/d) at HRT's of 32 and 24 h, respectively.

These results indicate that high nitrification rates of swine wastewater can be attained using enriched nitrifying populations immobilized in polymer resins. This is an attractive approach to biological ammonia removal because the capacity of the nitrification tank can be increased by increasing the nitrifiers' population independent from the wastewater retention time. Higher ammonia removal rates are critical for development of nitrification units to treat animal waste because aeration cost is often a limiting factor.

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