# Biodenitrification in Sequencing Batch Reactors

**Final Report**

**Los Alamos National Laboratory Subcontract No. 2554K0014-3Y**

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**January 23, 1996**

# REPORT
# DEPARTMENT OF ENERGY/LOS ALAMOS NATIONAL LABORATORY
# SPONSORED RESEARCH PROJECT

## BIODENITRIFICATION IN SEQUENCING BATCH REACTORS

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ABSTRACT

**Objectives.** One plan for stabilization of the Solar Pond waters and sludges at Rocky Flats Plant (RFP), is evaporation and cement solidification of the salts to stabilize heavy metals and radionuclides for land disposal as low-level mixed waste. It has been reported that nitrate ($NO_3^-$) salts may interfere with cement stabilization of heavy metals and radionuclides. Therefore, biological nitrate removal (denitrification) may be an important pretreatment for the Solar Pond wastewaters at RFP, improving the stability of the cement final waste form, reducing the requirement for cement (or pozzolan) additives and reducing the volume of cemented low-level mixed waste requiring ultimate disposal. A laboratory investigation of the performance of the Sequencing Batch Reactor (SBR) activated sludge process developed for nitrate removal from a synthetic brine typical of the high-nitrate and high-salinity wastewaters in the Solar Ponds at Rocky Flats Plant was carried out at the Environmental Engineering labs at the University of Colorado, Boulder, between May 1, 1994 and October 1, 1995.

**Approach and Tasks.** Experiments were conducted in bench-scale SBRs, with a 30-liter working volume to determine the importance of control of the mixed liquor pH during the biological denitrification reaction. Also smaller-scale flask experiments were done to determine the effect of the intermediate metabolite, nitrite ($NO_2^-$), on denitrification at varying mixed liquor pH values. Finally, flask experiments with SBR-adapted activated sludge were conducted to determine the effect of salinity on denitrification activity in activated sludge. These latter experiments included a study of the bacterial species active in varying salinity conditions in the activated sludge mixed liquor.

**Results.** In the bench-scale SBR tests, it was found that Brines with nitrate concentration of 2,700 mg/l nitrate-nitrogen, or $NO_3$-N (12,000 mg/l as $NO_3$) could be denitrified by activated sludge in a Sequencing Batch Reactor. The denitrification reaction proceeded more quickly if the mixed liquor pH was controlled at 8.5, compared with the lower pH value which had been used in previous investigation. From associated flask activated sludge tests, it was concluded that the higher pH condition was more effective for denitrification because the toxicity of the intermediate metabolite,

nitrite ($NO_2^-$), was reduced, probably by decreasing the amount of undissociated $HNO_2$, which has been reported to be a strong bacterial inhibitor by other investigators. We also found that the SBR would support a biomass density as high as 18,000 mg/l mixed liquor suspended solids, or MLSS, (1.8% dry weight) because of a novel mechanical mixing system for the SBRs. In previous research we had found that a non-denitrifying activated sludge from a local municipal wastewater treatment plant could be adapted to denitrify wastewaters as high as 1,350 mg/l $NO_3$-N and an ionic strength of 0.17 (approximately 10,000 mg/l total dissolved solids, or TDS, which is equivalent to 1% TDS, by weight). The results of the salinity experiments with activated sludge reported here are that a non-denitrifying activated sludge from a wastewater treatment plant with relatively low ionic strength could be adapted within 1 to 2 days to denitrify wastewater brines with an ionic strength as high as 1.5 (approximately 100,000 mg/l, or 10%, TDS). Furthermore, within 14 days the activated sludge could be adapted to denitrify a brine with ionic strength of 3.0 (approximately 200,000 mg/l, of 20%, TDS).

**Deliverables.** Rocky Flats Environmental Technology Site (RFETS) and the Department of Energy have access to the benefits below.

1. A Sequencing Batch Reactor process was developed which can be scaled up for on-site treatment of high-nitrate, high-salinity wastes at the Rocky Flats site (and other sites appropriate within the DOE facilities).
2. The SBR process can be operated optimally be means of pH control, which can be implemented at full scale using commercially-available sensing and control equipment
3. The SBR denitrification process uses ordinary municipal activated sludge as an inoculum. Adaptation of a salinity tolerant (halotolerant) bacterial culture from municipal activated sludge is rapid and repeatable, so the SBR process may be initiated easily and a stable biomass maintained throughout operation.

OBJECTIVES, TASKS AND ACCOMPLISHMENTS

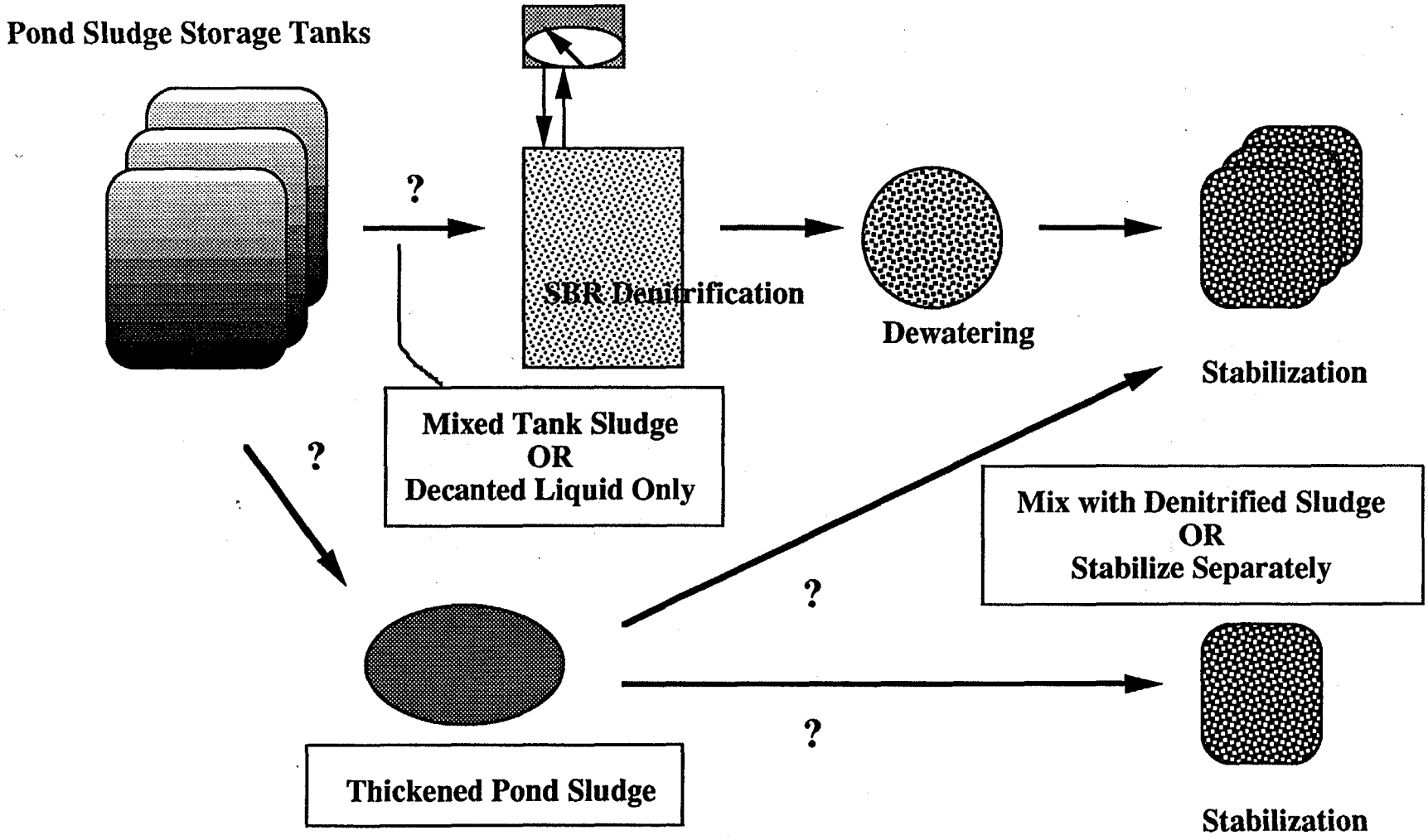
The goal of this research was to develop a biological treatment process for removing nitrates from high-nitrate, high-salinity brines at the Rocky Flats Plant site (now RFETS). Figure 1 is a process flow chart showing how biological denitrification would be used in this way. A brief review of the relevant scientific and technical literature is presented in the Background section below to motivate the research objectives, tasks performed and accomplishments.

**Background.** It has been reported that nitrate salts can interfere with the stability of cemented hazardous wastes (Napier, 1989). With a significant reduction in nitrate ($NO_3^-$), evaporated liquid wastes and sludges can be solidified at higher waste-to-cement ratios and still stabilize hazardous waste compounds, especially heavy metals, for mixed waste disposal in landfills (Cook, 1995). Higher waste-to-cement ratios mean lower addition of additives cement, lime and pozzolans, and, probably more important, means that the final volume of waste requiring disposal is reduced.

*Nitrate/Nitrite Inhibition.* It has been reported that high nitrate wastes can be biologically denitrified, resulting in reduction of $NO_3^-$ to harmless $N_2$ gas. In the process an organic carbon source must be added for the denitrifying bacteria which produce cells and mineral end products: $CO_2$ and $H_2O$ (Francis and Callahan, 1975; Francis and Mankin, 1977; Abeling and Siefried, 1992; Cook, et al., 1993). However, it also has been reported that very high concentrations of nitrate can inhibit biological denitrification (Abeling and Siefried, 1992; Veydovec, et al., 1994). Most investigators have found that nitrate concentrations in excess of 1,000 to 1,500 mg/l nitrate-nitrogen ($NO_3$-N) either partially or completely inhibit biological denitrification. Several researchers have suggested that it is the accumulation of the denitrification intermediate metabolite, nitrite ($NO_2^-$), which inhibits denitrification in high nitrate environments. Particularly it is the undissociated acid species, nitrous acid ($HNO_2$) has been reported to be inhibitory at concentrations as low as 0.13 mg/l $HNO_2$-N (Abeling and Siefried, 1992). Nitrous acid is a weak acid, with an log acid dissociation constant ($pK_a$) equal to 3.4 at 12° C. (ACS, 1975) This means that at neutral pH (7.0) and 1,000 mg/l $NO_2$-N, there will be approximately 0.3 mg/l $HNO_2$-N, greater than the reported inhibitory concentration.

*Salinity.* In general, bacterial growth can be determined by the salinity of the environment. Truper and Galinski (1986) have classified bacteria by their ability to grow in increasingly saline waters: non-halophilic bacteria tolerate 0 to 1.5% NaCl; marine bacteria, 1.5 to 6%; moderately halophilic, 3 to 15%; halophilic *sensu stricto*, 9 to 24%; and extreme halophiles, 18 to 30%. Although the later organisms have been found in highly specialized environments like salt ponds, Kushner reported that halo-tolerant bacteria were isolated from domestic activated sludge that could grow on as much as 15% NaCl.

# Figure 1




**Pond Sludge Denitrification/StabilizationTreatment Process Schematic**

*Bacterial Adaptation.* Researchers have found that bacteria can adapt to high-salinity environments, by either changing their cell membrane lipid composition to resist increasing osmotic pressure in saline environments or by increasing the ionic composition of their cytoplasm contents (Vreeland, 1987; Kogut, 1991).

In summary, biological denitrification of very high nitrate wastewaters has been found to be problematic, possibly due to the presence of the metabolic intermediate, undissociated nitrous acid. In addition, high salinity may restrict bacterial growth in some waters. In order to develop an activated sludge biological denitrification process for RFETS wastewaters, it was necessary to investigate the effects of nitrate, nitrite and dissolved salt concentrations on adaptation and growth of the denitrifying bacteria.

## Investigation of Nitrate Strength, Effect of Nitrites and pH

### Objectives.

- Develop a Sequencing Batch Reactor (SBR) process to study activated sludge denitrification and to provide the basis for future scale-up for application at RFETS.
- Investigate the mechanism of high-nitrate inhibition of activated sludge.
- Determine the water quality factors which allow for denitrification of maximum concentration of nitrate in wastewater, especially pH.

### Tasks and Results.

Individual activities and the associated results for each activity have been reported together, to maintain logical continuity of the report.

TASK Develop a synthetic wastewater brine for use in the laboratory denitrification experiments that simulates the Solar Pond water in Pond 207C at RFETS. Pond 207C was chosen because that water has the highest concentration of both nitrate and total dissolved solids (TDS) of the ponds.

RESULT The composition of the synthetic wastewater brine used for the SBR experiments was selected based on the major inorganic ions reported in the Pond 207C water characterization. Table 1 shows the brine developed for the SBR experiments compared with average water characteristics from Solar Pond 207C at RFETS.

Table 1: SBR and Pond 207C Wastewater Characteristics

| Ion | SBR Waste (mg/l) | Pond 207C (mg/l) |
|---|---|---|
| $NO_3^-$ | 60,200 | 61,500 |
| $Na^+$ | 90,620 | 122,000 |
| $HCO_3^-$ | 57,300 | 28,000 |
| $CO_3^{2-}$ | 0 | 28,000 |
| $Cl^-$ | 120,700 | 21,650 |
| $K^+$ | 46,800 | 37,250 |
| $SO_4^{2-}$ | 12,500 | 9,600 |
| $PO_4^{3-}$ | 270 | 0 |
| % salt | 20 | 30 - 50* |
| ionic strength | 3 | 4 - 7.5 |
| pH | 8.0 - 8.3 | 10 - 11.5 |

* average specific ion concentrations do not support the high reported salinity

The differences between the chemical composition of the synthetic wastewater brine and the reported characteristics for Pond 207C water stems from the pH difference. For biological treatment to occur, the high Pond water pH (> 10) was lowered with hydrochloric acid (HCl), resulting in higher concentration of chloride ($Cl^-$) by a factor of over 5. The lower concentration of carbonate ($CO_3^{2-}$) in the synthetic brine that resulted from the pH shift was balanced by a higher concentration of the bicarbonate species ($HCO_3^-$).

TASK Design a bench-scale Sequencing Batch Reactor activated sludge bio-reactor for denitrification of high-nitrate and high-salinity wastewater which can readily be scaled up for application at RFETS.

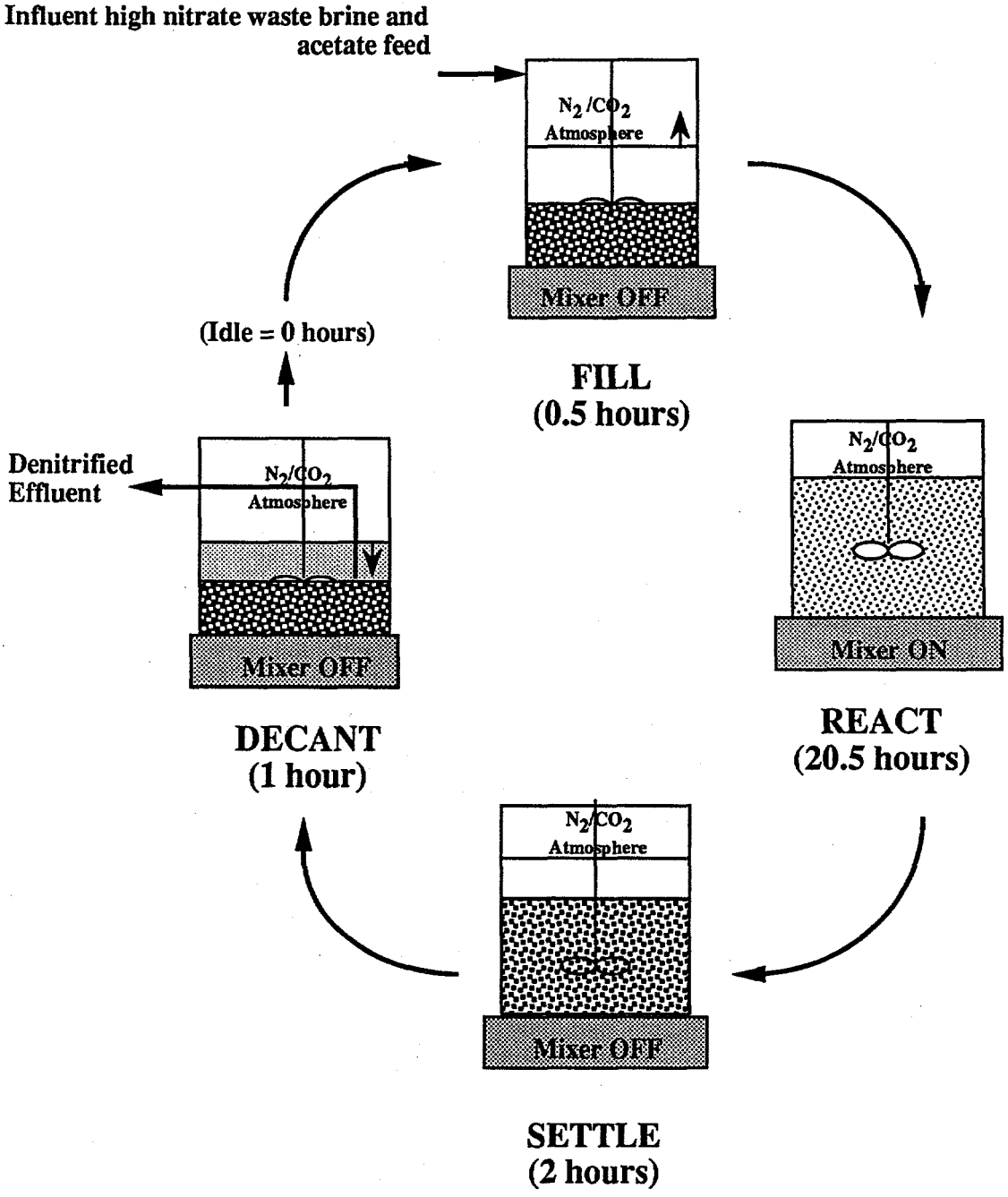
RESULT Figure 2 is a process flow diagram for the Sequencing Batch Reactor Process as developed for denitrification of high-nitrate wastewater. The SBR process is operated on a 24 hour cycle, including 0.5-hour for filling, 20.5 hours for the denitrification reaction, 2 hours for settling the activated sludge biomass and 1 hour for decanting the treated wastewater. The

Influent volume is 15 liters per cycle, with 15 liters containing concentrated activated sludge recycled.

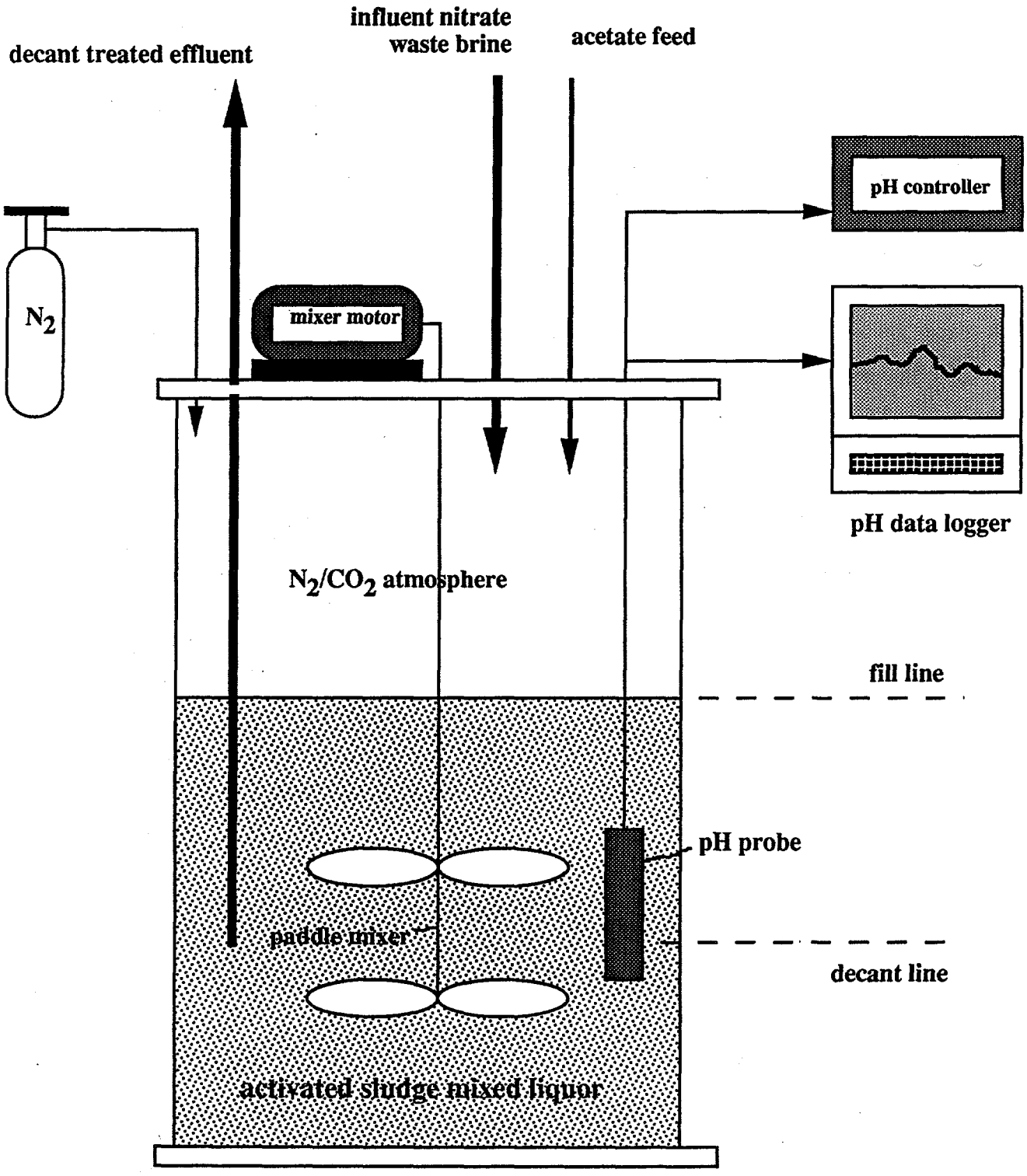
Figure 3 is a schematic of the bench-scale SBR used in the experiments. The reactor is made from acrylic tubing 35.6 cm. (14 in.) in diameter and 76.2 cm. (30 in.) high. The working volume of the reactor is 30 liters (8 gallons). The reactor is sealed and the head space atmosphere is a combination of $N_2$ gas, from a combination of an exterior source and biological activity and $CO_2$ gas from biological activity. A small amount of nitrogen gas is added continuously to insure anoxic conditions required for biological denitrification. The activated sludge suspension is mixed by an array of two paddles with the vanes oriented in opposite directions to maximize turbulence at low speed. The paddle shaft is turned at 40 - 50 rpm by an electric motor. Waste brine and acetate carbon feed are pumped through the top of the reactor and treated effluent is decanted by a siphon tube extending to the decant line. The pH of the SBR mixed liquor is controlled by a pH probe (Broadley-James) held in a rigid stainless steel shaft, connected to a proportional pH controller (Cole Parmer Model 5656-00). pH probe voltage signals are converted to digital data using a data logger (Fluke Hydra) and the pH data and then the data are stored on a computer (Zenith 386 Model).

For the experiments, two SBRs were operated in parallel. Figure 4 is a schematic of the entire SBR system, including feed tanks and pumps. The SBR system is controlled by programmable timers (Chrontrol).

# Figure 2

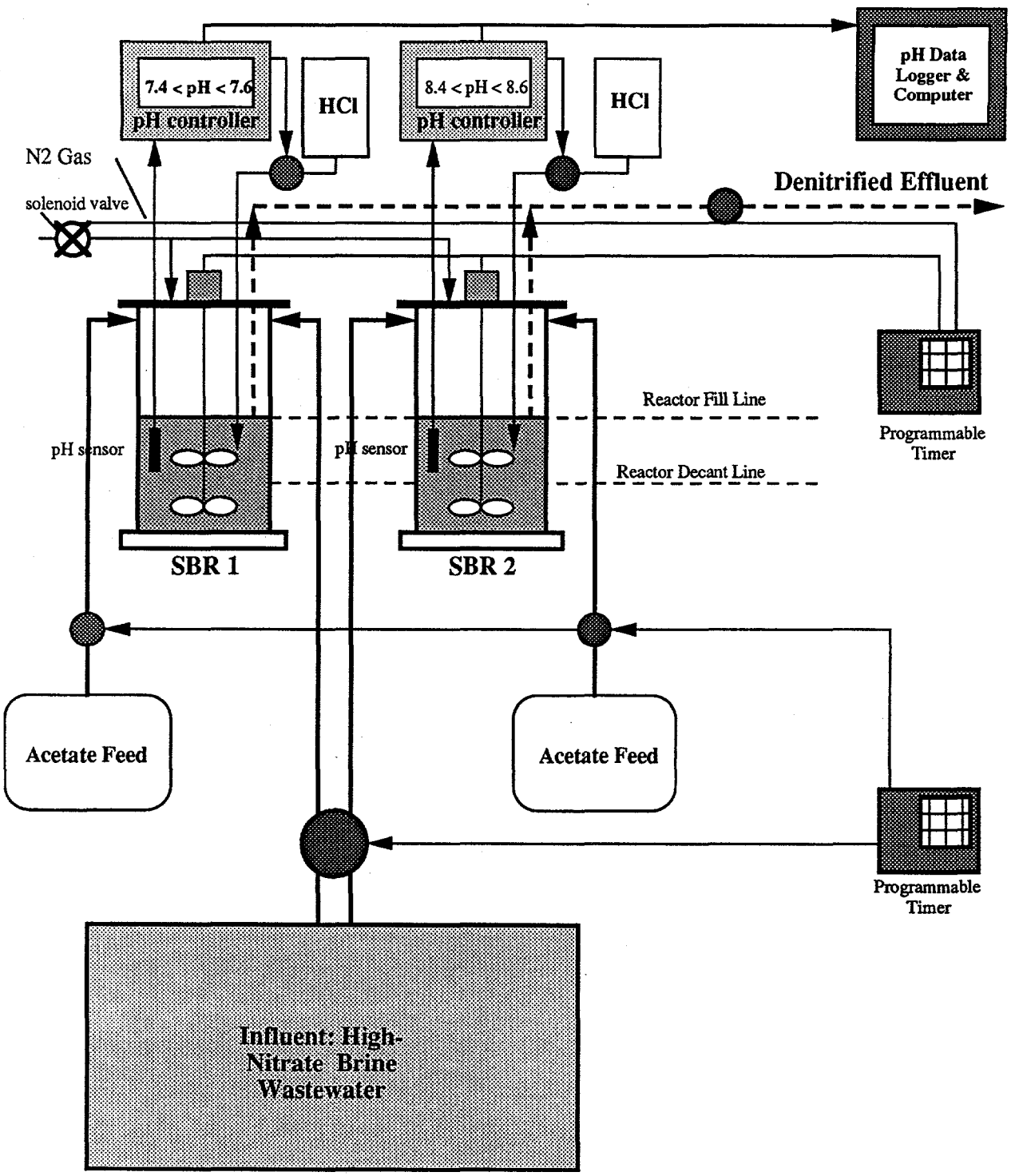



**SBR Denitrification Process Cycle for pH Control Experiments**




# Figure 3

## Bench-Scale SBR Schematic




nitrate waste brine influent, acetate feed, HCl pH control and effluent pumps

# Figure 4

## Bench-Scale SBR Denitrification System with pH Control

TASK Investigate nitrate inhibition of denitrification at wastewater concentrations ranging from 1,350 mg/l to 5,400 mg/l $NO_3$-N. The range was selected because the wastewaters at the lower end have been reported to be susceptible to denitrification, but also to occasionally be inhibitory. The high extreme of nitrate concentration, 5,400 mg/l $NO_3$-N, is well outside the reported feasibility range for denitrification, but is close to the ambient nitrate concentration in Pond 207C. Therefore that value provides a meaningful upper bound for the experiments.

The biological denitrification reaction tends to increase water pH. Research was done previously that indicated that activated sludge denitrification in an SBR was more stable if the pH was controlled with a range of 7.4 to 8. However, it was also noted that even at pH values as high as 9.6, denitrification occurred rapidly for weeks. That is high pH values were not shown to have an inhibitory effect. For these experiments each of the two SBRs was controlled at a single pH value, pH = 7.5 in SBR 1 and 8.5 in SBR 2. pH control was effected by addition of hydrochloric acid whenever the reactor pH rose above the set point due to the denitrification reaction. Acid addition stopped as soon as the mixed liquor pH dropped back to the set pH value.

Table 2 shows the experiments to test the effect of wastewater nitrate concentration and controlled pH on denitrification. In order to control for adaptation of the biomass, the SBRs were inoculated with activated sludge from a local municipal wastewater treatment plant (Broomfield, Colorado) where there was no denitrification in the treatment process at the beginning of each experiment. Thus each experiment started with an un-acclimated activated sludge biomass.

Table 2. Bench-Scale SBR Denitrification Experiments

| | SBR 1<br>pH = 7.5 | SBR 2<br>pH = 8.5 |
|---|---|---|
| Experiment 1 | | |
| $NO_3$-N (mg/l) | 1,350 | 1,350 |
| ionic strength | 0.6 | 0.6 |
| Experiment 2 | | |
| $NO_3$-N (mg/l) | 5,400 | 5,400 |
| ionic strength | 2.4 | 2.4 |
| Experiment 3 | | |
| $NO_3$-N (mg/l) | 2,700 | 2,700 |
| ionic strength | 1.2 | 1.2 |

RESULTS

Figure 5 shows the SBR mixed liquor pH profile during the denitrification reaction for SBRs 1 and 2, with pH set points 7.5 and 8.5, respectively during experiment 1. Figures 6 and 7 are profiles of nitrate ($NO_3$-N), nitrite ($NO_2$-N) and acetate-carbon during the first 10 hours of the 20.5-hour reaction period. Note that all the nitrate and nitrite are consumed after approximately 7 hours reaction. There was no significant difference in the rate of nitrate/nitrite disappearance for the wastewater with nitrate concentration of 1,350 mg/l $NO_3$-N. Biomass density, as mixed liquor suspended solids (MLSS) was similar for the two SBRs: 3,600 mg/l in SBR 1 and 4,500 mg/l in SBR 2.

In experiment 2, wastewater with an extremely high nitrate concentration, 5,400 mg/l $NO_3$-N, was fed to the SBRs. Little denitrification was found to occur in either reactor., as is shown in Figures 8 and 9, which are nitrate and nitrite-nitrogen and acetate-carbon profiles during reactions in SBRs 1 and 2 respectively. By day 7 of experiment 2, effluent suspended solids had risen from approximately 700 mg/l to over 3,000 mg/l, equal to the mixed liquor biomass concentration. This indicated that the activated sludge biomass was washing out of the reactor, which correlated with the chemical data that no significant denitrification was occurring.

At the beginning of experiment 3, no denitrification was observed in the newly inoculated reactors, as shown in the profiles for day 2 or the experiment in Figures 10 and 11 for SBRs 1 and 2 respectively. By day 10, SBR 1, with pH controlled at the lower value of 7.5 still was not denitrifying, as shown in Figure 12. However SBR 2 was completely denitrifying the influent nitrate and intermediate metabolite nitrite after approximately 6 hours, as shown in Figure 13. This may be explained by the fact that although both SBRs were inoculated with 3,000 mg/l MLSS, by day 5, solids were washing out of SBR 1, but not SBR 2. As a result MLSS was significantly lower SBR 1: 1,700 mg/l versus 4,400 mg/l in SBR 2. By day 30 of experiment 3, both SBRs were completely denitrifying all influent nitrate. SBR 1, at pH = 7.5, completed the reaction in approximately 8 hours, as shown in Figure 14; while in SBR 2 denitrification was complete in less than 2.5 hours, as is shown in Figure 15. The gap between reactor MLSS concentrations had narrowed with 9,350 mg/l MLSS in SBR 1 and 16,511 mg/l MLSS in SBR 2. It was found that the difference in biomass concentration did not account for the faster denitrification in SBR 2, with the pH controlled at 8.5. Also of interest is the fact that substantial nitrite accumulated in SBR 2, over 500 mg/l $NO_2$-N, although nitrite accumulation at the higher pH did not appear to inhibit denitrification. However no nitrite accumulation was observed in SBR 2 with lower pH (7.5). Possibly two different populations of denitrifying bacteria existed in the two SBR activated sludges. In SBR 1 the biomass slowly reduces nitrate, perhaps in a single cell. On the other hand, two populations of bacteria may coexist in the activated sludge in SBR 2: one rapidly reducing nitrate to nitrite which is

excreted and reduced by a different population of denitrifying bacteria. Apparently the result is a more efficient 2-step reaction. However, it was thought that the higher pH value was necessary in order to adapt to nitrite accumulation without inhibition. To investigate that, a series of flask experiments was conducted under more controlled conditions.

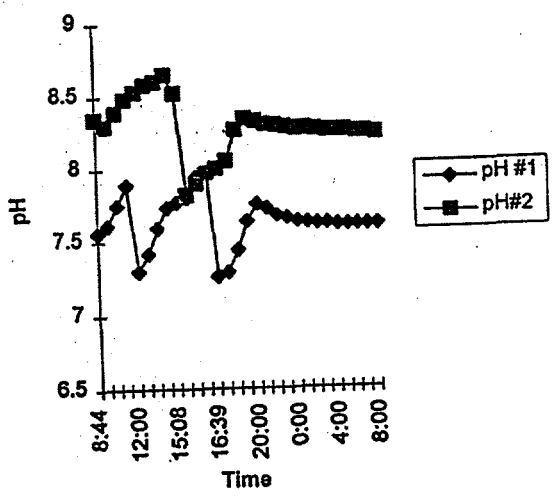



**Figure 5: typical; pH profiles for SBRs 1 and 2 during experiment 1**

**Figure 6** Exp. 1 Day 40, pH 7.5

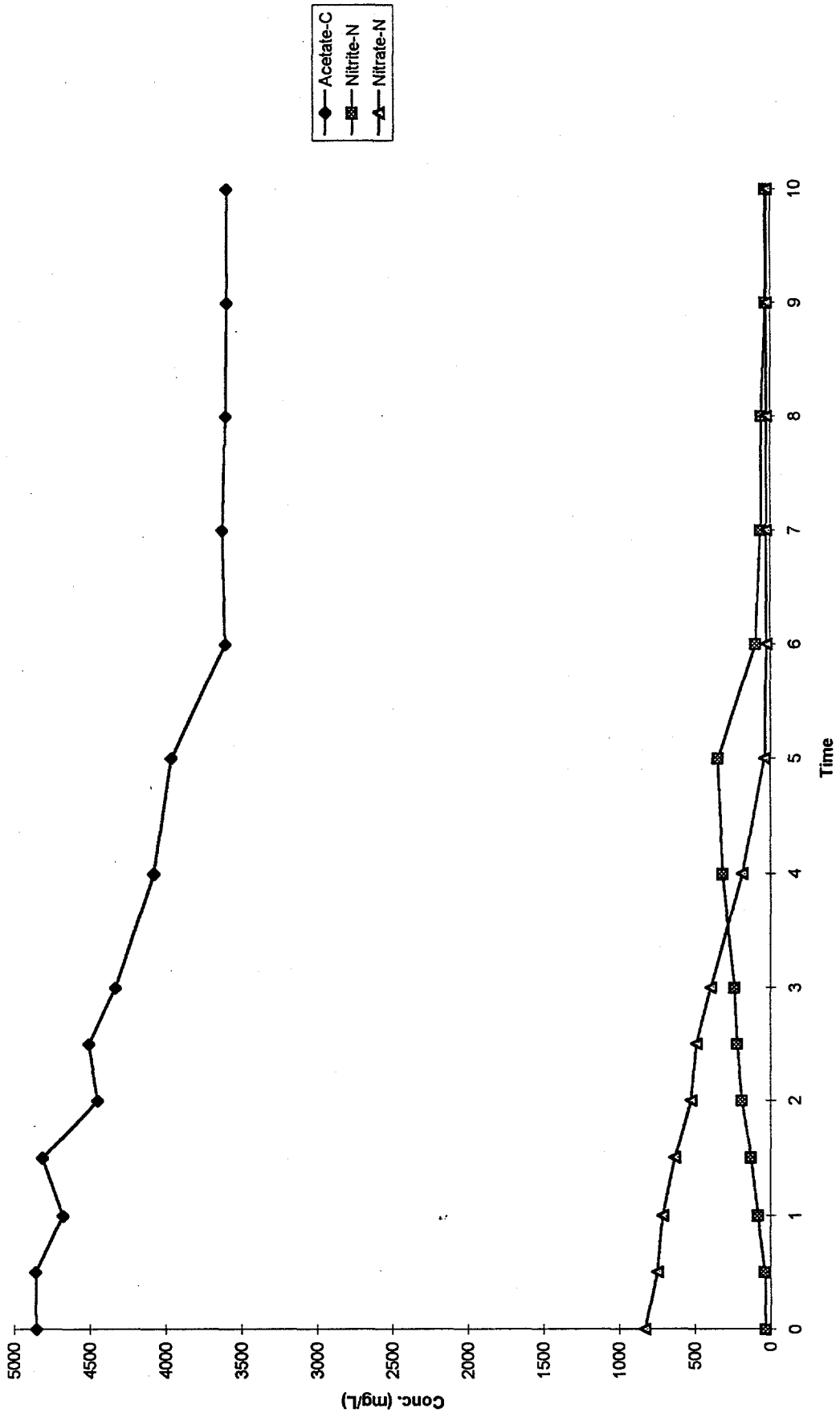


**Figure 7** Exp. 1 Day 40, pH 8.5

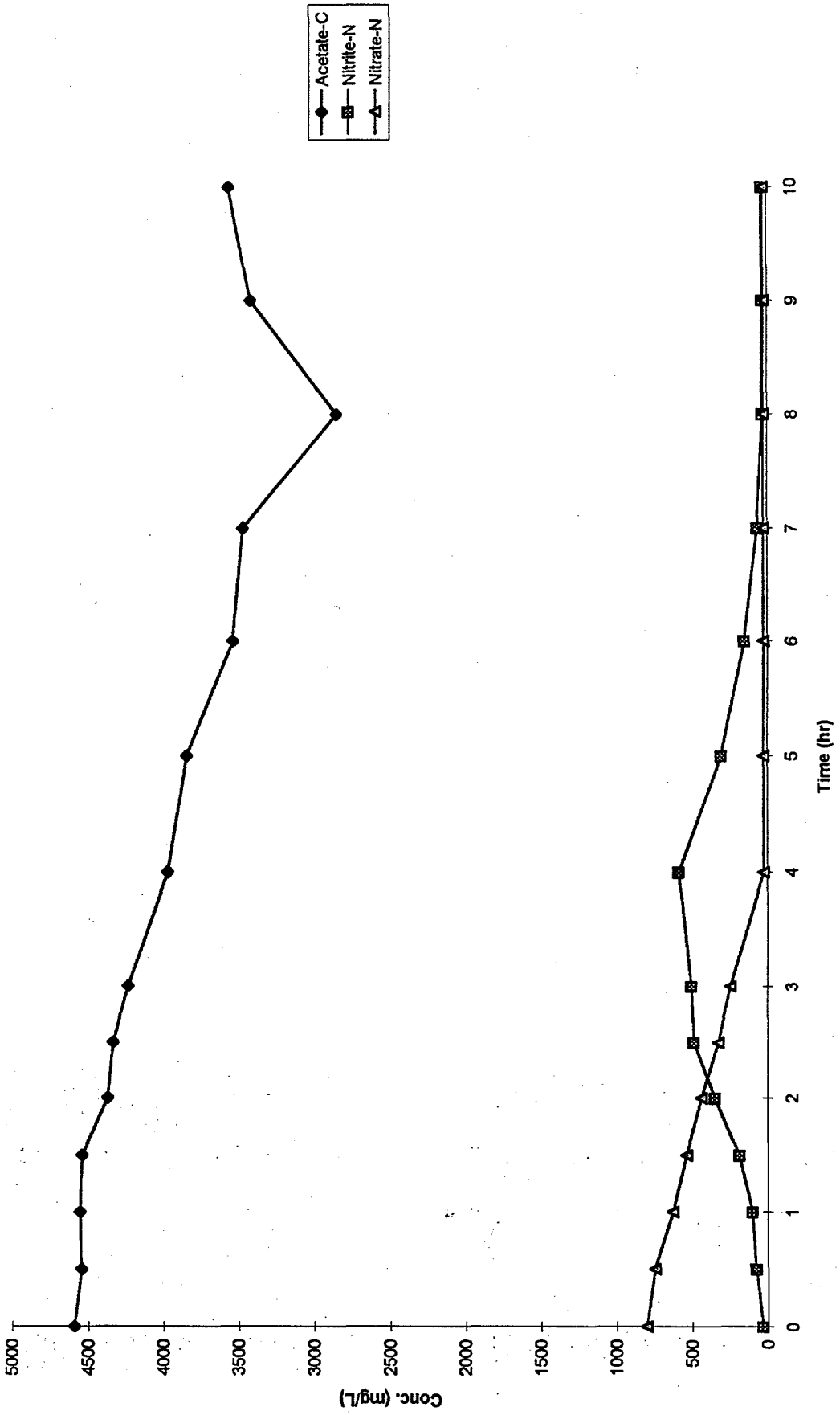


**Figure 8** Exp. 2 Day 3, pH 7.5

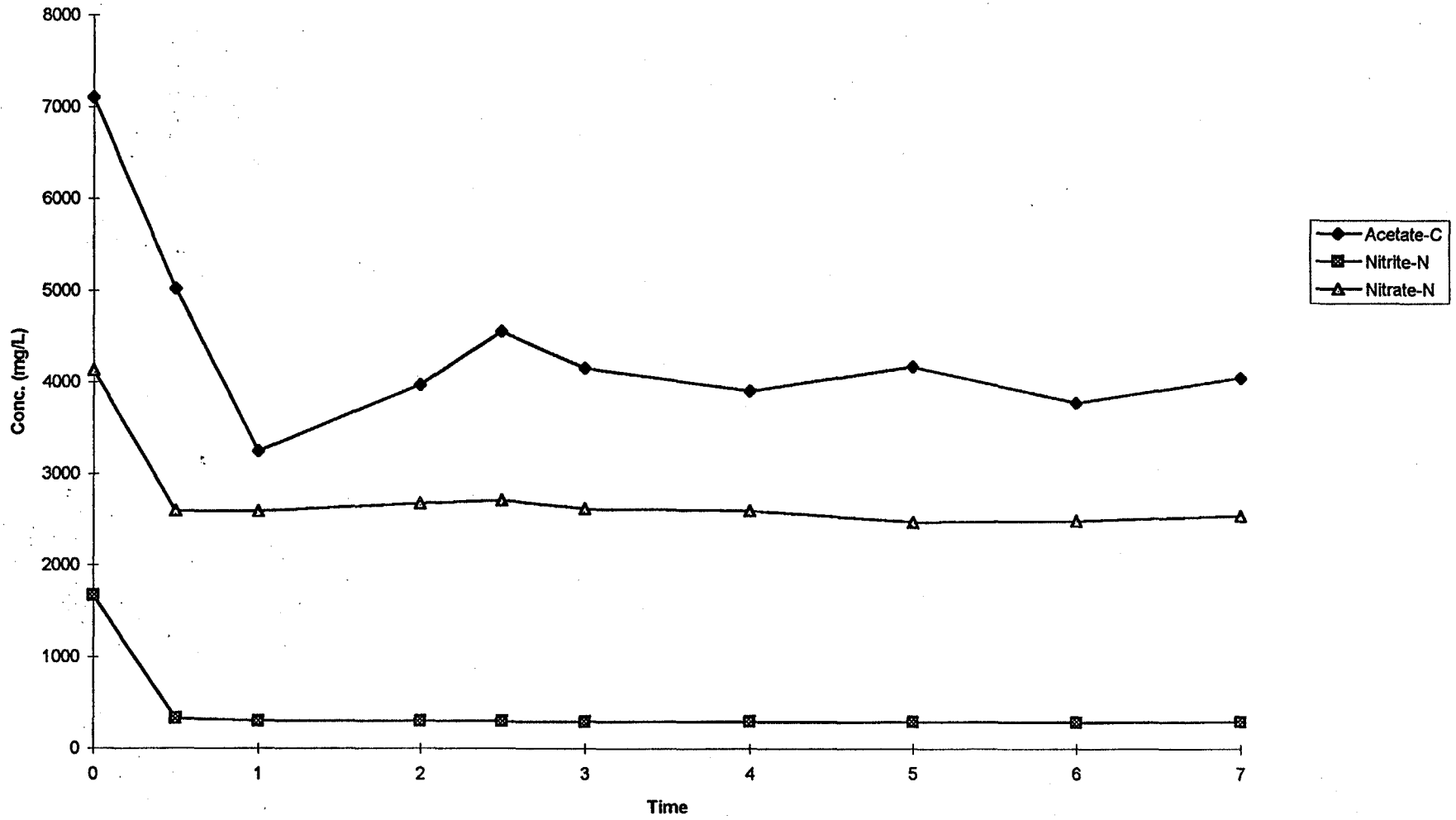


**Figure 9** Exp. 2 Day 3, pH 8.5

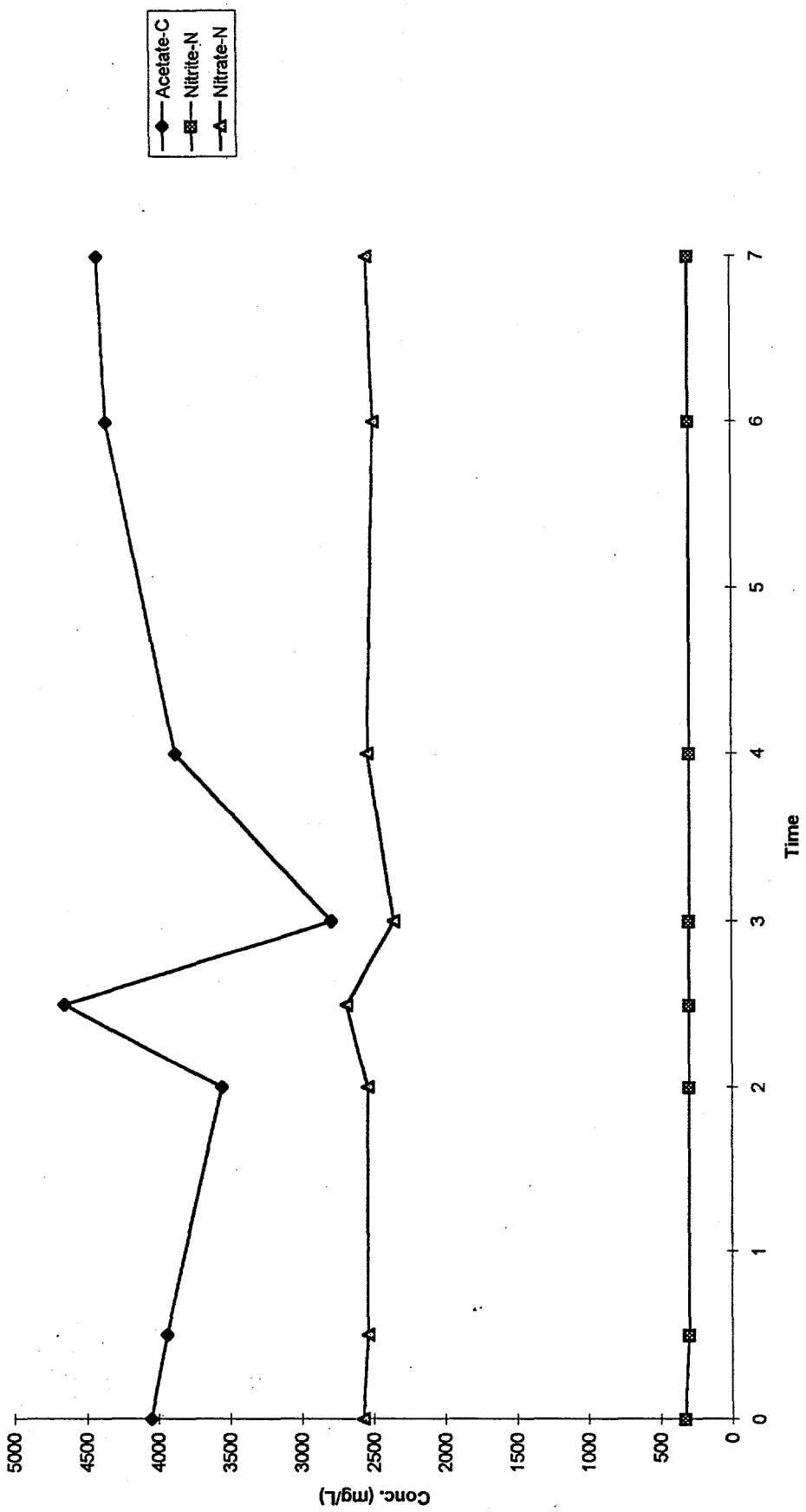


**Figure 10** Exp. 3 Day 2, pH 7.5

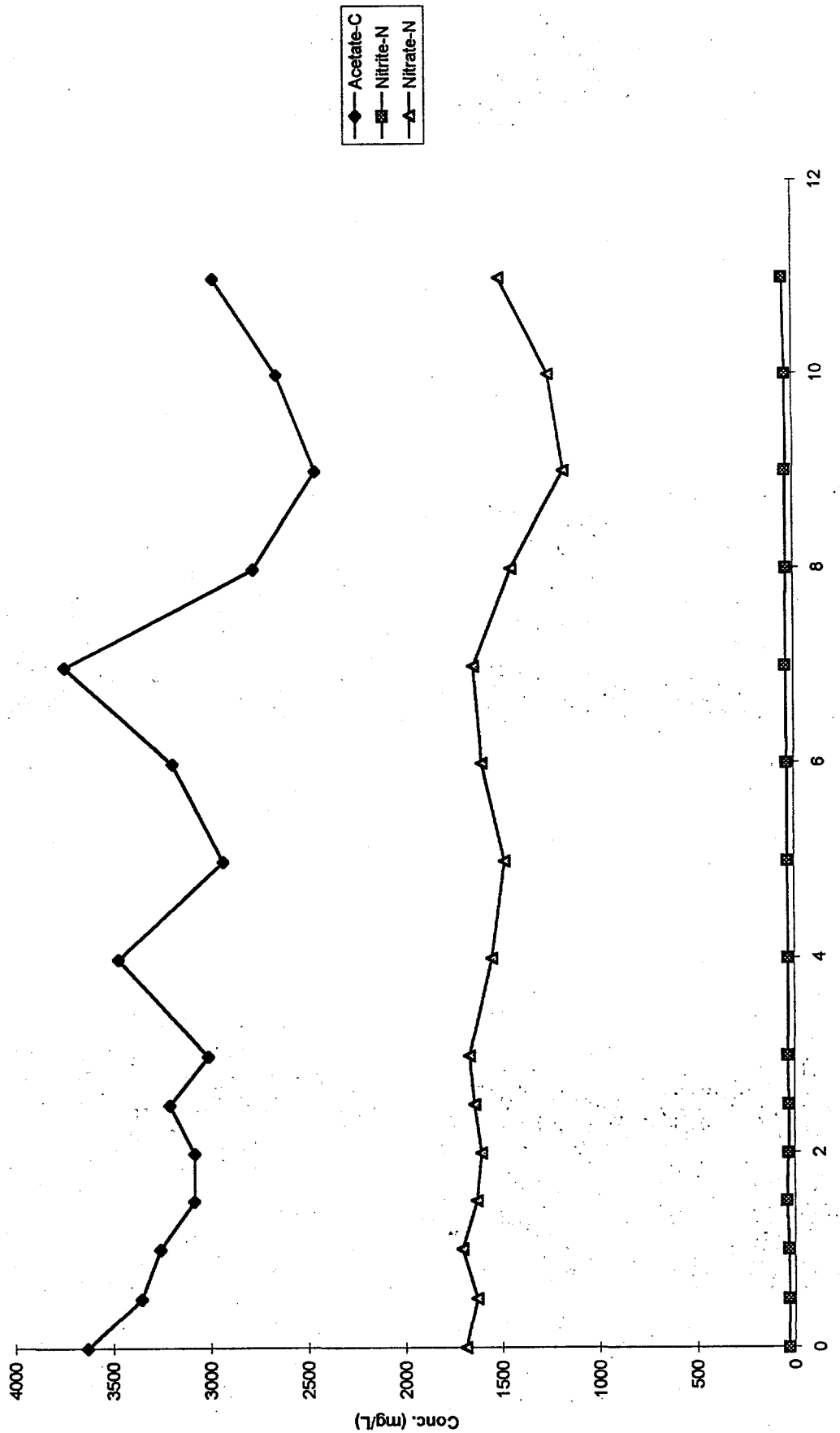


**Figure 11** Exp. 3 Day 2, pH 8.5

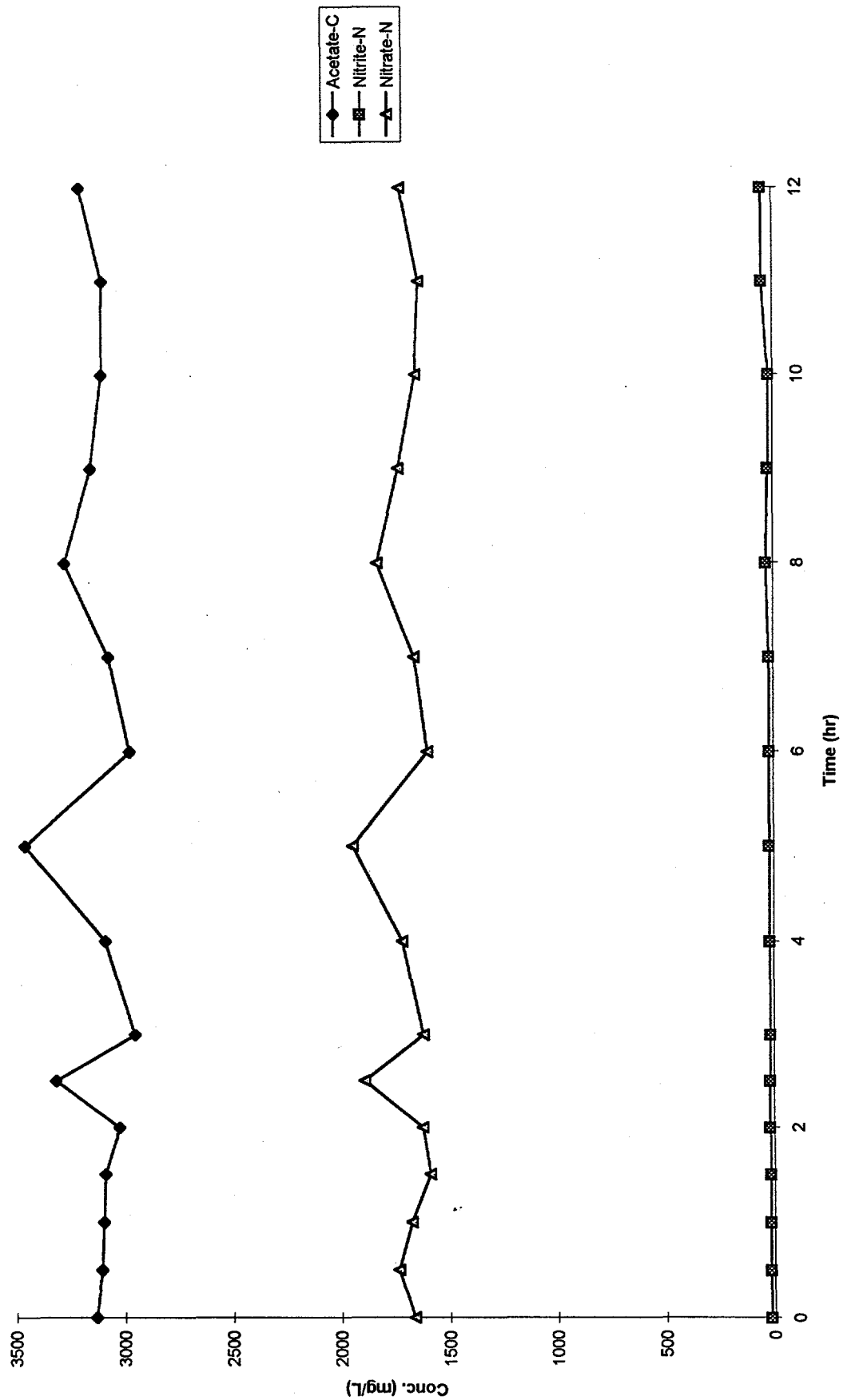


**Figure 12** Exp. 3 Day 10, pH 7.5

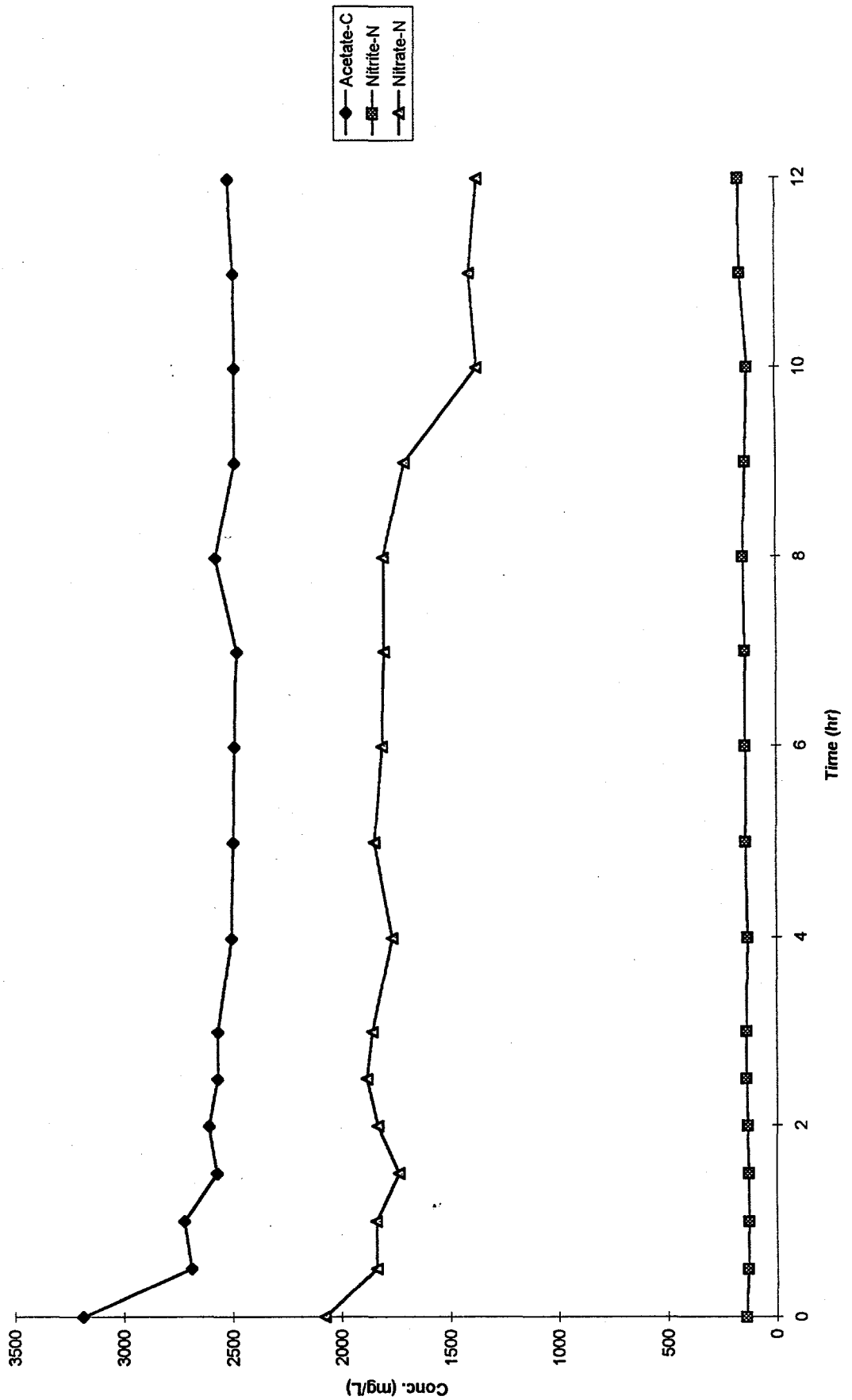


**Figure 13** Exp. 3 Day 10, pH 8.5

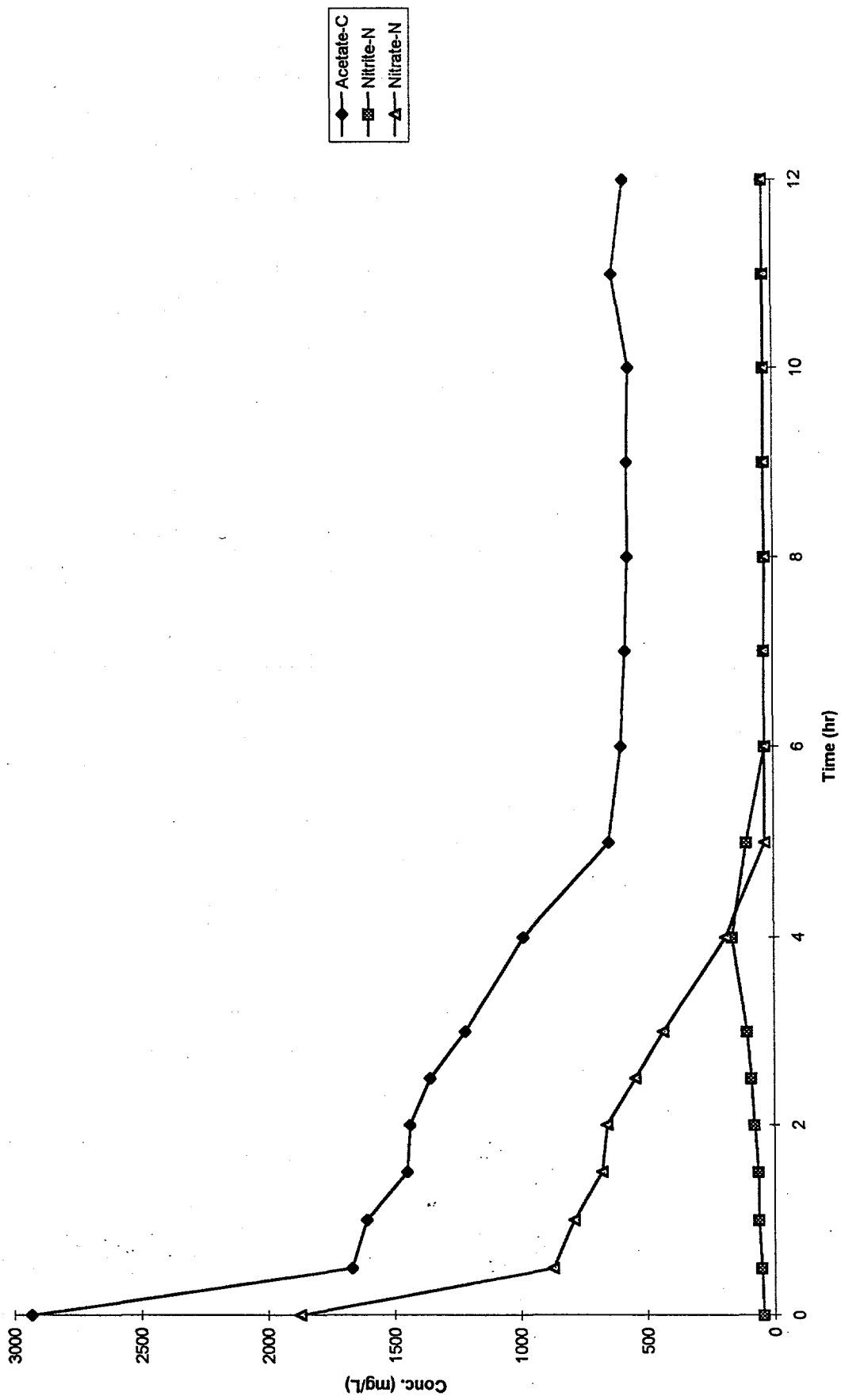


**Figure 14** Exp. 3 Day 30, pH 7.5

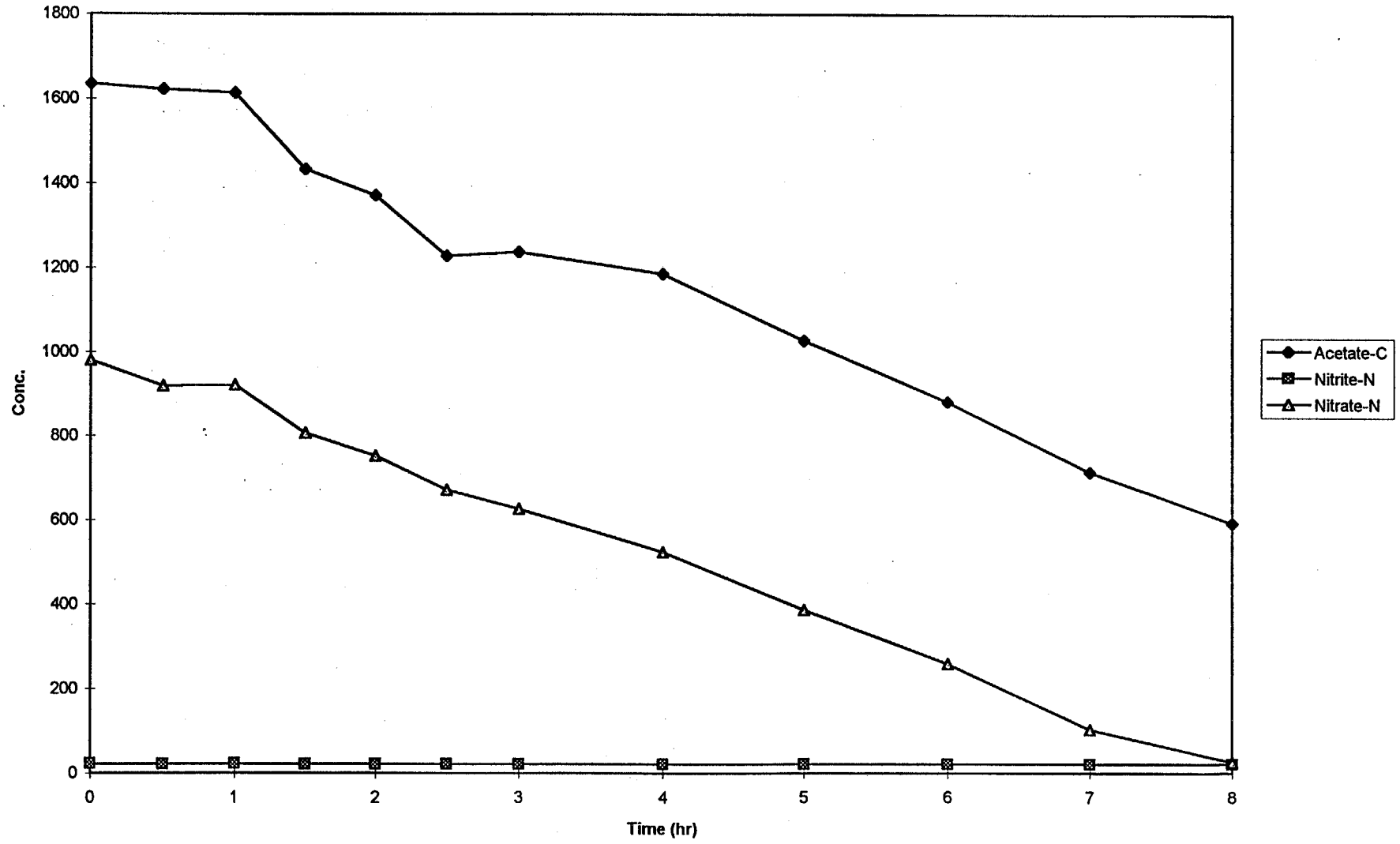
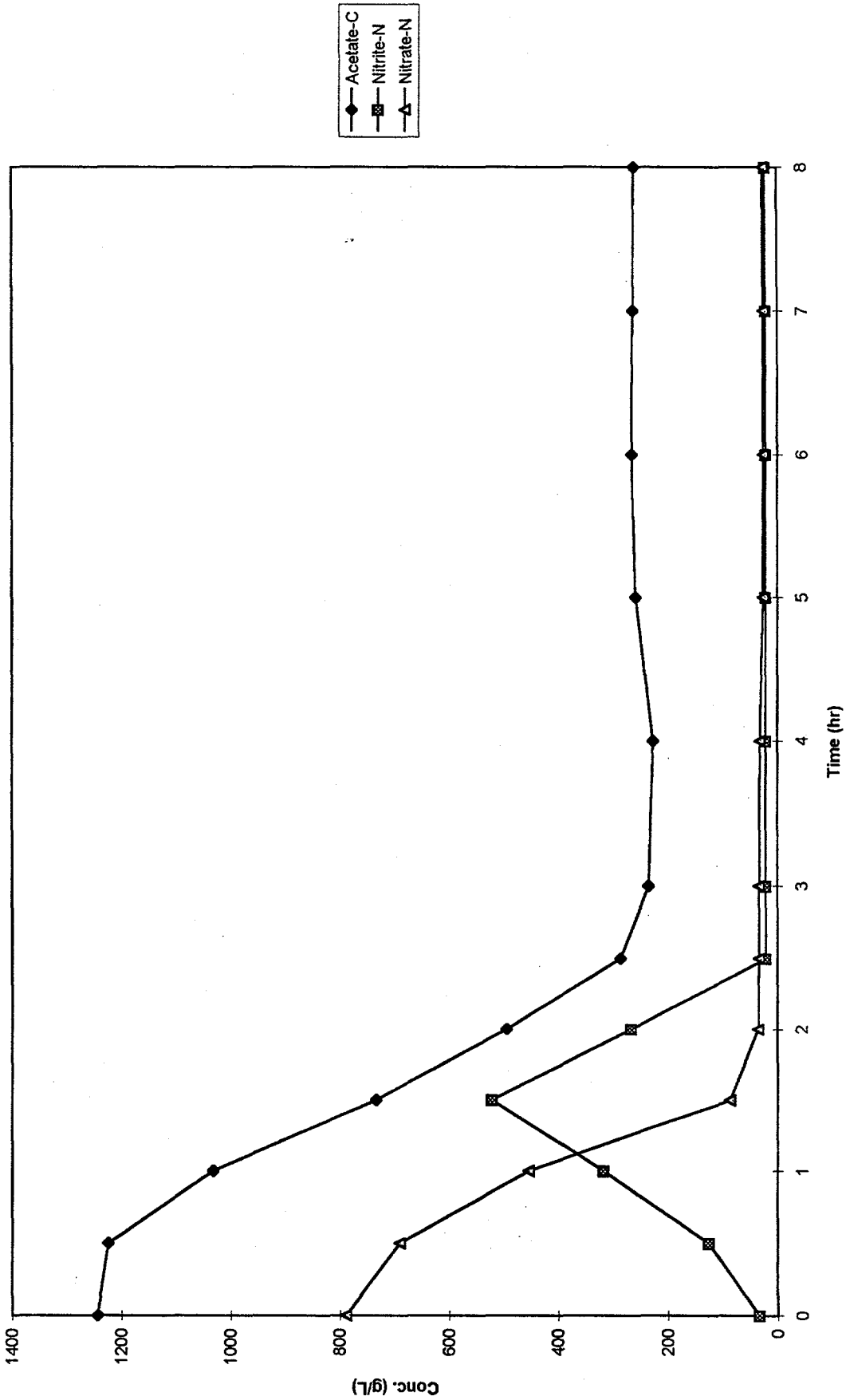


**Figure 15** Exp. 3 Day 30, pH 8.5



TASK Directly investigate nitrite inhibition of denitrification in flask experiments using activated sludge adapted to denitrification of the 2,700 mg/l $NO_3$-N ionic strength = 1.2 wastewater. The activated sludge concentration in these short-duration experiments of less than 24 hours was controlled at between 1,100 and 1,250 mg/l MLSS. The ionic strength of the water was less than 0.1, containing only varying concentrations of nitrite, between 20 and 2,200 mg/l $NO_2$-N. pH of the flask suspensions was maintained at one of the three experimental values, 6.0, 7.0 and 8.0 using a phosphate buffer. Duplicate flasks were run for each nitrate concentration and pH combination. The upper limit of initial flask nitrite concentration was chosen in order to reach inhibition of denitrification. Table 3 is a summary of the flask nitrite experiments.

Table 3. Flask denitrification of nitrite by activated sludge cultures

| pH = 6.0 Initial $NO_2$-N (mg/l) | pH = 7.0 Initial $NO_2$-N (mg/l) | pH = 8.0 Initial $NO_2$-N (mg/l) |
|---|---|---|
| 15 | 50 | 30 |
| 30 | 140 | 50 |
| 50 | 270 | 125 |
| 55 | 570 | 250 |
| 170 | 830 | 550 |
| 230 | 1,130 | 850 |
| | | 1,150 |
| | | 1,600 |
| | | 2,100 |

In addition flasks experiments for all pH values and initial nitrite concentrations were performed for MLSS concentrations ranging from 2,500 to 5,500 mg/l.

RESULTS Figure 16, 17 and 18 show denitrification for flasks with the pH maintained at 6.0, 7.0 and 8.0, respectively. As expected from previous reports of nitrous acid toxicity, significant denitrification of nitrite at pH 6 did not occur within 14 hours, even at the relatively low initial concentration value of 30 mg/l $NO_2$-N. In the flasks held at pH = 7.0, complete denitrification was observed with an initial concentration of 50 mg/l $NO_2$-N within 14 hours. At initial concentrations of 140 mg/l $NO_2$-N approximately 40% of the nitrite was consumed. Approximately 10% was denitrified at 270 and 570 mg/l $NO_2$-N, and no denitrification was

observed at the higher nitrite concentrations of 830 and 1,130 mg/l $NO_2$-N. For the flasks with a pH of 8.0, the activated sludge was able to denitrify influent nitrite concentrations up to 850 mg/l $NO_2$-N within 14 hours. It was only when the initial nitrite concentration exceeded 1,150 mg/l $NO_2$-N was significant inhibition found. The effect of pH on denitrification of nitrite is summarized in Figure 19, comparing the denitrification reaction in flasks at pH values of 6.0, 7.0 and 8.0 for an initial nitrite concentration of 230 - 270 mg/l $NO_2$-N.

A linear regression was done on all profiles to derive a rate constant for statistical comparison of the pH effect on denitrification of nitrite. This assumes that the denitrification reaction can be modeled as a zero-order reaction. This was thought to be an acceptable model, given that uninhibited denitrification at that range of nitrate concentration has been found to be a zero-order reaction:

$$\frac{r_N}{X} = -k$$

where $r_N/X$ = specific rate of denitrification per unit biomass concentration (time $^{-1}$), X = MLSS concentration and k is the zero-order reaction coefficient (time $^{-1}$).

For all initial nitrite concentrations where denitrification was observed, k values were found from the slope of the linear regression for data sets from each combination of pH and initial $NO_2$-N concentration. These are summarized in Table 4.

Table 4. Flask activated sludge zero-order nitrite denitrification reaction coefficients

| | pH = 6 | pH = 7 | pH = 8 |
|---|---|---|---|
| MLSS (mg/l) | k ($hr^{-1}$) | k ($hr^{-1}$) | k ($hr^{-1}$) |
| 1,100 - 1,250 | 0.00231 | 0.0135 | 0.0645 |
| 2,500-3,300 | 0.00328 | 0.0194 | 0.0667 |
| 4,900-5,500 | 0.00268 | 0.0140 | * |
| average | 0.00276 | 0.0157 | 0.0656 |
| standard deviation | 0.0005 | 0.00321 | 0.00156 |

Values for k for each MLSS range were compared and found not to differ significantly between MLSS ranges; therefore they have been averaged within each pH group. The average k values were found to be significantly different with greater than 95% confidence in increasing order from pH = 6 to pH = 8. A plot of log k versus pH is shown in Figure 20. Figure 21 is a plot of

all the individual k values from the flask experiments plotted against the theoretical concentration of $HNO_2$. A regression line has bee fitted to the data, but there actually appears to be a threshold effect. That is, as the $HNO_2$ concentration increases above 0.5 - 1 mg/l (0.15 - 0.3 mg/l N), the rate of denitrification drops more than an order of magnitude. This $HNO_2$ toxicity threshold is similar to the value reported by Abeling and Siefried (1992).

**Figure 16. Nitrite denitrification by activated sludge in flasks, pH = 6.0**

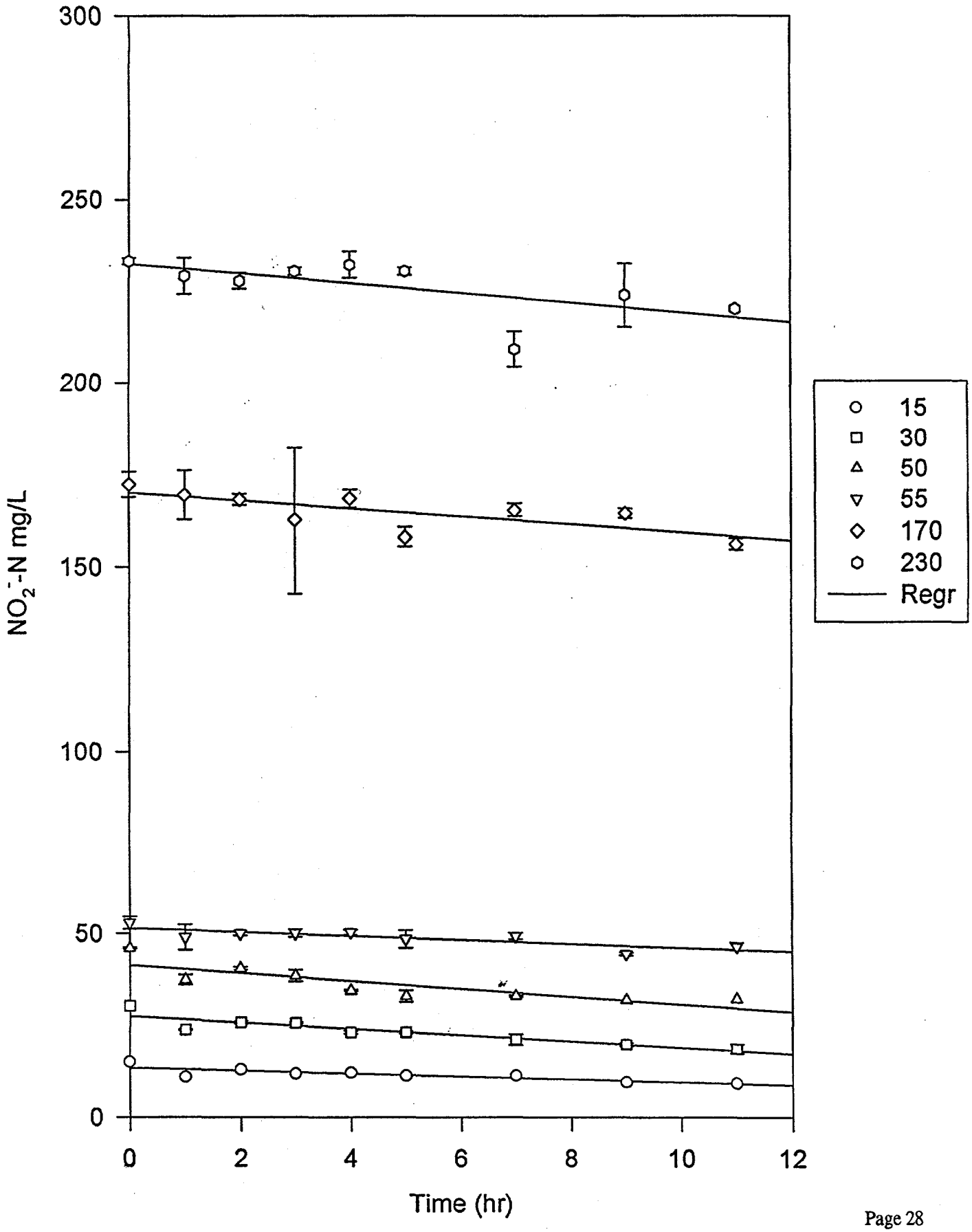


**Figure 17. Nitrite denitrification by activated sludge in flasks, pH = 7.0**

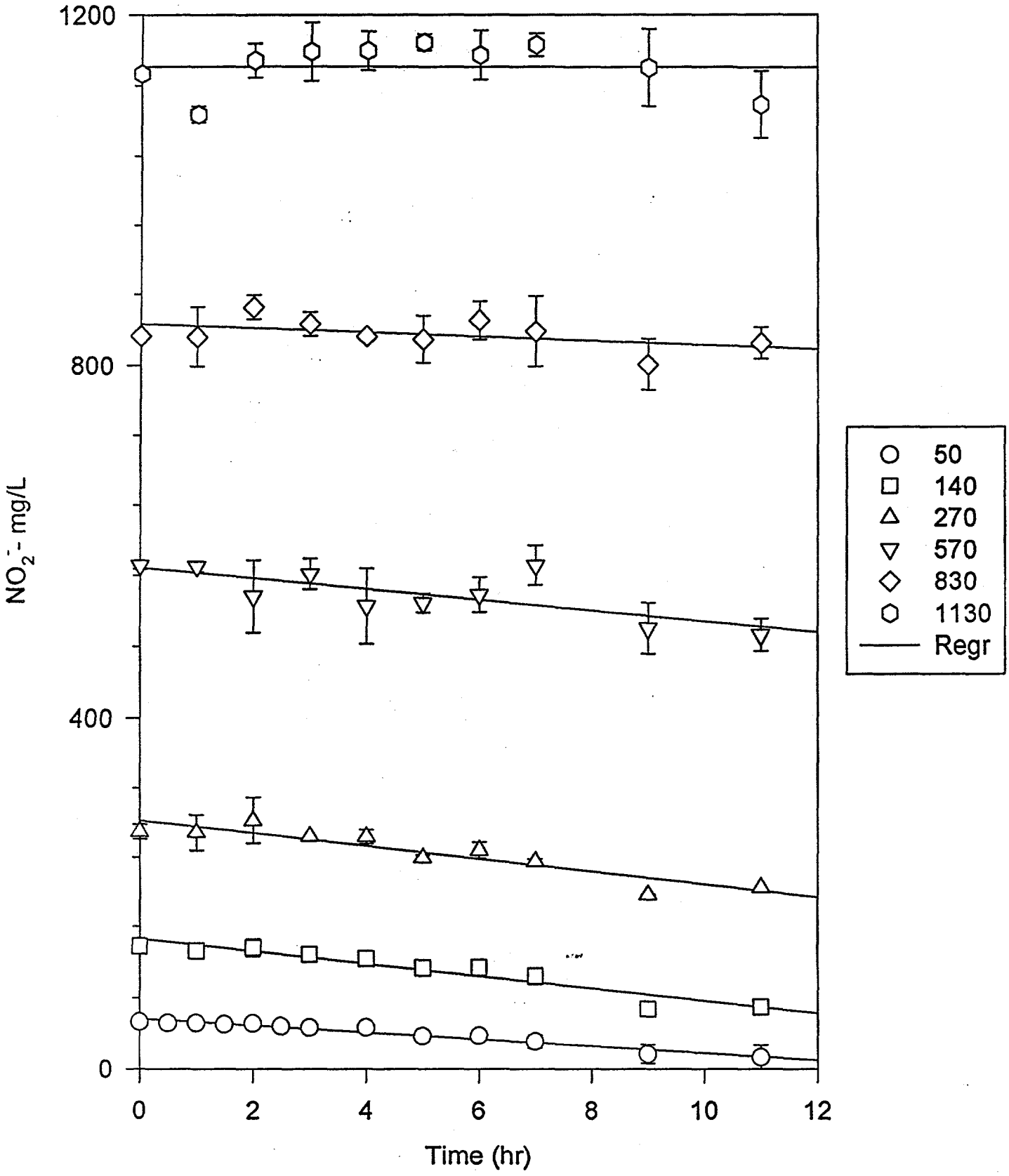


**Figure 18. Nitrite denitrification by activated sludge in flasks, pH = 8.0**

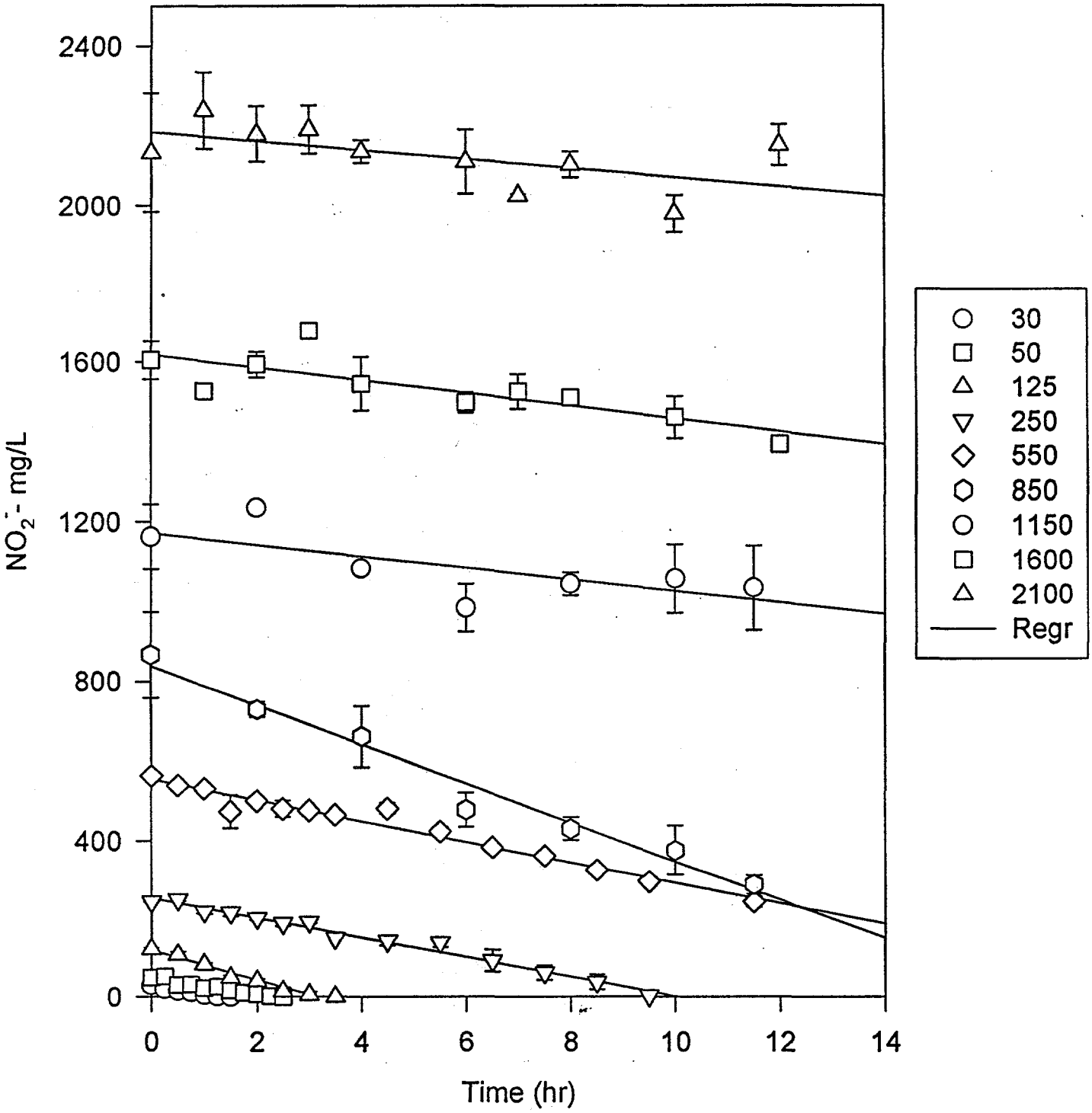


**Figure 19. Effect of pH on nitrite denitrification at initial concentration of 230 - 270 mg/l $NO_2$-N**

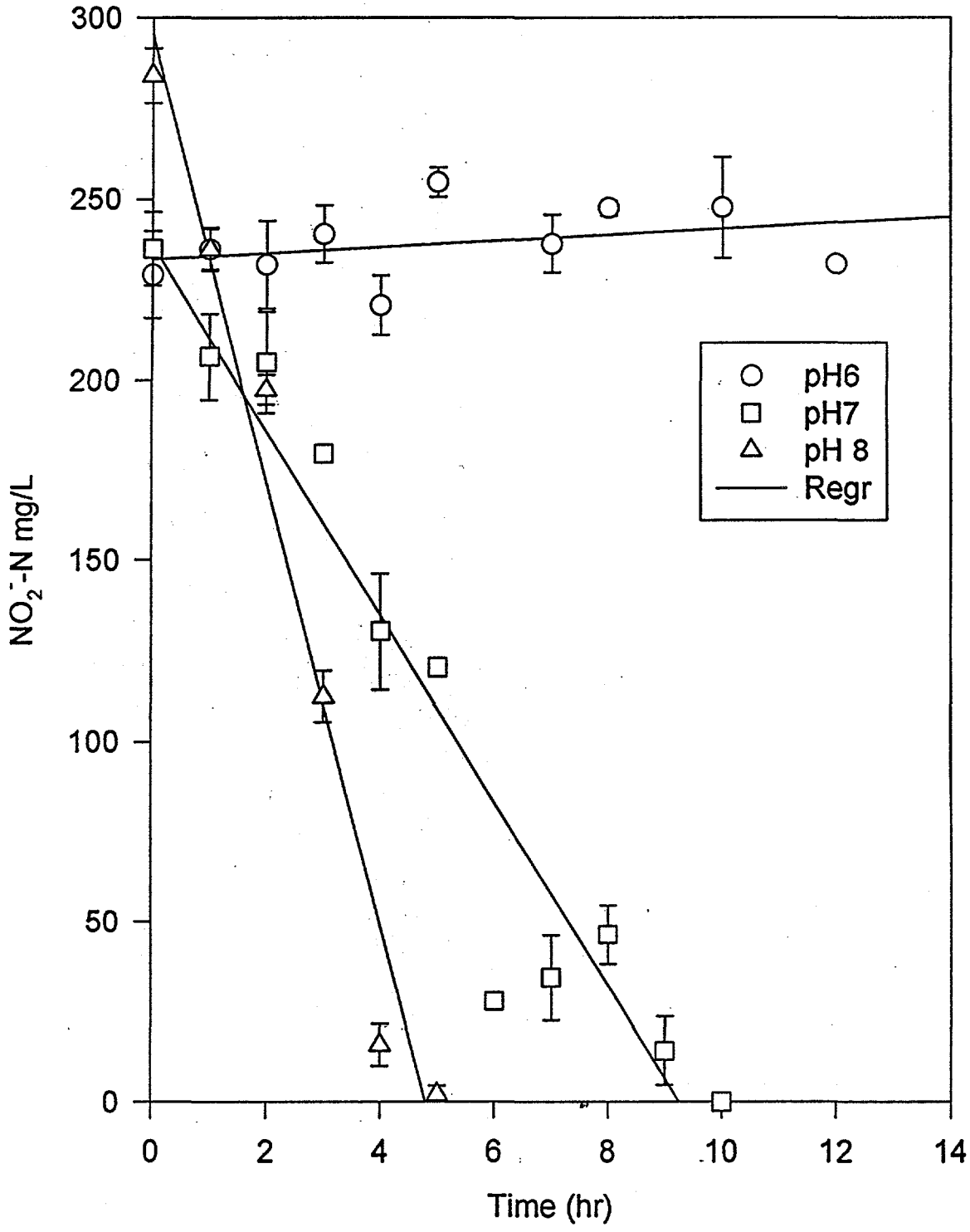


**Figure 20. Effect of pH on zero-order rate coefficient, k**

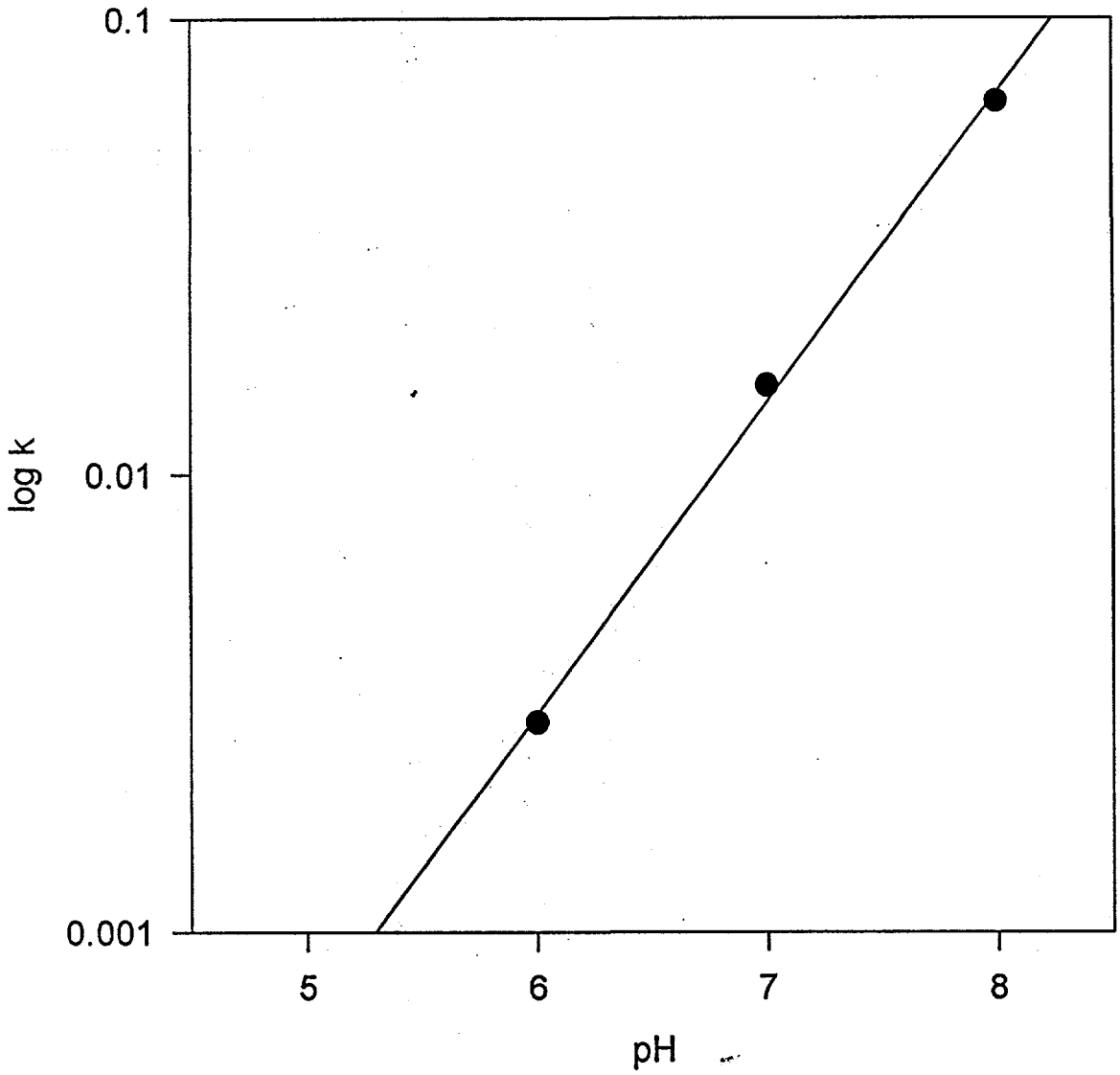
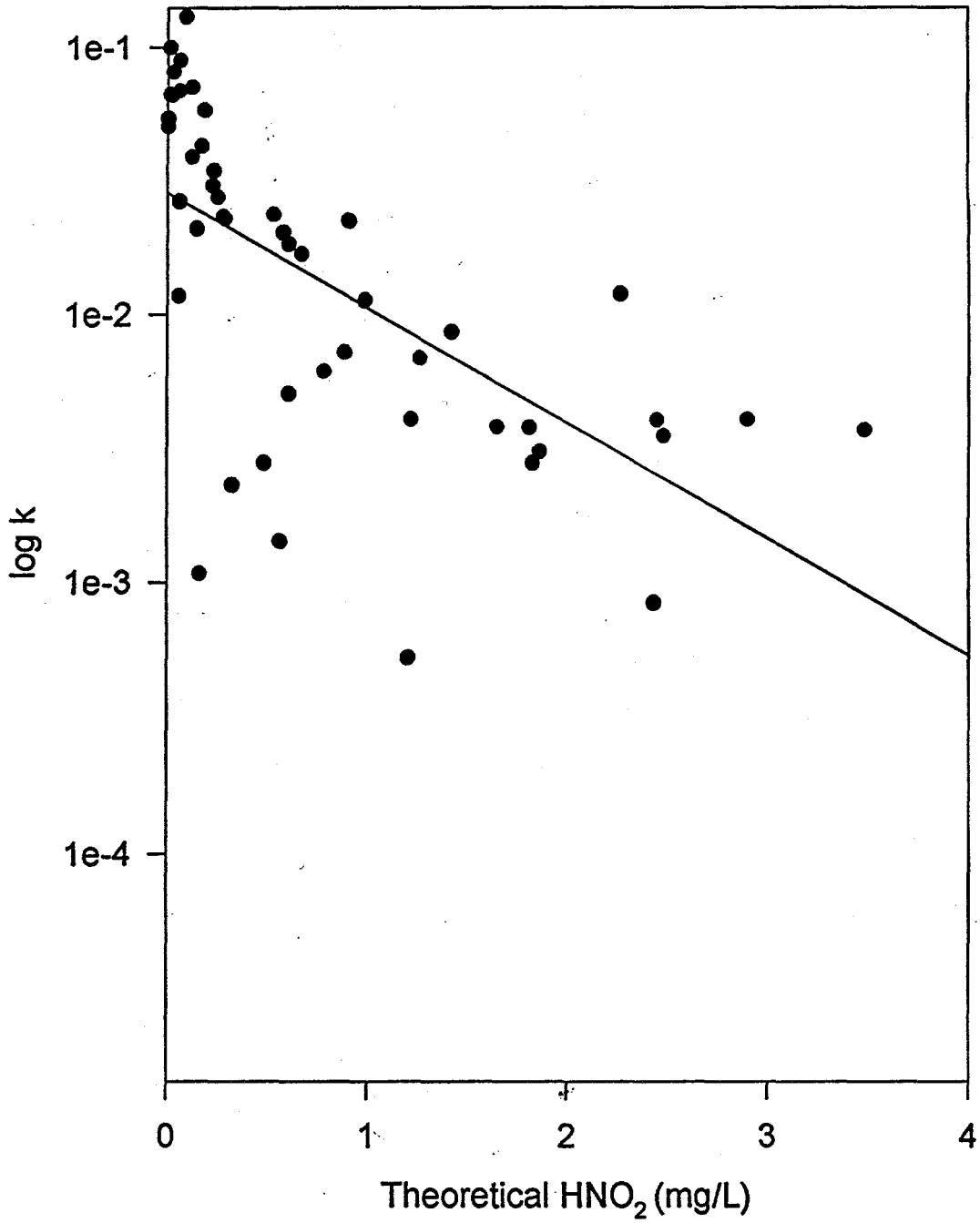


**Figure 21. Theoretical HNO2 concentration versus reaction rate coefficient, k**



## Investigation of Effect of Salinity on Denitrification

### Objectives

- Investigate effect of high salinity on denitrifying activated sludge
- Determine if SBR activated sludge process can be used to denitrify high-salinity and high-nitrate brines
- Determine if changes in salinity affect the activated sludge bacterial population

TASK Flask experiments using activated sludge acclimatized to denitrification at an ionic strength of 0.6 were inoculated into flasks containing brines with variable salinity values: 0.3, 0.6, 1.0, 1.5, 2.0 and 3.0 ionic strength, I (2, 4, 6.7, 10, 16.7 and 20% salt, respectively), and the initial nitrate concentration in all flasks held constant at 1,000 mg/l $NO_3$-N. Flask pH was maintained between 8.0 and 9.0 by phosphate buffer. The brine used in the flask experiments was similar in composition to the wastewater used in the SBR experiments, except the nitrate concentration was held constant and salinity increased by adding NaCl. As in the SBR and nitrite denitrification flask experiments acetate was used as the carbon source for the denitrifying bacteria. Each experimental flask contained approximately 3,000 mg/l MLSS. An abiotic control was used to insure that biological activity was responsible for denitrification, even at very high salt concentrations. The batch reactions were run for 11 days, with samples for nitrate, nitrite, acetate and pH taken at 0.5, 2, 3, 4, 5 and 11 days.

RESULTS Figures 22 - 28 are the chemical profiles for nitrate, nitrite, acetate and pH in the activated sludge flasks for ionic strength values of 0.3, 0.6, 1.0, 1.5, 2.0, 3.0 and 3.0 for the abiotic control, respectively. The control flask results indicated that there were no abiotic reactions in the flasks. For ionic strength values of 0.1 to 1.5 no appreciable lag in the onset of denitrification was observed; although nitrite was found to accumulate for ionic strength values starting at 0.6. At ionic strength values of 2.0 and 3.0 there was a significant lag period before the onset of denitrification. Nitrite continued to accumulate in both these flasks. Unfortunately the lag at the ionic strength = 3.0 flask was so large that the nitrite peak was missed. However, by 11 days, all the nitrate and nitrite in the flasks, regardless of ionic strength, had been consumed. This is a significant result. No other investigators have reported activated sludge activity in such high salinity environments. Table 5 is a summary of denitrification lag periods and nitrite accumulation in the salinity experiments.

Table 5. Effects of salinity on denitrification by activated sludge

| ionic strength | time required for complete denitrification (days) | lag (days) | nitrite peak (mg/l $NO_2$-N) | time to nitrite peak value (days) |
|---|---|---|---|---|
| 0.3 | 2 | 0 | 100 | 0.5 |
| 0.6 | 2 | 0 | 400 | 0.5 |
| 1.0 | 2 | 0 | 500 | 0.5 |
| 1.5 | 5 | 3 | 600 | 0.5 |
| 2.0 | 5 | 3 | 1,.000 | 3 |
| 3.0 | >5, <11 | 3 | * | * |

TASK Additional flask experiments were conducted to identify bacterial populations active in the denitrifying activated sludges at various ionic strengths. The activated sludge cultures assayed were: the activated sludge inoculum from the Broomfield (Colorado) municipal wastewater treatment plant, activated sludge from the SBR denitrifying at an ionic strength of 0.6, flask activated sludge grown under conditions in the above salinity experiments at ionic strength values of 1.0, 1.5, 2.0 and 3.0. There were two kinds of bacterial population assays. Bacterial species were identified by cell wall lipid analysis (Microbial Identification System) and by community fatty acid analysis done without isolation of individual bacterial strains (Microbial Insights, Knoxville, Tennessee).

RESULTS No true denitrifying bacteria were isolated from the municipal wastewater plant activated sludge, although 8 strains were identified that are known to reduce nitrate to nitrite, the first step of denitrification. These species were mostly from *Enterobacter* genuses. Three true denitrifying strains were isolated from the SBR activated sludge culture: *Paracoccus denitrificans, Pseudomonas stutzeri* and *Alcaligenes faecalis*.. At ionic strength equal to 1.0, *Pseudomonas stutzeri* and *Pseudomonas pseudoalcaligenes* were identified. Although bacteria grew on nitrate-agar in an anaerobic environment, these colonies could not be identified by the Microbial Information System database. One investigator has suggested that because cell wall lipid rearrangement is a known method of bacterial adaptation to saline environments, that changed cell wall lipids prevent identification by the lipid database (Russell, 1995). Figure 29 shows the summary results from community lipid analysis of four of the activated sludges:

activated sludge from the municipal wastewater treatment plant ("RAS from WWTP") is compared with activated sludge grown at ionic strengths of 0.6 and 3.0. It is interesting that the most dramatic change in microbial community structure appears to occur in the adaptation from the non-denitrifying aerobic conditions of the municipal activated sludge to the anaerobic denitrification conditions in the lab studies. If the lipid composition shift indicates a population shift in the activated sludge, apparently increasing salinity has little effect on microbial population composition, even at the high salinity value of ionic strength = 3.0.

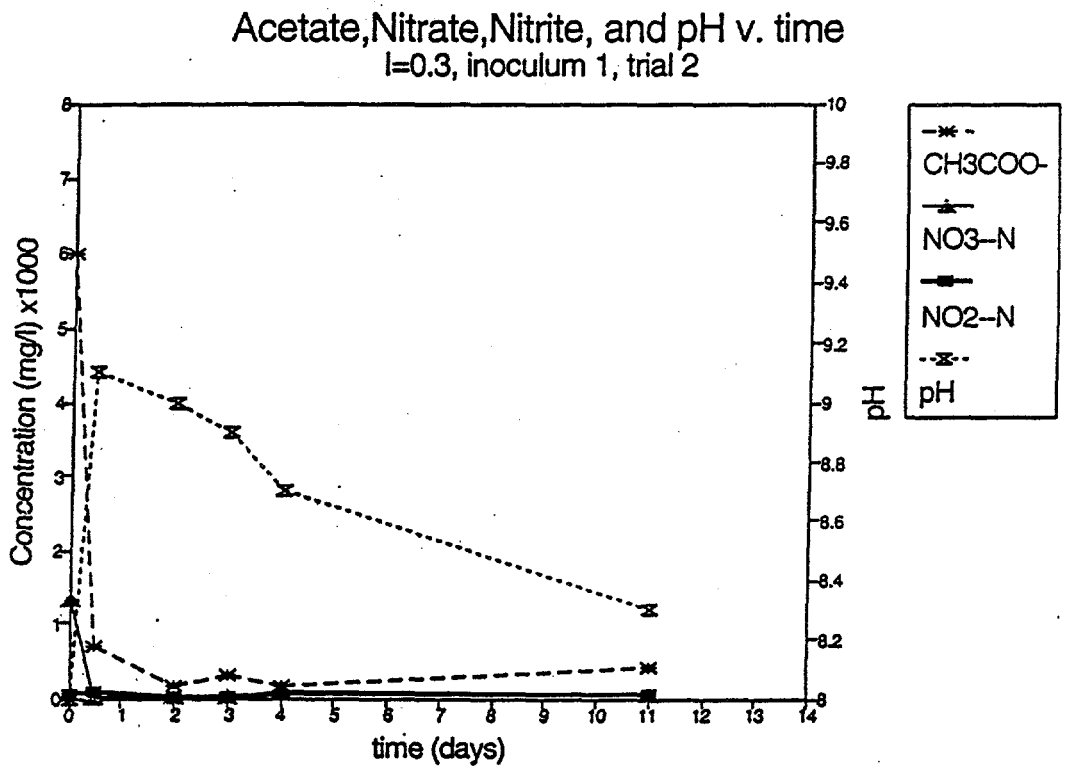



Figure 22 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=0.3, inoculum 1 flask, trial 2.

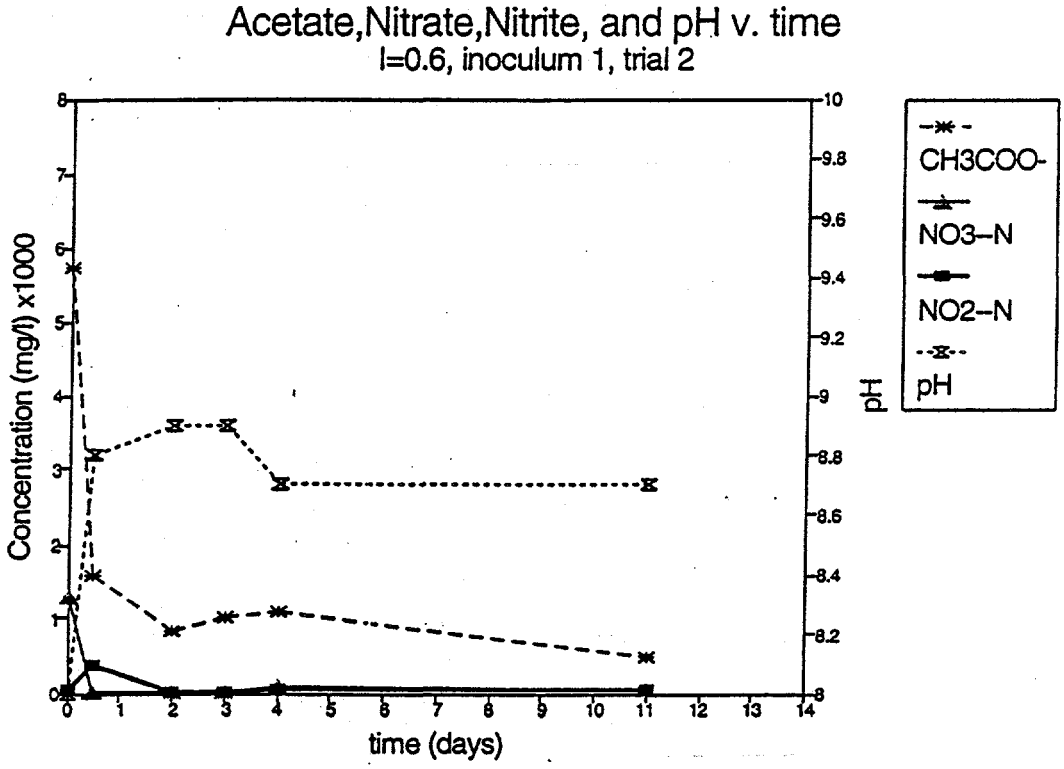



Figure 23 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=0.6, inoculum 1 flask, trial 2.

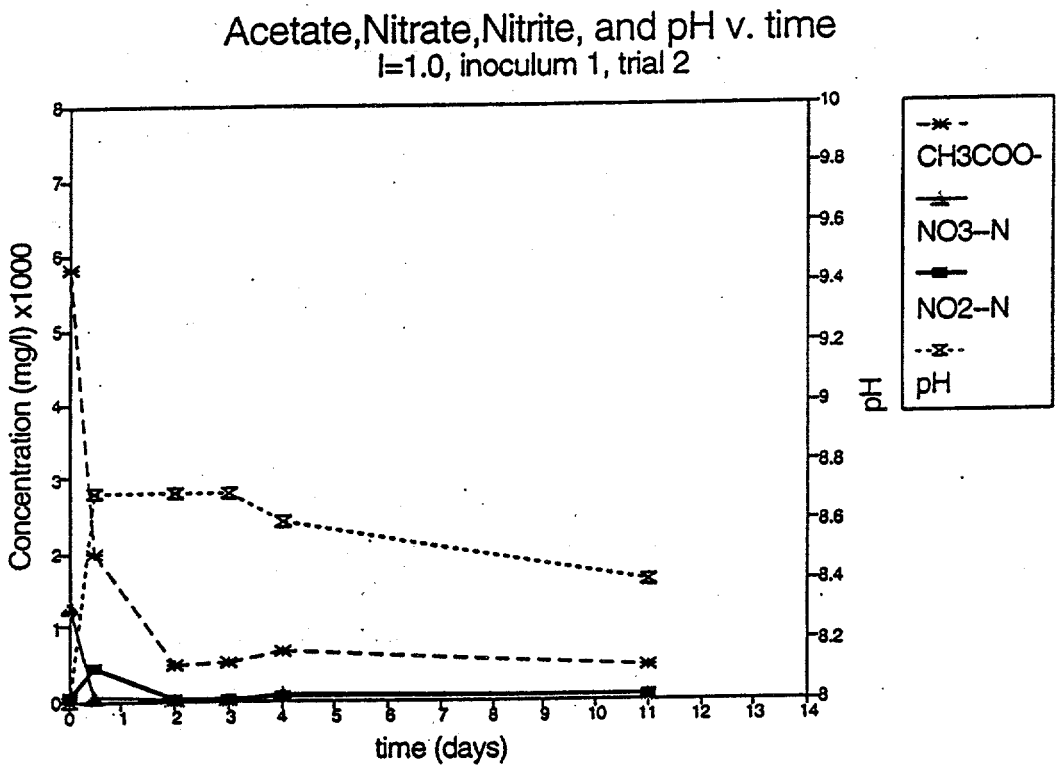



Figure 24 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=1.0, inoculum 1 flask, trial 2.

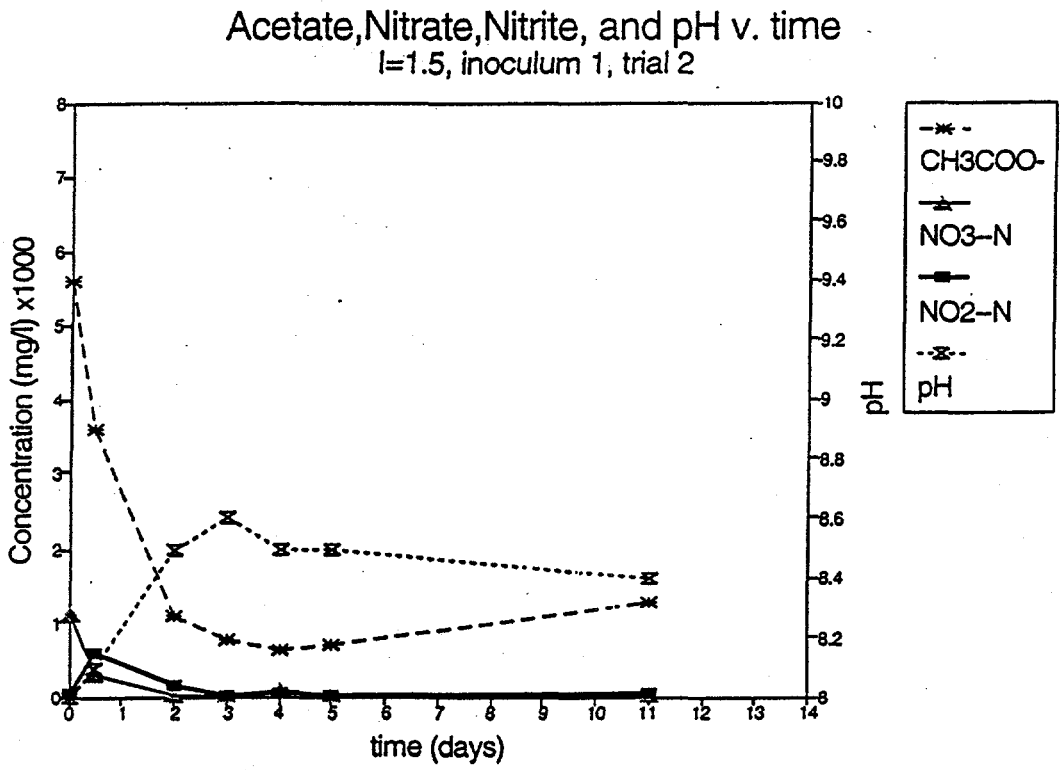



Figure 25 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=1.5, inoculum 1 flask, trial 2.

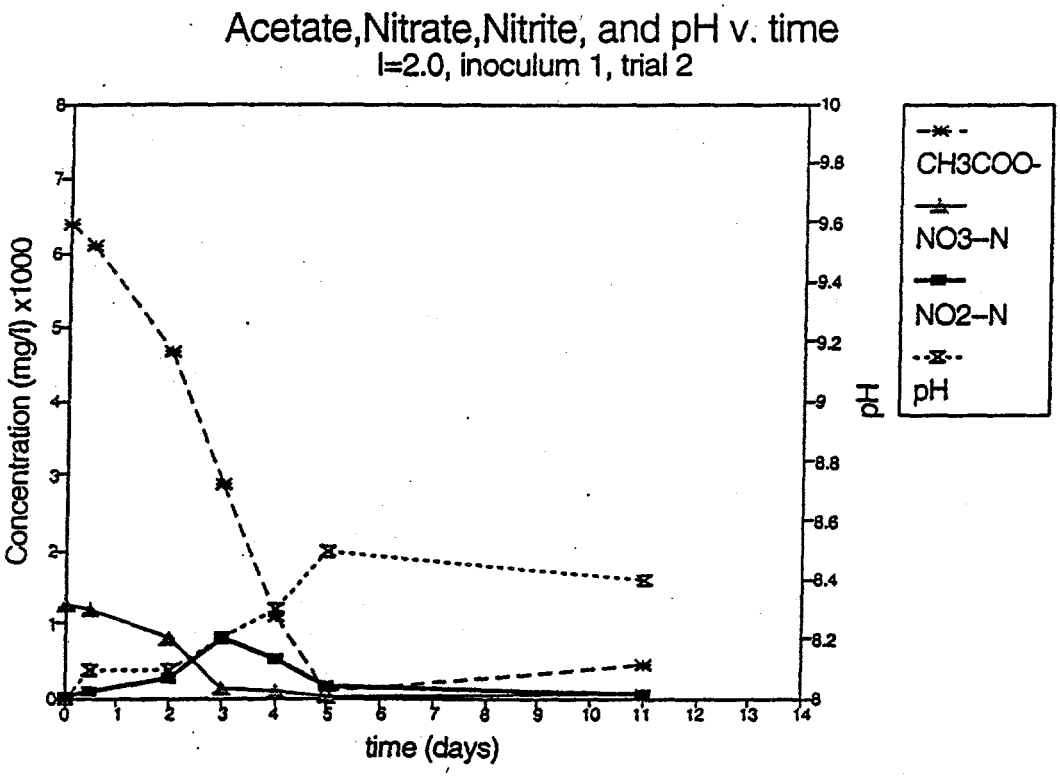
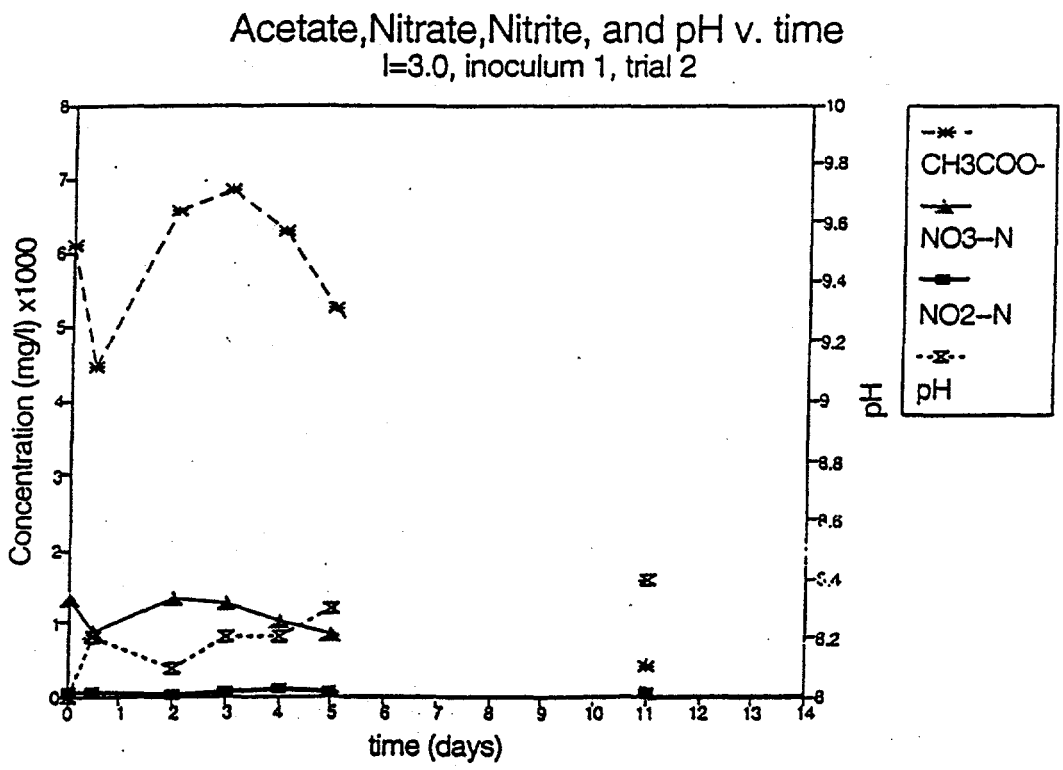



Figure 26 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=2.0, inoculum 1 flask, trial 2.




Note: Lines connecting data points between days 5 and 11 are not shown to emphasize that nitrite concentration probably peaked between these days, consistent with the pattern of increasing magnitude and time delay of nitrite peak with increasing ionic strength apparent in the lower ionic strength flasks(see Figures 18-22 and Table 9).

Figure 27 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=3.0, inoculum 1 flask, trial 2.

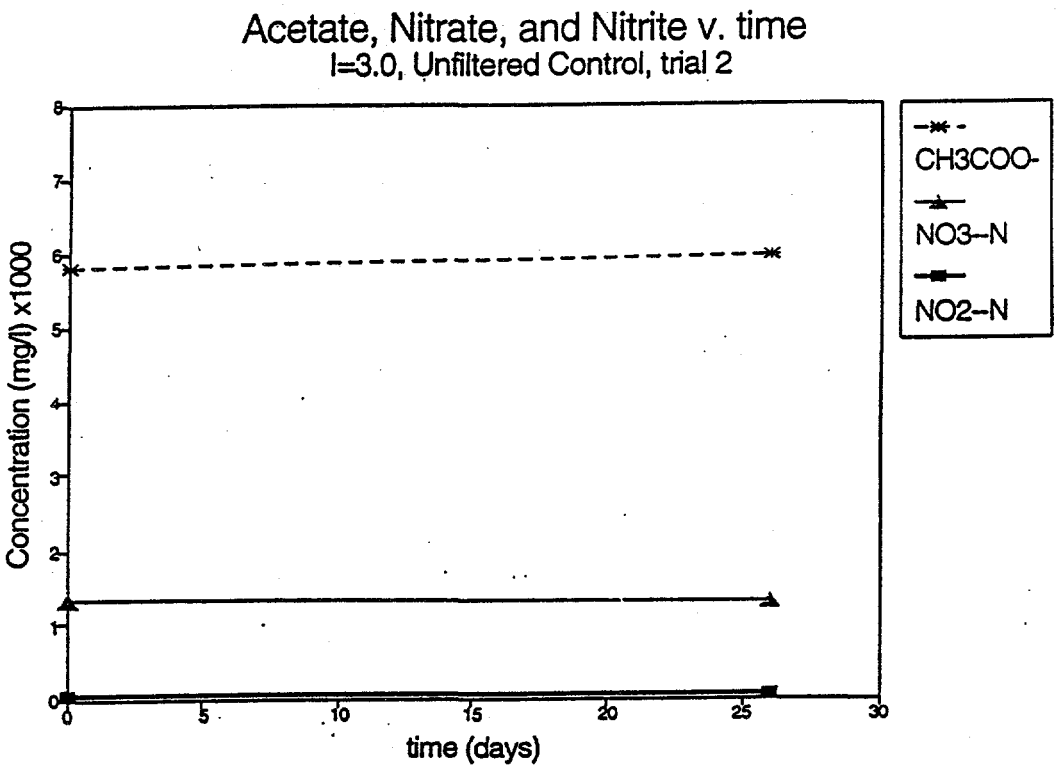



Figure 28 Acetate, nitrate, and nitrite concentration and pH vs. time plot for the I=3.0 control flask, uninoculated, filter sterilized (abiotic), trial 2.

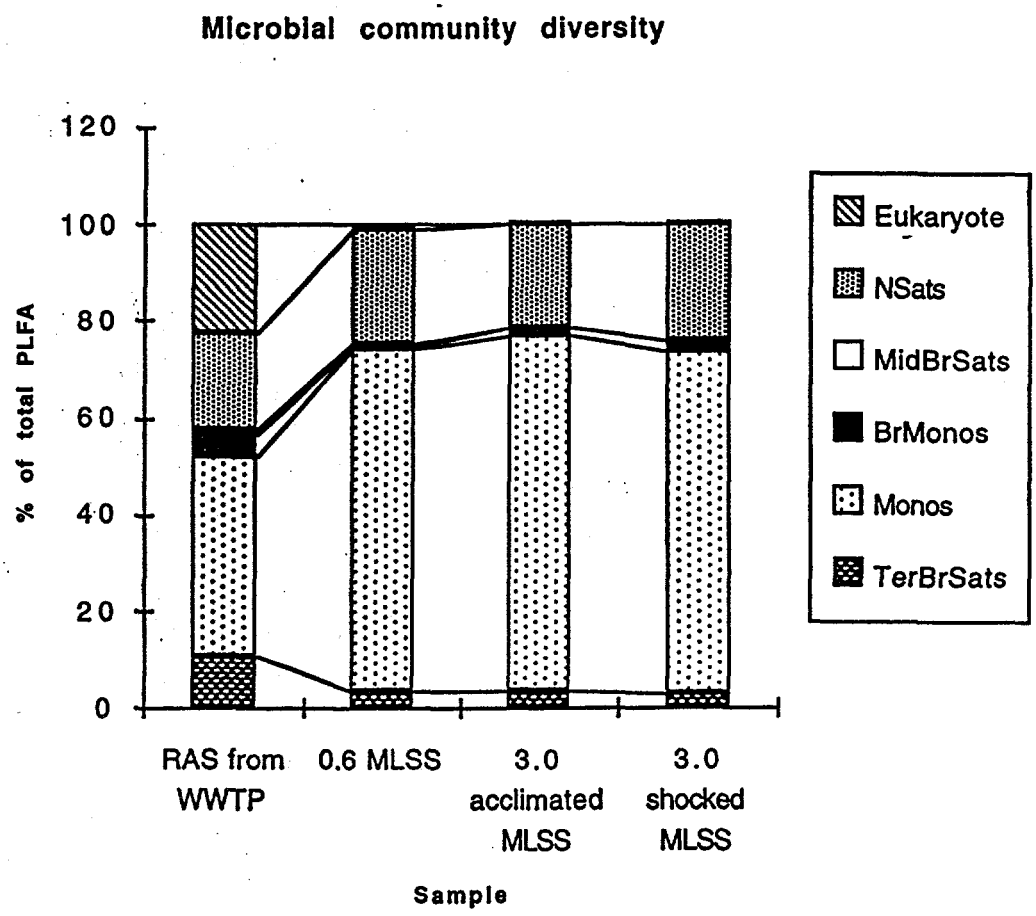



**Figure 29. Microbial Community Diversity with Varying Conditions.** Illustrates total phospholipid composition of four seperate samples. Shows that RAS from WWTP has the most diverse community structure. The 0.6 MLSS, 3.0 acclimated MLSS and 3.0 shocked MLSS have very similar phospholipid compositions. Legend identifies lipid type: eukaryote, normal saturate (NSats), mid-chained branched saturates (MidBrSats), branched monoenoics (BrMonos), monoenoics (Monos), and terminally branched saturates (TerBrSats).

**PUBLICATIONS**

Two publications are in preparation:

"Inhibition of Denitrification in Activated Sludge by Nitrite"

"Effect of Salinity on Denitrification and Population Dynamics in Activated Sludge."

**OTHER RESULTS**

This research has supported the work of two Ph.D. students, one receiving a stipend from research funds, and two masters students in the Environmental Engineering program at the University of Colorado, Boulder. Currently, experiments are continuing on denitrification of high-nitrate and high-salinity wastewaters like those at RFETS, especially on the development of an effective adaptation strategy which can shorten the lag that seems to be required for denitrification at very high ionic strength values of 3.0 (20% salinity).

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