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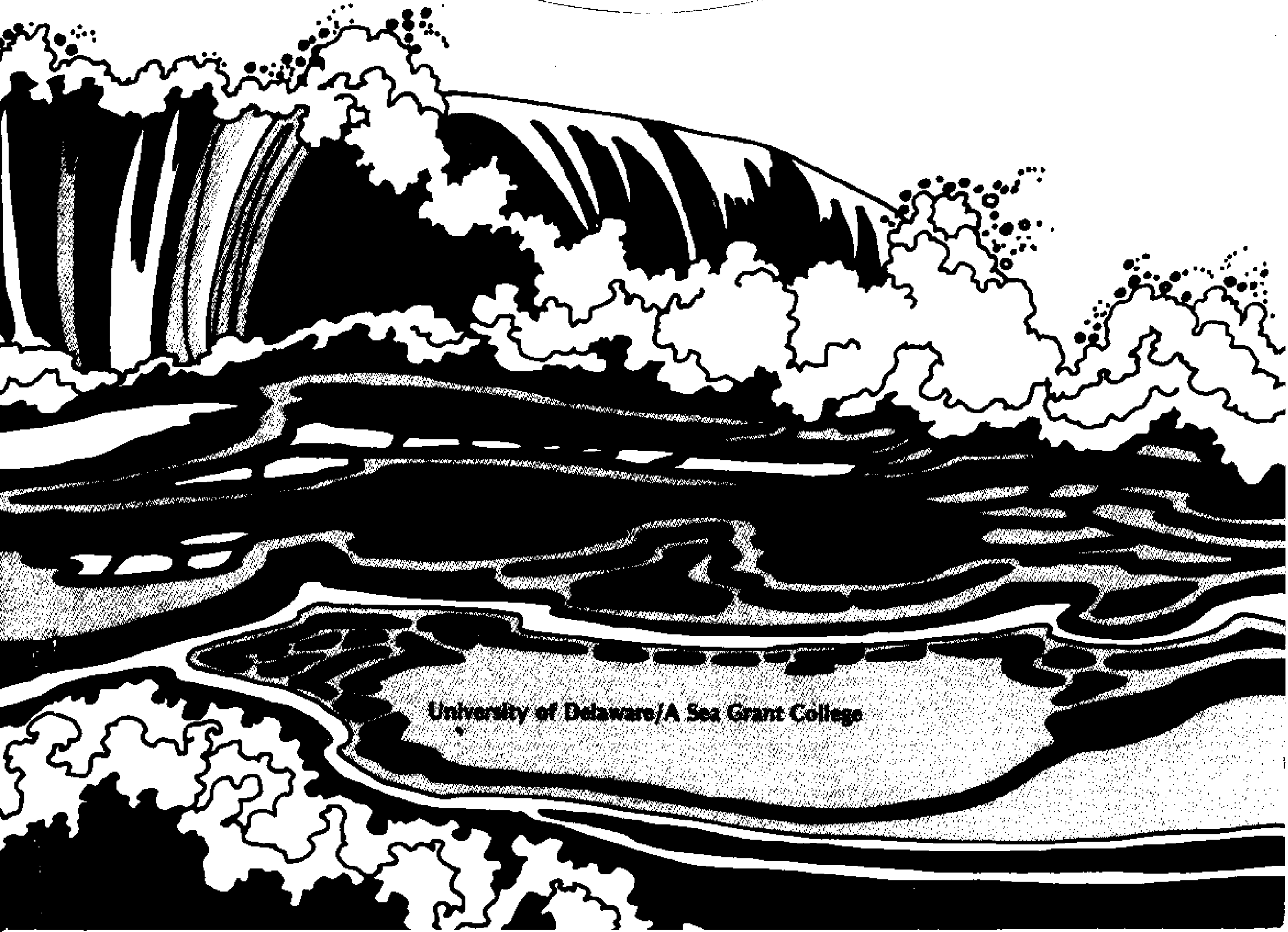

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ABSTRACT

The scope and significance of the problem of maintaining the quality of the water in recirculating mariculture systems is discussed. A quantitative study of the kinetics of important nutrient cycles is proposed as a means of establishing minimum system volumes, optimization of design and the requisite environmental controls. Significant results in a study of inorganic nutrient nitrogen cycling and toxicity in recirculating systems are reported. A modular filter design suggested by a study of the kinetics of nitrification in marine systems demonstrates how a knowledge of kinetic and chemical factors can lead to more efficient water treatment hardware.

ACKNOWLEDGMENT

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INTRODUCTION

We are engaged in a project designed to demonstrate the economic feasibility of raising hard clams (*Mercenaria mercenaria*) and oysters (*Crassostrea virginica*) in a recirculating mariculture system. The advantages of a system which recirculates a high percentage of the culturing water over an open (flow-through) system in which the water is used once and then discharged include such factors as the ability to control and to optimize growth conditions, freedom from competition with natural predators, less competition with other coastal marine activities, and lower harvesting costs (Epifanio et al., 1973). However, it is clear that the quality of water as measured by its ability to sustain growth is a critical factor when a high degree of water reuse is required.

The ecosystems used in a mariculture effort characteristically involve higher organism densities than those typically observed in nature, the possibility of controlling the culturing environment, and the necessity for active participation by man to insure the long-term stability of the culturing system.

Therefore, we have found it useful to attempt to quantify the relationships which exist in a man-made ecosystem with the objectives of:

1. enhancing the rate at which desirable water-quality-related reactions occur;
2. learning about the effect of reduced water volume on the necessity for control of environmental parameters such as changes in temperature, salinity, oxygen, etc.;
3. devising inexpensive reliable monitoring equipment;
4. aiding in the design of practical hardware systems.

One important sequence of reactions which falls into a category readily amenable to study can be represented by:

$$R_1C\text{-}N \xrightarrow{K_0} NH_4^+ \xrightarrow{K_1} NO_2^- \xrightarrow{K_2} NO_3^- \xrightarrow{K_3} R_2C\text{-}N \tag{1}$$

This is a simplified representation of the nitrogen cycle as it would be expected to occur in a recirculating system for shellfish production. The value of K_0 represents the rate constant which describes the rate of conversion of animal nitrogen to the nitrogenous waste produced NH_4^+. The value of K_1 is the rate at which the toxic ammonium ion is oxidized to toxic nitrite ion by bacteria. Similarly, the value of K_2 describes the rate of conversion of nitrite ion to the algal nutrient nitrate ion. The nitrate ion can in turn be used by phytoplankton at a rate represented by K_3 to produce organically bound plant nitrogen, which can then be fed to the cultured herbivores.

Three factors supplement this simplified reaction sequence:

1. The individual values for K, the rate constants, are not truly constant, but are often strong functions of environmental parameters.
2. The concentrations of the intermediate materials cannot be allowed to increase without limit since these substances may be toxic to organisms in the mariculture system.
3. The consecutive nature of these reactions means that small perturbations in one part of a system may be added cumulatively to a chain of undesirable events, producing a breakdown in the overall function of the cycle.

In this context we have evaluated analytical methods of detecting ammonia in seawater (Srna et al., 1973), and measured the rate of production of ammonia by oysters and clams (Srna, 1974), the toxicity of ammonia and its oxidation products (Epifanio and Srna, 1974), and the rate of oxidation of ammonia by nitrifying bacteria to the nitrite and nitrate ion (Srna and Baggaley, 1974).

In each of the kinetic studies we have further determined how small rapid changes in fundamental operating variables such as pH, temperature, salinity, oxygen, and sulfide affect the mathematical rate expressions which we derived.

These mathematical expressions have been combined to reflect their interdependence (Equation 1) and are yielding information about the factors affecting the stability of a culturing system, minimum system volumes, and monitoring parameters.

In this report, we discuss how the results of our study of the effect of environmental factors on the kinetics of the stepwise oxidation of ammonia to nitrite and then to nitrate ion in a batch nitrification filter contributed to the design of a continuous (flow-through) nitrification filter. Our study of a batch process utilizing a subgravel type filter (Srna and Baggaley, 1974) yielded kinetic information about the characteristics and rates of the reactions:

$$NH_4^+ + 3/2\ O_2 \xrightarrow{K_1} NO_2^- + 2H^+ + H_2O \qquad (2)$$

$$NO_2^- + 1/2\ O_2 \xrightarrow{K_2} NO_3^- \qquad (3)$$

where K_1 and K_2 are the rate constants. These processes are accomplished by bacteria of the genera Nitromonas and Nitrobacter respectively.

We learned that:

1. A relatively long time (40 days) was required to generate a substantial population of nitrifying bacteria.
2. The nitrification capacity of the filters could be defined in terms of the amount of nutrient added per day.
3. The rate of oxidation of nutrient ammonium or nitrate ion in the filters can be described in terms of a first order rate equation given by

$$\ln \frac{C_o}{C_o - x} = -Kt \qquad (4)$$

   where C_o is the initial concentration, x is the amount reacted after time t and K is a first order rate constant.
4. The pH affects the values of the rate constant for oxidation of ammonium and nitrite ions. This is shown in Figure 1. It is evident that the rate of ammonium oxidation is increased by high pH values and depressed at low values. Conversely, the rate of oxidation of nitrite ion is slightly enhanced at lower values of pH.
5. By considering the consecutive nature of the overall reaction sequence

$$NH_3 \xrightarrow{K_1} NO_2 \xrightarrow{K_2} NO_3 \qquad (5)$$

it was possible to derive a set of equations which predict the concentration of ammonium, nitrite, and nitrate in the filter medium at any time, t, after the introduction of ammonia to the filter. At constant pH, temperature, salinity, and oxygen levels, the rate constants for K_1 and K_2 can be determined from Figure 1 and the change in concentration in the filter of ammonium and nitrite ion with time can be calculated. For example, Figure 2 shows the calculated and measured concentration of ammonia and nitrite ion for an experiment performed at a pH of 7.80.

6. The rate constants for nitrification are not totally independent of initial concentration of ammonium or nitrite ion in the filter. At input concentrations greater than about five times the normal daily input the measured rate constant decreased. The rate of oxidation of excess inorganic nitrogen ions is slow and independent of the concentration of substrate ion in the water.
7. Oxygen is consumed during nitrification and the pH of the filter medium tends to decline in accordance with Equations 2 and 3.
8. Low sulfide ion concentrations (0.1 ppm) severely inhibit nitrification.
9. Nitrification filters utilizing a gravel substrate tend to become less porous as solid material accumulates in them. This tends to reduce the efficiency of these filters.

The modular nitrification filter design based on these considerations is shown in Figure 3. It is a low-water-volume (4-liter) flow-through system in which culturing water containing a high concentration of ammonia and a relatively high pH is air lifted to the top of a box containing silicate gravel. As the water trickles through the first bed of gravel, the action of the nitrifying bacteria attached to the gravel will cause conversion of some of the ammonium ions to nitrite ions. We would also expect that the oxygen and pH levels would drop in accordance with the stoichiometry of Equations 2 and 3. After passing through the first filter module, the water is air lifted to the top of the second module. Thus, well-aerated water containing a mixture of ammonium and nitrite ions at a somewhat reduced pH will trickle through the second module where further oxidation of ammonium to nitrite ion occurs and where conversion of nitrite to nitrate ion should begin. This process is repeated through the remaining two stages to yield an effluent of reduced ammonia concentration, a higher nitrate concentration, and a reduced pH.

We have constructed a nitrifying filter using a modular design and have evaluated its performance in order to determine whether information generated by our physical-chemical study of nitrification chemistry in a simple sub-gravel nitrification filter could be utilized in the design of a more practical flow-through nitrification filter.

MATERIALS AND METHODS

The modular nitrification filter was constructed by joining a series of wooden boxes (5.0 cm x 12.5 cm x 16.0 cm) by an air-lift pump constructed of 2.54 cm i.d. dark grey pvc pipe. Each box was filled with approximately 1200 grams of washed silicate pebbles which were about 18 mm in diameter.

A flow of water through the boxes of 200 ml/minute was maintained by the action of the air-lift pumps. A battery jar containing 16 liters of artificial seawater was used as a water reservoir. The whole apparatus, which contained 20 liters of artificial seawater (salinity 32 ppt), was a closed system in which water was continuously air lifted from the battery jar reservoir through the filter modules, and then the effluent from the last filter module was returned to the battery jar reservoir. A peristaltic pump was used to add continuously 1.0×10^{-3} moles of ammonium chloride/day which served as a nutrient for the nitrifying bacteria.

After about thirty days, the concentration of ammonium ion in the reservoir stabilized at a concentration of 1.0×10^{-4} moles/liter.

When this occurred, we initiated a series of experiments to determine if some of the results derived from our study of the batch sub-gravel filter could be transferred to our modular filter design which is a continuous process.

The kinetic order of reaction, the value of the rate constant, the effect of pH, the distribution of inorganic nitrogen ions in the filter and the ability of the filter to function after having a large portion of its nitrifying bacteria removed were investigated.

The kinetic order of the nitrification reactions and the nitrification rate constants associated with the modular filter were determined by adjusting the concentration of ammonium chloride in the battery jar and monitoring the decrease in the concentration of ammonium ion in the battery jar over time as the water was continuously circulated through the filter system.

Similarly the effect of pH on the rate constants was measured by adding ammonium chloride or sodium nitrite to the battery jar reservoir and adjusting the pH to high or low values.

The distribution of the degradation products of ammonia (nitrite and nitrate ions) was also measured as well as the pH in different modules of the filter. This was accomplished by drawing samples for analysis of ammonium, nitrite, nitrate and pH from taps located on the outlet of each filter box.

Finally the effect on the rate constant of bypassing the nitrifying bacteria from the first filter module was measured by removing the gravel from the first module and measuring the rate of oxidation of ammonium ion and nitrite ion under these circumstances.

For each experiment performed on the filters, samples were taken for analysis from the battery jar and from the effluent from each filter module. The analytical techniques and methods of data treatment were the same as employed previously (Srna and Baggaley, 1974).

RESULTS

Figure 4 shows the decrease in ammonium ion in the battery jar reservoir of the nitrification system over time. The logarithmic decrease in concentration indicates a first order reaction. The correlation coefficient of the data to the first order expression (Equation 4) is 97% and yields a rate constant equal to 0.74 hr^{-1}. This compares to a value of .19 hr^{-1} which is the rate constant that we obtained in our previous study, using a daily ammonium chloride aliquot which was one-fourth that used in the present study. This information suggests that within the limits of experimental error in the measurements (see Srna and Baggaley, 1974) the rate constant for a nitrifying filter can be defined and quantified in terms of the daily nutrient input which the filter receives.

Figure 5 shows the effects of high and low pH on the oxidation of ammonium ion in the filter. The rate constant for ammonium oxidation at a pH of 7.13 is about one-third of the rate of a pH of 7.78. This change in rate compares favorably with the pH effect demonstrated in the batch subgravel filter.

The measured relative rates of oxidation of nitrite ion in the filter at a pH of 8.20 and 6.18 are about the same. The data are plotted in Figure 6. It can be seen that a reduced pH has little effect on the ability of the Nitrobacter to convert nitrite ion to nitrate ion. This conclusion is also in accordance with the results from our previous study.

Figure 7 shows the pH, ammonium, nitrite, and nitrate data obtained from samples drawn from each of the filter modules and the battery jar. The ammonium ion concentration decreased logarithmically as the water passed through the filter. The level of nitrite ion increased and then decreased, while the concentration of nitrate ions, the final product, increased. The pH of the influent water was decreased as the water passed through the successive nitrification stages in accordance with Equation 2.

This pattern of nitrification in the filter modules is very similar to the pattern of stepwise nitrification observed in the batch process (Figure 2), except that in this case, the time axis has been exchanged for an axis which could be labeled distance.

Finally, we determined the rate constant of the filter before and after removing the pebbles from the first filter module (Figure 8). The values of pH were about the same for both experiments (pH = 7.8 and pH = 7.7) but the initial concentration of ammonium ion was higher for the experiment with the four complete nitrification modules present. The relative rate constant for the complete filter was .25 and for the filter without the first module the rate constant was .26. Thus, it can be seen that the remaining bacteria in the filters are able to oxidize a large amount of the ammonium ion entering the filter.

DISCUSSION

The results of this evaluation of the modular nitrification filter point out some of the advantages of careful quantification of kinetic processes in a closed ecosystem. We were able to determine that we could greatly reduce the volume of water residing in a nitrifying filter, thus making it possible to reduce the overall volume of a mariculture system without a loss in the rate constants for nitrification. The rate constants which described nitrification in a 100-liter subgravel filter which received a single daily ammonium aliquot were essentially the same as those determined in a four-liter system which received the same daily amount of ammonium ion continuously. The pH affected the nitrification rate constants of the continuous system in the same way as in a batch system of much larger volume. We have taken advantage of the fact that bacteria which oxidize ammonia and need a high pH to function efficiently will be located predominantly on the pebbles in the first two modules where the pH of the water is high. Nitrobacter in turn will tend to concentrate on the pebbles in the last two modules, where they function most efficiently at reduced values of pH and high concentrations of nitrite ions.

The equations used to predict the concentration of inorganic nitrogen as a function of time which were written to describe the batch nitrification process (Srna and Baggaley, 1974) appear to have value in predicting the distribution of ions in a continuous flow filter. This means that the residence time (or flow rate) through a continuous filter necessary to accomplish a certain level of oxidation of both ammonium and nitrite ions can be calculated and incorporated into a system design.

The air-lift pumps located between the individual modules serve two functions; restoring oxygen to the water and transporting the water through the filter. These extra aeration steps may also help oxidize sulfide ions generated in a previous module due to a high oxygen demand.

Clogging problems often exhibited by nitrification filters can be dealt with without total interruption of service since the first module would be expected to clog first and it can be removed, back-flushed, and placed on the end of the filter sequence for gradual restoration of its nitrifying bacterial population. The remaining modules accept the excess ammonium ion load.

The characteristic pH drop of the culturing water as nitrification occurs can be easily monitored in our modular filters and it may provide an easy way to determine when the filter's capacity has been severely degraded.

Figure 1. The rate constants for ammonium and nitrite oxidation at different values of pH

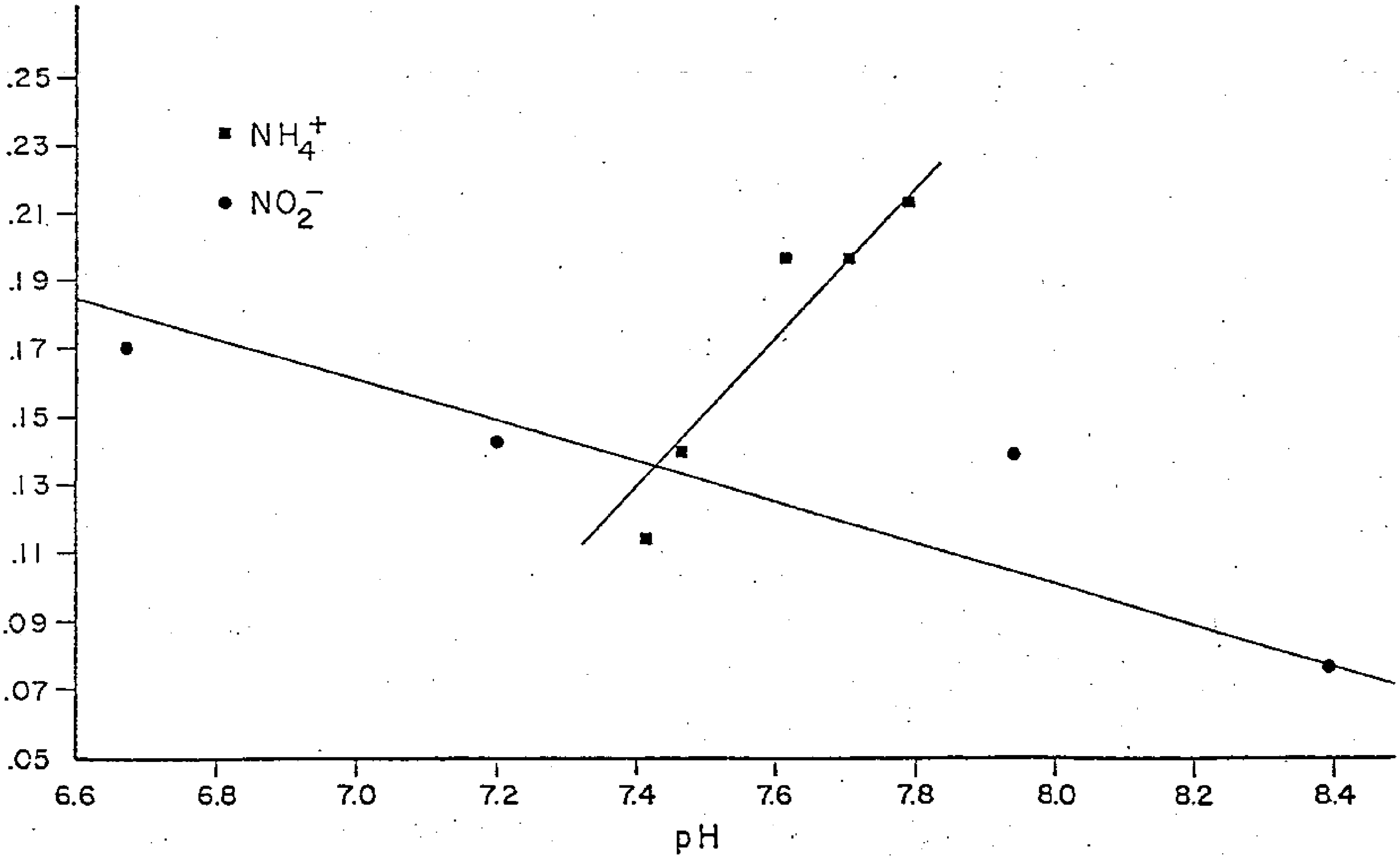


Figure 2. The experimental and calculated nitrification sequence

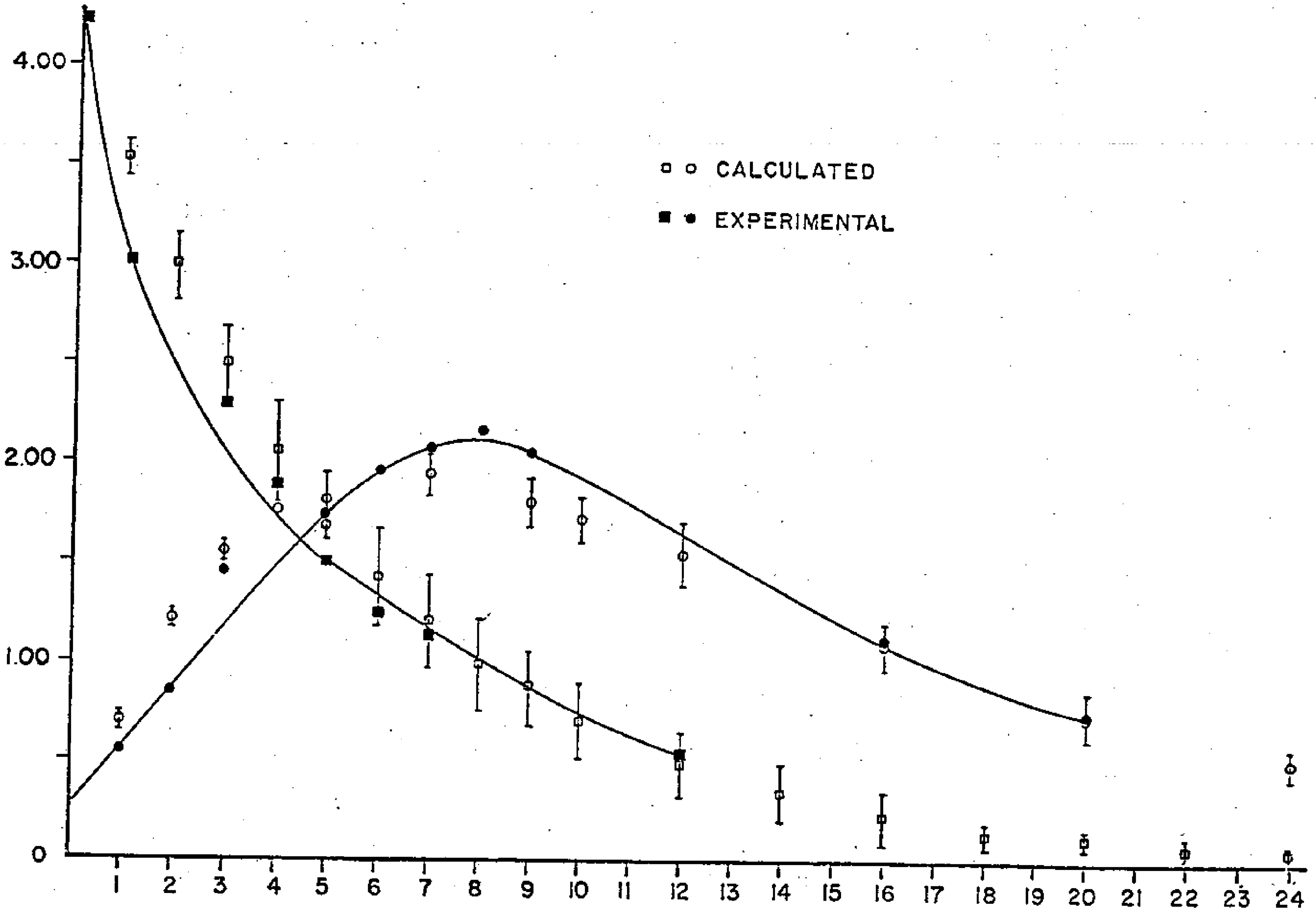


Figure 3. The modular nitrification filter

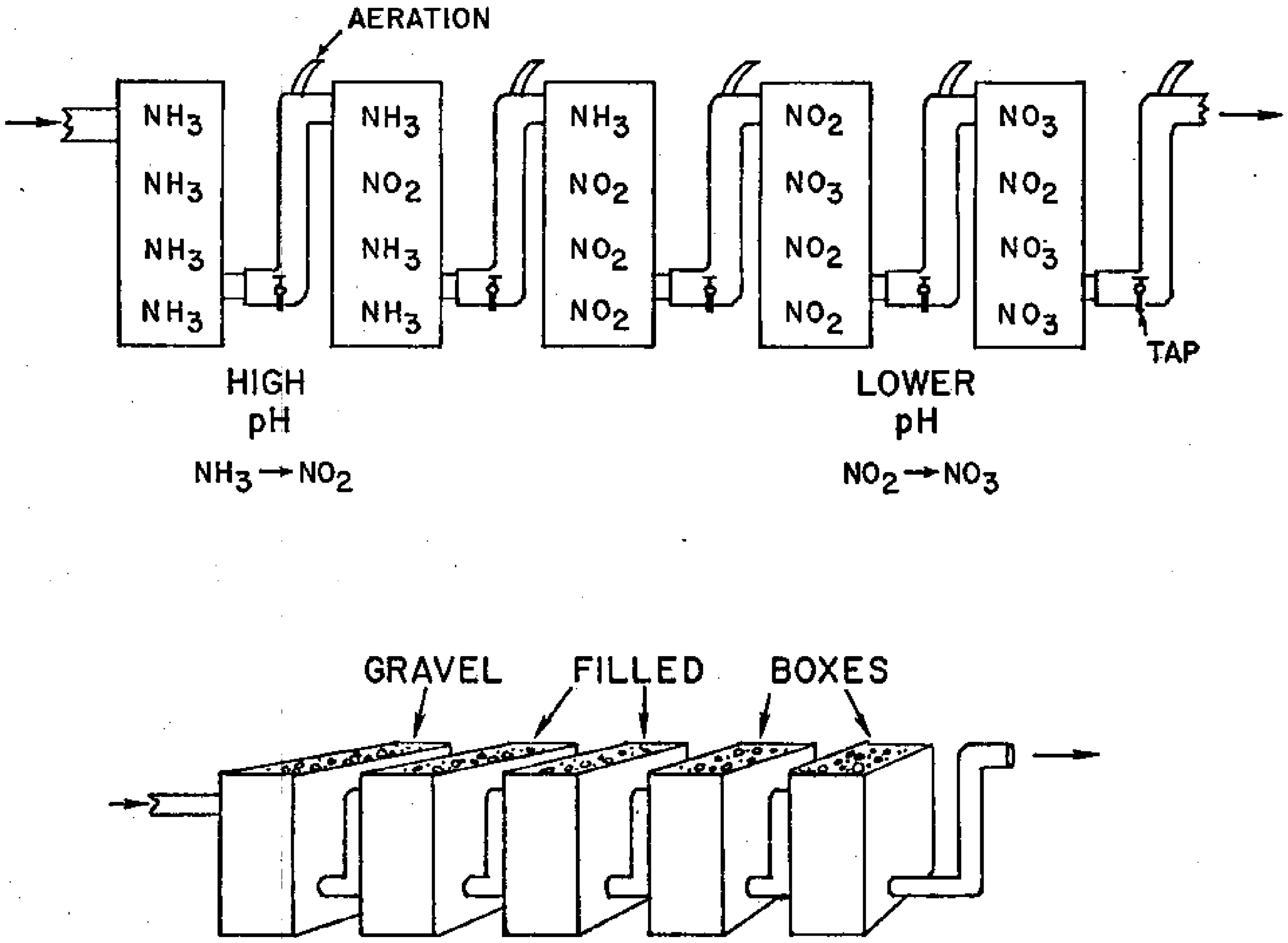


Figure 4. The decrease in ammonia concentration in the battery jar reservoir

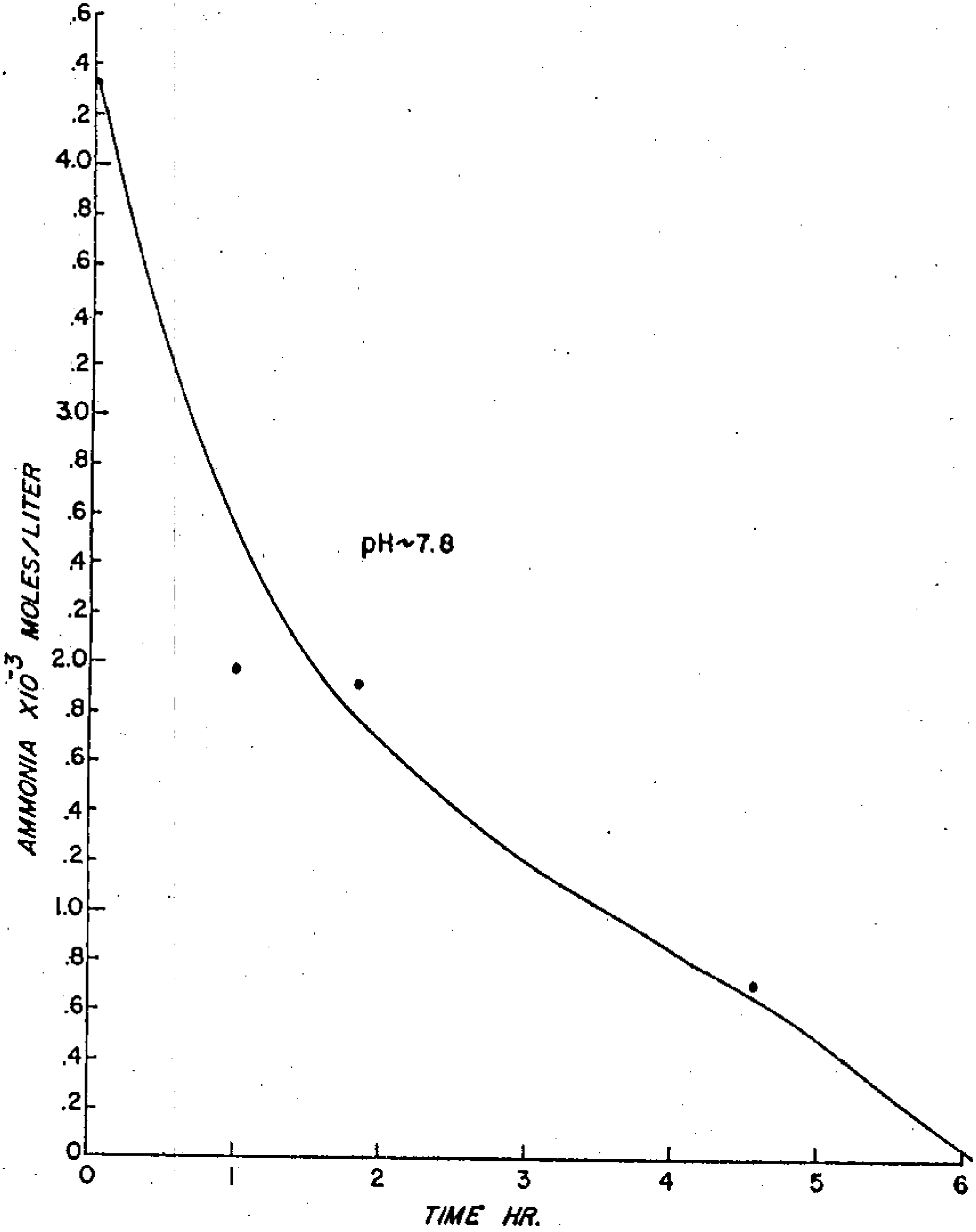


Figure 5. The relative rate of oxidation of ammonia at a pH of 7.13 and at a pH of 7.78

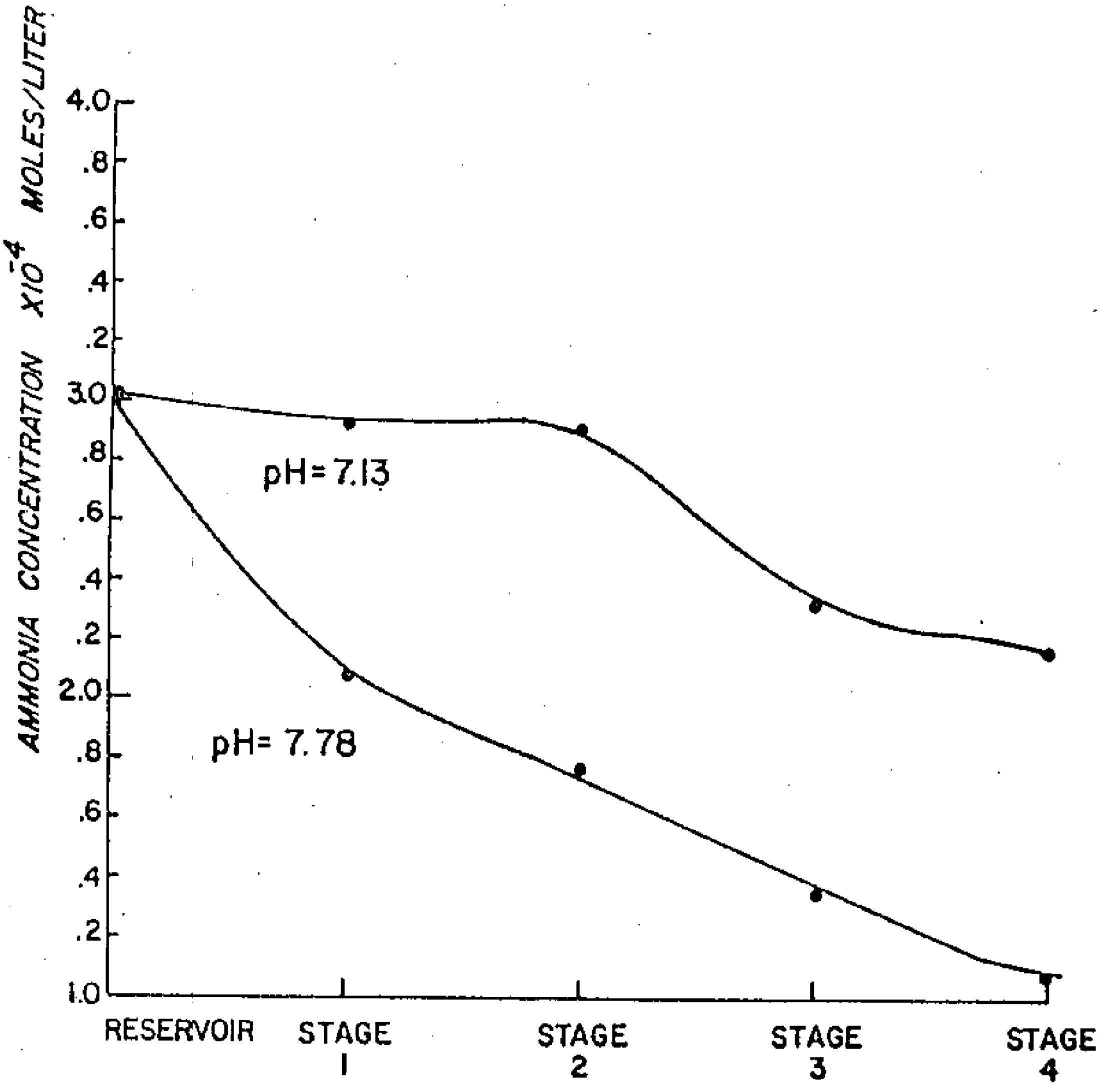


Figure 6. The relative rates of oxidation of nitrite at a pH of 8.20 and at a pH of 6.18

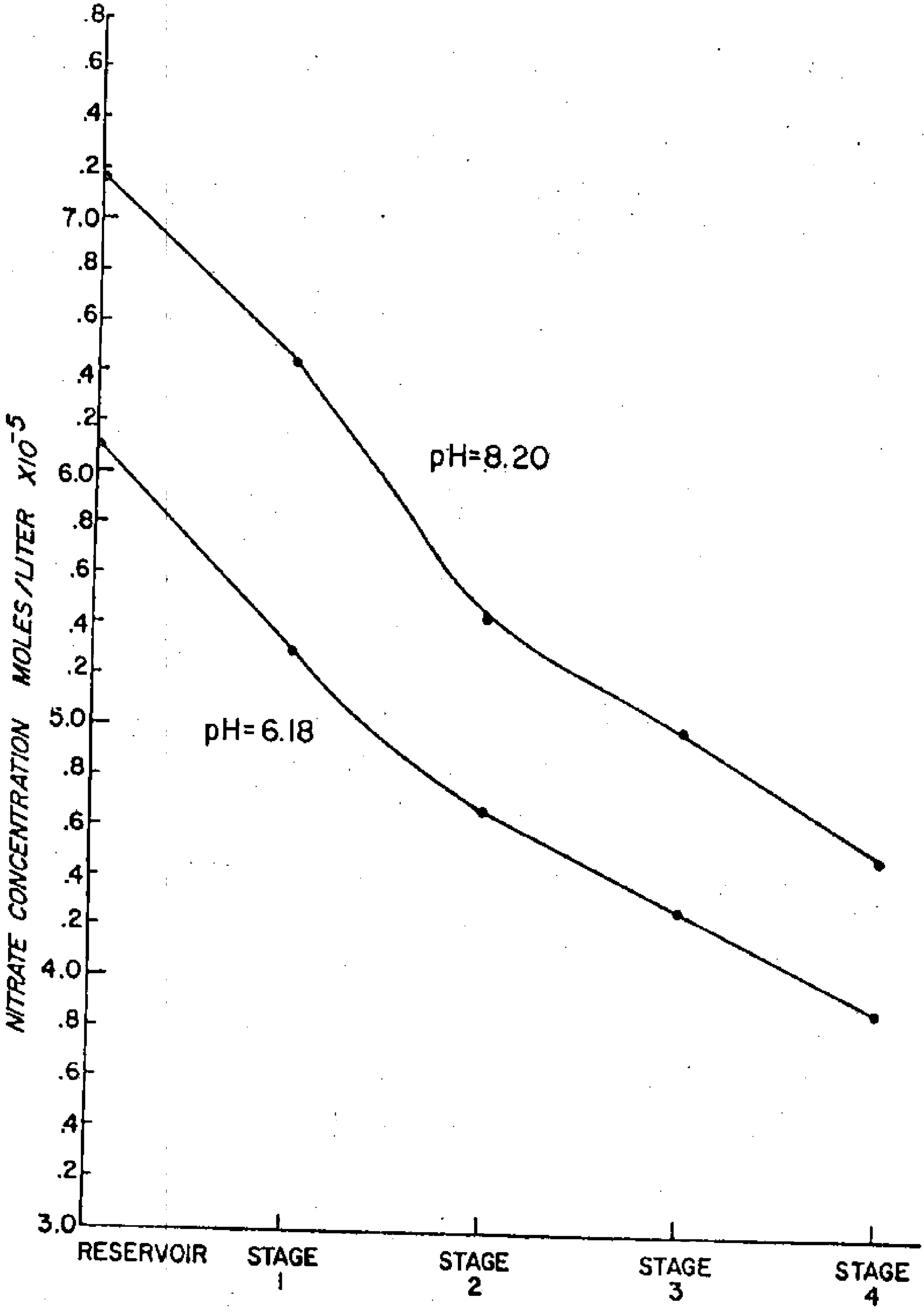


Figure 7. The distribution of ions and the pH in the filter modules

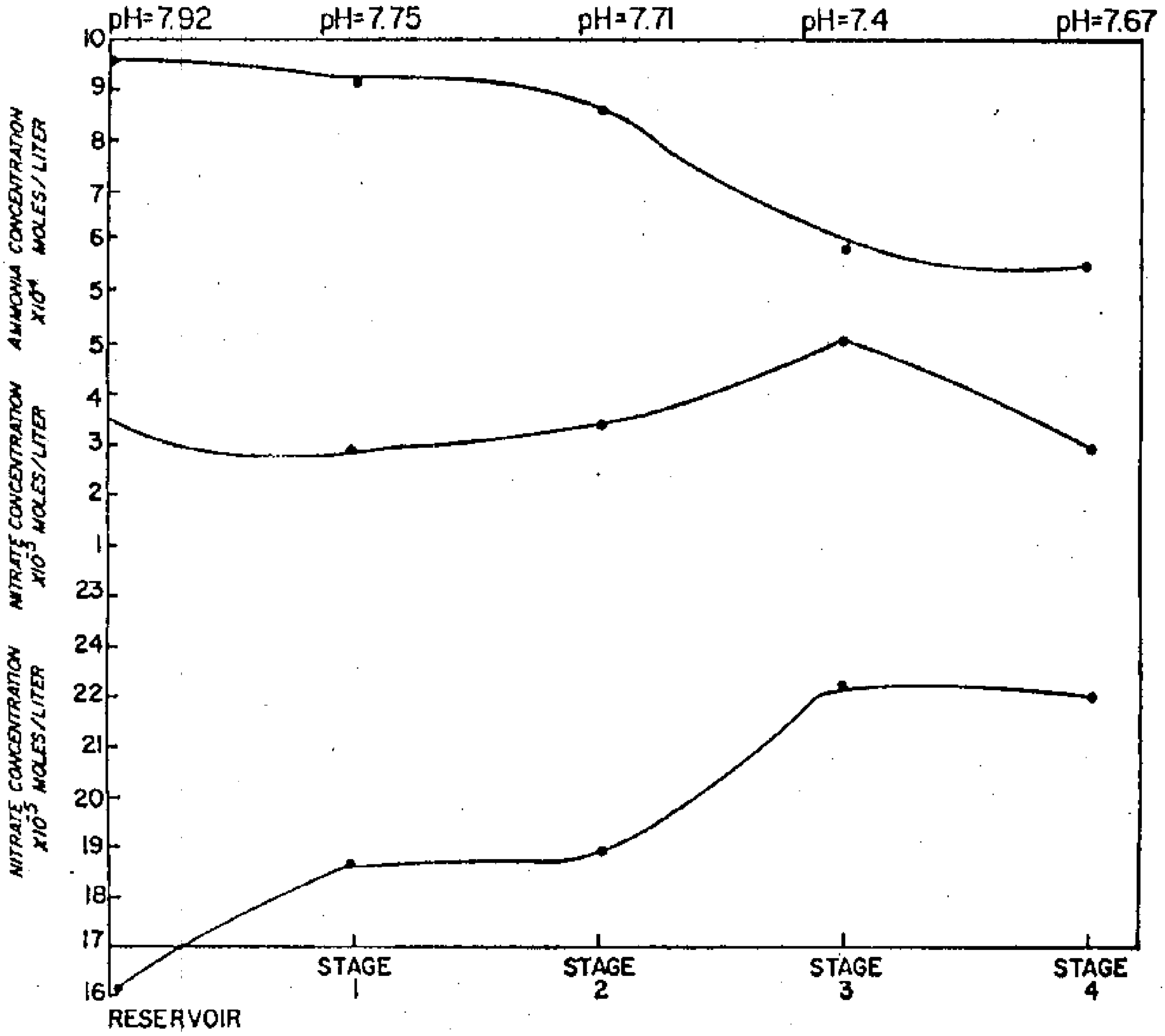
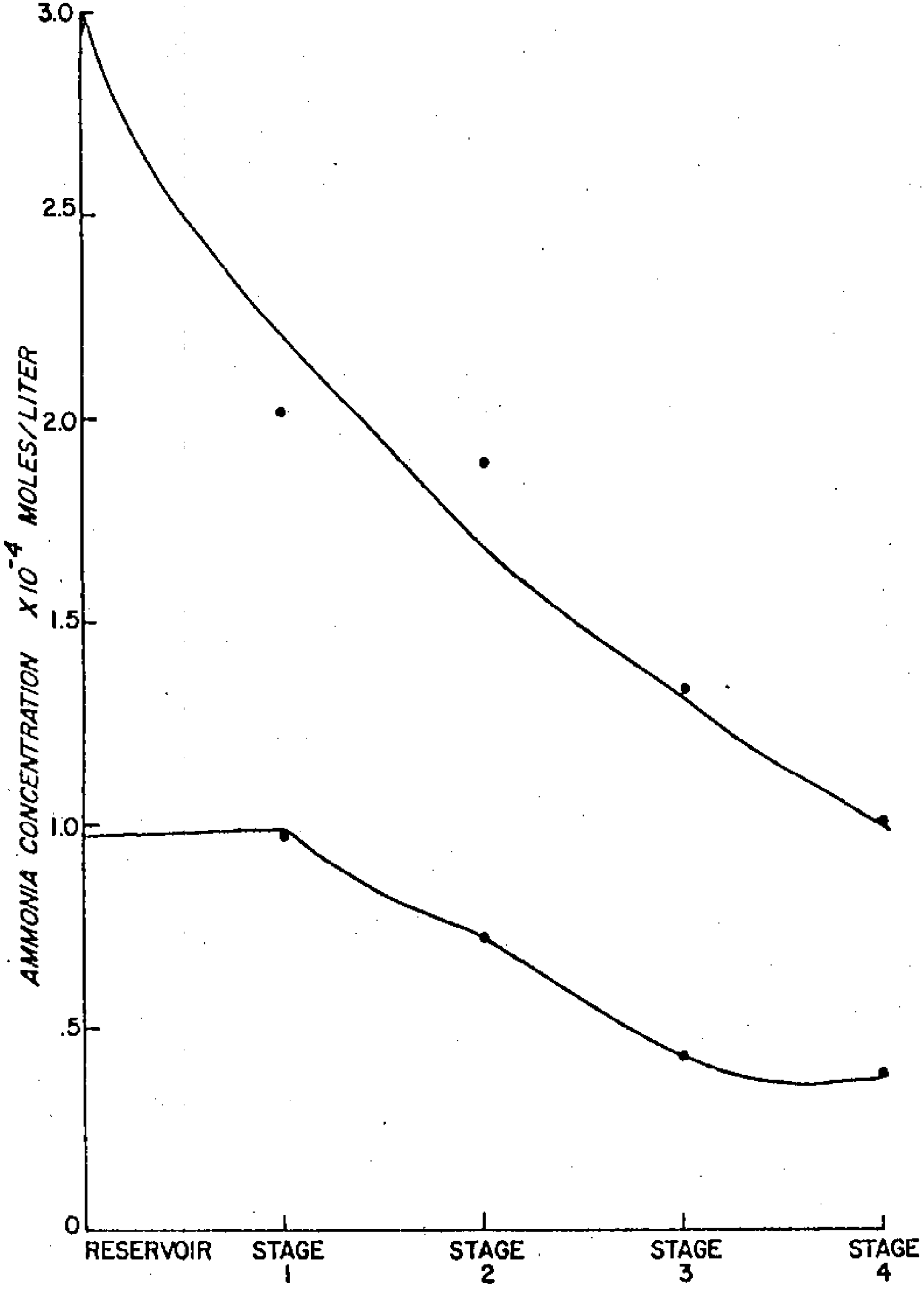


Figure 8. Nitrification in the filter with the first module removed



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