THE EFFECT OF INITIAL FOOD TO MICROORGANISM RATIOS ON AMMONIA SPECIFIC UTILIZATION RATES IN BATCH STUDIES AT VARYING TEMPERATURES AND pHs

By

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k	- Ammonia removal rate, in mg NH_3/liter/minute
U	- Specific utilization rate, in mg NH_3/minute/mg suspended solids
F/M	- Initial food to microorganism ratio, in mg NH_3/mg suspended solids
MLVSS	- Mixed liquor volatile suspended solids
COD	- Chemical oxygen demand
BOD	- Biochemical oxygen demand

CHAPTER I

INTRODUCTION

Organic nitrogen in the form of unassimilated protein matter, such as that found in animal feces, is converted to ammonia by the action of saphrophytic bacteria under either aerobic or anaerobic conditions. The conversion process is called "deamination" (1). The ammonia produced in turn exerts an oxygen demand in oxidation from ammonia to nitrite and nitrate, much the same as the oxygen demand exerted by organic carbon in oxidation to carbon dioxide, called the "nitrogenous oxygen demand" or "NOD." This nitrogenous oxygen demand can and does lower dissolved oxygen levels in streams receiving unnitrified effluents from wastewater treatment plants.

For years, sanitary engineers dismissed the nitrogenous oxygen demand because:

1) Nitrification is caused by special organisms, the population of which is minimal in surface waters.
2) The reaction constant for nitrogenous oxidation is small in relation to the constant for carbonaceous matter.
3) Oxidation of ammonia to nitrates simply converts dissolved oxygen to a form in which it is still available to prevent development of anaerobic conditions (2).

More recently, however, serious attention has been given to the nitrogenous oxygen demand as a critical factor in the overall

maintenance of dissolved oxygen levels in receiving streams.

There are currently three methods found to be feasible for removal of ammonia nitrogen from domestic wastewater: air stripping, ion exchange, and biological nitrification followed by denitrification (3). In the biological treatment process, ammonia is aerobically converted to nitrite then nitrate, and the nitrate is anaerobically converted to free nitrogen. This study is concerned with the biological ammonia removal process, and more specifically with only the aerobic nitrosification process.

Ammonia removal by nitrification will proceed at varying rates. The rate is dependendent upon several parameters, among which are pH and temperature. In this study, ammonia utilization rates were found for various temperatures and pHs in an attempt to assess the effect of temperature and pH on the ammonia utilization rate as a function of the initial ratio of ammonia to suspended solids.

CHAPTER II

LITERATURE REVIEW

The biological nature of nitrification was first demonstrated by Schloesing and Muntz in 1877, when they showed that living organisms were responsible for the conversion of ammonia to nitrate. Pure cultures of the nitrifying bacteria were first isolated by Winogradsky in 1890 (4).

Nitrification takes place in two steps: the conversion of ammonia to nitrite by the Nitrosomonas group, and the conversion of nitrite to nitrate by the Nitrobacter group. Both microbial groups are very small rods that obtain their carbon from carbon dioxide. Many kinds of organic matter are toxic to them, including nitrates (5).

The conversion of ammonia to nitrite is called "nitrosification," and is carried out by the Nitrosomonas as follows:

$$2NH_3 + 3O_2 \xrightarrow[ADP \quad ATP]{} 2HNO_2 + H_2O$$

The conversion of nitrite to nitrate is called "nitrification," and is carried out by the Nitrobacter as follows (6):

$$HNO_2 + 1/2\ O_2 \xrightarrow[ADP \quad ATP]{} HNO_3$$

These reactions provide the nitrifying organisms with energy to

synthesize new cell substances from CO_2 autotrophically. Certain heterotrophic organisms can carry out the oxidation of ammonia to nitrate, but the amounts produced are always very small (1).

It is generally agreed that biological nitrification is, from technical and economic viewpoints, the most feasible method of removing ammonia from wastewater (7), but there are many contradictions and discrepancies regarding conditions necessary for nitrification. Several operating and design parameters have been investigated in attempts to ensure the occurrence of nitrification in a biological wastewater treatment system. There are many discrepancies in results, and still more unanswered questions about the nitrification process.

Downing, Painter, and Knowles (8) report that, from work with mixed cultures, the growth of the nitrifying organisms conforms to the general kinetic equations developed by Monod. They find, however, that predicted rates are somewhat higher than those observed. They also state that in treatment plants it is likely that nitrification will be either virtually complete or will not occur at all; that this phenomenon is due to the extremely rapid decrease in predicted concentration of ammonia in the effluent with increase of aeration time above the minimum value.

The latter statement is debated by Johnson and Schroepfer (9), who note a possible contradiction may be found in actual full-scale plant operation data where partially nitrified effluents are found consistently. They do concede that low night-time loading could conceivably cause this phenomenon.

In their discussion of activated sludge control by mean cell residence time, Jenkins and Garrison (10) state that if "the reciprocal

growth rate of an organism is greater than the mean cell residence time, the organism will not be able to maintain a stable population in the activated sludge flow." They point out that the growth rates of the genus Nitrosomonas spp. are far lower than most of the bacteria in the activated sludge floc, and that nitrification cannot be expected to proceed in a system whose mean cell residence time is less than the reciprocal growth rate of Nitrosomonas spp.

Johnson and Schroepfer (9) also found inconsistent results when operating a continuous flow reactor in the load factor range of 0.35 to 0.80, and maintain that operating in this zone would very likely not yield highly nitrified effluents. There are also likely to be sludge bulking problems in this range, and they recommend 0.25 to 0.35 as a reasonably good range.

In 1972, Prakasam and Loehr (11) found a loading factor range of 0.15 to 0.84 lb COD/day/lb MLVSS resulted in 45 to 60 percent removal of the total Kjeldahl nitrogen in a continuous flow study with solids retention time greater than two days. However, Balakrishnan and Eckenfelder (3) have reported complete nitrification when the organic load factor did not exceed 0.36 to 0.4, providing all other optimum conditions were satisfied; and Beckman Avendt, Mulligan and Kehrberger (12) stated that food to microorganism ratios of 0.25 or less are required for optimum nitrification (note that here food to microorganism ratio pertains to carbon as the substrate rather than ammonia, as found later in this text). It is necessary to point out that these are all reported on combined carbon oxidation-nitrification facilities.

The seemingly most reasonable explanation of organic compound inhibition of nitrification comes from Downing, et al. (8). They

explain it with the fact that the presence of organic compounds tends to decrease concentrations of dissolved oxygen to below the threshold value for nitrification, at least locally in the sludge tank.

Beckman, et al. (12) conclude that nitrification is attainable in a combined carbon oxidation-nitrification sludge facility with dissolved oxygen maintained above 2.0 mg/l, but Ludzack and Ettinger (13) find that relatively high BOD:N ratios decrease the overall process advantages.

Wild, Sawyer, and McMahon (2) conclude that it will be mandatory to accomplish nitrification in a separate biological system, so that the reciprocal growth rate can be kept less than the mean cell residence time at all times. This will mean that a large part of the normal BOD will have to be removed before the wastewater enters the nitrification unit. They consider the minimum dissolved oxygen level to be 1.0 mg/l, and note no apparent inhibition of nitrification for various BOD concentrations. It is pointed out, however, that the average BOD concentration of the feed would have an effect on the percent MLVSS composed of nitrifiers, and therefore available for nitrification.

In the literature explored, it was generally agreed that a minimum sludge age of three days would be required for nitrification (3)(12). Balakrishnan and Eckenfelder found the minimum sludge age to be three to four days at 23°C, and Beckman, et al. found it to be three days at 18.3°C. Prakasam and Loehr (11) find a sludge retention time of two days to be adequate for 45 to 60 percent removal of total Kjeldahl nitrogen.

There is a marked reduction in the minimum period of aeration required to achieve nitrification as the concentration of sludge is

increased, as documented by Downing, et al. (8). Balakrishnan and Eckenfelder's (3) work supports a minimum hydraulic retention time of eight hours, but Johnson and Schroepfer (9) maintain retention times as low as two to three hours may be adequate, providing the load factor was of the 0.25 to 0.35 magnitude. Their work shows that the detention time is not in itself a complete parameter for establishing limits of operation in order to obtain highly nitrified effluents.

Reports on the optimum temperature for biological nitrification vary. Ludzack and Ettinger (13) make the general comment that low-temperature operation results in lower sludge density and reduced nitrification. Downing, et al. (8) state that results from a continuous flow reactor study show the growth rate constant roughly doubles for each ten degrees Centigrade increase in temperature from six to 25°C. Wild, et al. (2) maintain their results are in reasonable agreement with the van't Hoff-Arrhenius law in a temperature range of five to 30°C, and Beckman, et al. (12) report an optimum temperature of 18.3°C.

According to the microbiologists, the optimum pH for the nitrifying organisms is vague but should lie somewhere on the alkaline side of neutrality. The literature reviewed upholds the "vagueness" of the optimum pH.

Beckman, et al. (12) report no effect in a pH range from seven to eight. Wild, et al. (2) report optimum to be 8.4 with 90 percent of the maximum rate occurring in the 7.8 to 8.9 range, and less than 50 percent of the optimum outside the 7.0 to 9.8 range.

Prakasam and Loehr (11) maintain that pH control is unnecessary. Nitrification was maintained at a pH of 4.9, and in adjusting the units to values varying from five to 11, the degree of nitrification did not

increase.

Stankewich reports that these discrepancies could be attributable to acclimation of the organisms: "....although nitrifying organisms initially are preferential to a pH environment of 7 to 8, they will acclimate to lower pH environments and reestablish their maximum growth rate" (7).

Barth, Mulbarger, Salvotto, and Ettinger (14) made a series of field studies to determine whether deliberate modifications might increase nitrogen removal in municipal waste water treatment plants, and concluded that removal of nitrogen by the conventional treatment processes is erratic and is not correlated with carbon or solids removal. This report was published in 1966. In 1971, Mulbarger (15) did some further work in this area, and concluded that a three-sludge system was the best for nitrogen removal. While nitrification and denitrification could be achieved and maintained by other processes, only the three-sludge system provided optimum, dependable, and consistent performance; he feels its superior performance is directly attributable to the use of isolated, optimized sludge cultures. He recommended a 10 to 15-day mean cell residence time and a 2.5 to 3.0-hour hydraulic retention time. "Mixing carbon removal with nitrification not only results in a loss of soluble carbon removal efficiency but necessitates a longer aeration time for nitrification" (15).

CHAPTER III

MATERIALS AND METHODS

Laboratory Apparatus

The biological reactor consisted of a two-liter pyrex Erlenmeyer flask, the liquid volume of which was maintained at approximately 1500 ml. Oxygen and carbon dioxide were supplied to the reactor through two glass rod gas diffusers placed near the bottom of the flask and attached through a cotton filter to a compressed air supply in the laboratory. (Figure1 is a schematic of the laboratory apparatus.) A magnetic stirring mechanism with a 1-5/8-inch teflon-coated steel bar stirring rod in the reactor was used to ensure complete mixing in the tests at room temperature. For tests at other than room temperature, the flasks were placed in water baths with a water level as nearly as possible the same as the liquid level in the flask.

Culture Media

Stock solutions of ammonia, salts, and buffer were prepared and stored in a refrigerator at 40^{o}F until needed. The ammonia stock solution consisted of 3.88 grams $(NH_4)_2SO_4$ in one liter distilled water; the salt solution of 20.0 g $MgSO_4 \cdot 7H_2O$, 0.1 g $FeCl_3 \cdot 6H_2O$, 2.0 g $MnSO_4 \cdot H_2O$, and 1.5 g $CaCl_2$ diluted to two liters with tap water, and the buffer solution of 105.4 g KH_2PO_4 and 214 g K_2HPO_4 diluted to two liters

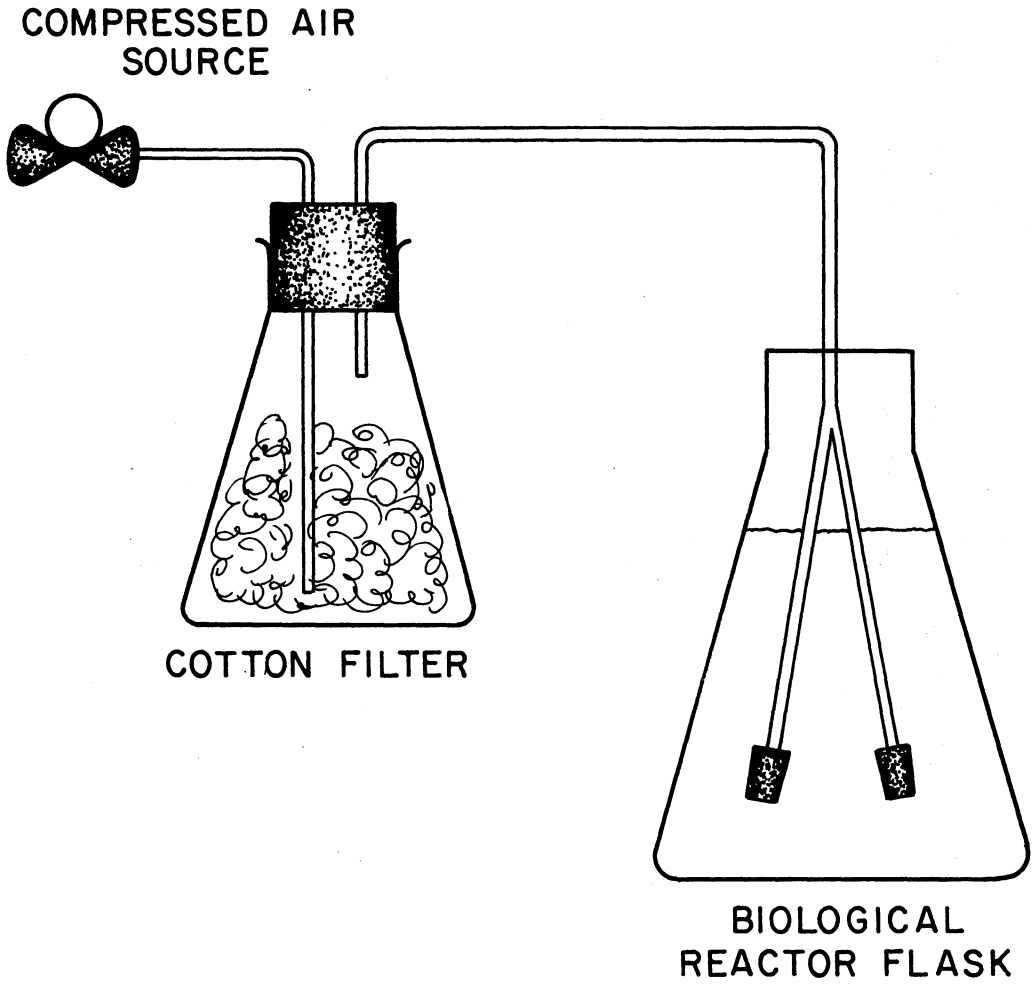

Figure 1. Schematic of Laboratory Apparatus

with distilled water. The feed solution was then mixed using 10 ml stock salt solution, 10 ml stock buffer solution, and 30 ml ammonia solution diluted to 1000 ml with distilled water. When required, pH adjustment was made by adding 0.1 N H_2SO_4 and NaOH to the feed solution titrimetrically with the aid of a magnetic stirring apparatus and a pH meter.

Experimental and Analytical Procedures

Seed organisms were obtained from the second stage of a two-stage activated sludge system in operation in the laboratory. Feeding the unit was accomplished by removing the air diffusers and allowing the solids to settle (for a time varying from 20 minutes to one hour), then pouring off approximately two-thirds of the original volume and adding feed solution. Typically, this meant decanting to 500 ml and adding 1000 ml feed solution.

The biological solids concentration was determined gravimetrically by filtration of 25 ml of sample through membrane filters (0.45 micron size by the Millipore Corporation) with the aid of a vacuum pump. Filters were placed in aluminum pans weighing approximately 1.5 g each and allowed to dry for one hour at 103°C, then cooled in a desiccator to a constant weight. The weights of the tares were recorded, and after use in filtration the filters were returned to the aluminum pans to once again be dried at 103°C to a constant weight (approximately two hours), cooled in a desiccator and weighed. Biological solids concentration was calculated using the weight differential.

The filtrate was stored in 20-ml vials at 40°F until the ammonia-nitrogen concentration determination was developed by Niss and described

by Ecker and Lockhart (16). Two reagents were employed:

Reagent A

4.7 g sodium citrate	96 g phenol
1.7 g citric acid	and distilled water to 480 ml

Reagent B

6.0 g boric acid	30 ml commercial Clorox bleach
8.0 g sodium hydroxide	and distilled water to 200 ml

The cell-free samples were diluted, if necessary, to the range of 2-20 mg/l of ammonia-nitrogen. To 1.0 ml of sample was added 5.0 ml of Reagent A and 2.0 ml of Reagent B. The samples were then mixed and heated in a boiling water bath for five minutes and cooled rapidly in ice water. Optical density was determined using a Bausch and Lomb Spectronic 20 spectrophotometer at a wave length of 615 against a distilled water blank. Optical density readings were compared to a standard curve plotted using known concentrations of $(NH_4)_2SO_4$ prepared immediately before the ammonia-nitrogen determinations.

A stock culture of microorganisms was maintained at $20^{o}C$ and a pH of 7.4 during the pH variation studies to serve as a partially controlled source of organisms for the varying conditions. When pH 8.0 was found to be closer to the growth optimum, the stock culture was changed to pH 8.0 for the duration of the temperature variability studies.

A seed culture was allowed to acclimate to the new environmental conditions (change in temperature or pH) for approximately one week before samples were taken. The biological reactors were fed once every

24 hours in the manner previously described. Time zero for the test runs was taken to be that instant the food was mixed into the decanting residual. Twenty-five milliliters of liquid was withdrawn at each designated sampling time and immediately filtered, also in the manner previously described. Samples were taken at time 0, 30 minutes, one hour, two hours, three hours, four hours, five hours, and eight hours.

Studies to determine optimum pH were run at the control temperature ($20^{o}C$). pHs tested include 6.5, 7.0, 7.4, 8.0, and 8.5. Those to determine optimum temperature were run at pH 8.0 and temperatures of 10, 20, 30, and $40^{o}C$.

It should be pointed out that the 25-30 mg/l ammonia concentration in the feed solution was not arbitrarily chosen--a series of tests with ammonia concentrations as high as 1000 mg/l were made at the initial control pH (7.4) and temperature ($20^{o}C$) to establish a workable feed concentration, and 25-30 mg/l proved to be the concentration at which removal to less than one mg/l residual ammonia concentration could be achieved in the batch system.

CHAPTER IV

RESULTS

Ammonia feed concentration was not arbitrarily chosen to be 20 mg/l; rather, tests were run to establish the best initial ammonia concentration for continued removal. Data from one such test is shown in Figure 2. The most rapid removal with the least residual NH_3 was at an initial NH_3 concentration of near 25 mg/l. The 50 mg/l initial concentration test flask had a higher residual NH_3 concentration, and the 100 mg/l flask showed no apparent removal. Specific utilization rates for the 25 and 50 mg/l tests are 0.0162 and 0.00282×10^{-3} mg NH_3/minute/mg suspended solids.

Ammonia concentration in mg/l was arithmetically plotted versus time in minutes for each batch test. The slope of the line plotted is the removal rate, k, in mg/l/min. A typical plot is shown in Figure 3.

Cell yield (mg organisms produced per mg substrate utilized) for the nitrifiers is low--in the range of 0.15 to 0.25. The low cell yield coupled with the low chosen initial ammonia concentration available for utilization by the microbes netted a very low potential change in suspended solids concentration in any of the given tests; hence, change in solids concentration was considered negligible and an average suspended solids concentration was used in calculating the specific utilization rate as well as the initial food to microorganism ratio.

The specific utilization rate, U, is equal to the ammonia removal

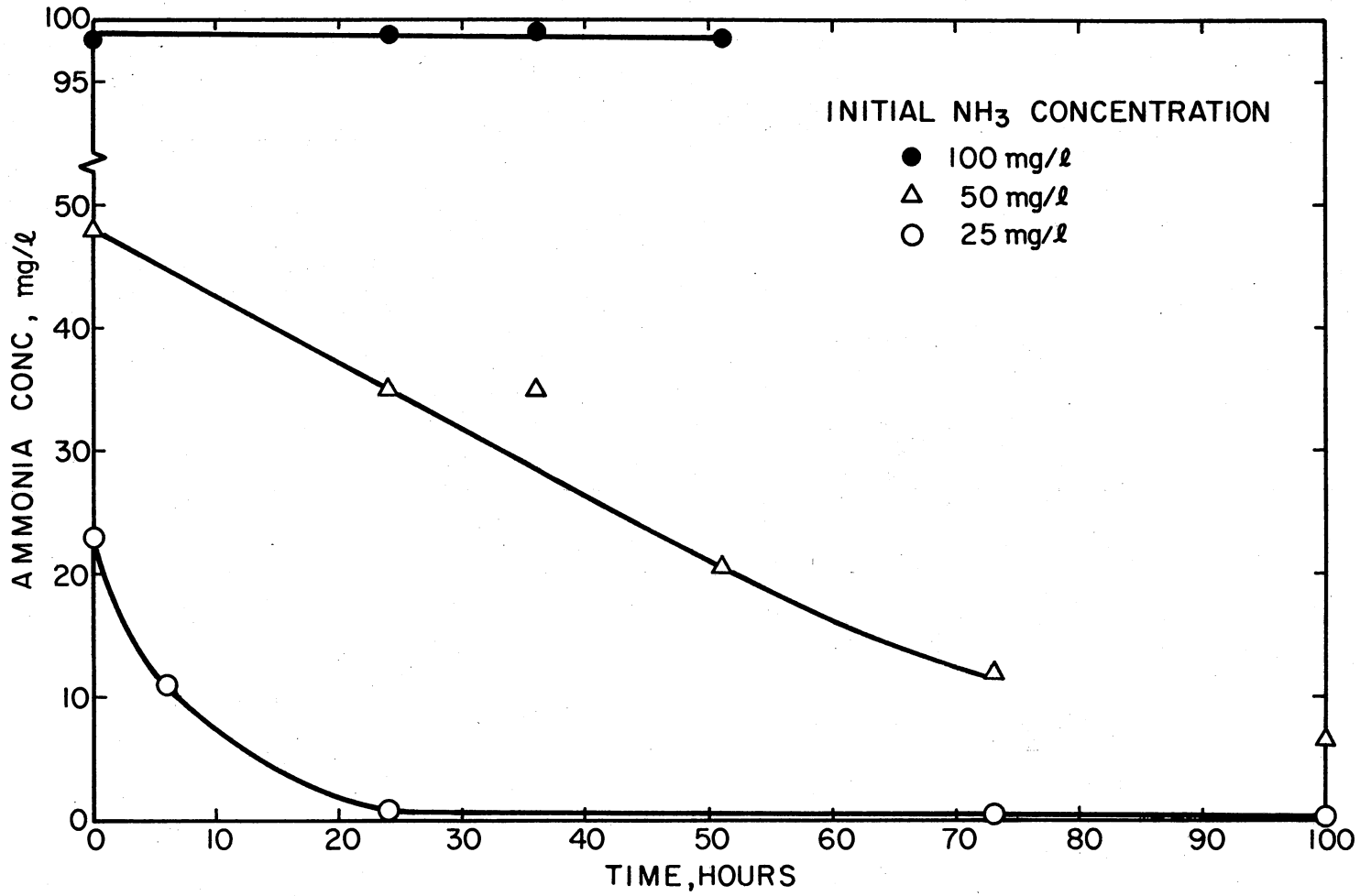

Figure 2. Three Test Runs - Ammonia Residual versus Time

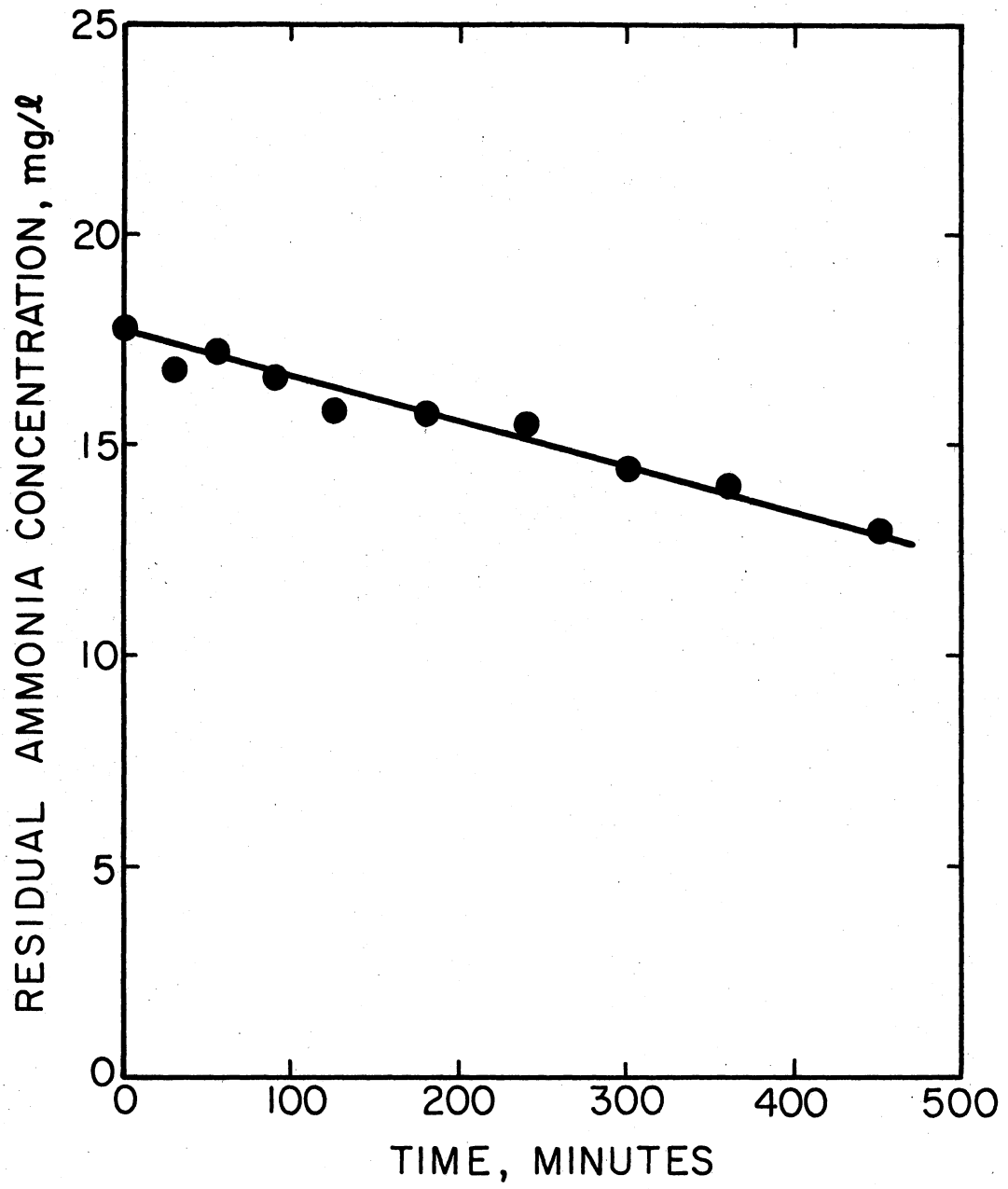

Figure 3. Residual Ammonia versus Time - Batch Study at 30°C and pH = 8.0

rate in mg NH_3/l/min divided by the suspended solids concentration in mg/l; hence, U has units of mg NH_3/min/mg suspended solids. The initial food to microorganism ratio (F/M) is found by dividing the initial ammonia concentration in mg/l by the suspended solids concentration in mg/l. Here F/M has units of mg NH_3/mg suspended solids.

In Tables I and II is presented a summary of the initial ammonia concentration, average suspended solids, and the calculated values of k, U, and F/M for each of the test runs.

Not only was there a low potential for solids concentration increase during the tests, but also for solids concentration increase in the batch unit while not being tested. With samples taken during test runs depleting the amount of solids in the flasks and a certain amount of solids inadvertently wasted during the feeding process, solids concentration generally stayed about the same or decreased over an extended period of time.

The change in the specific utilization rate (U) with the initial F/M for the pH values tested is shown in Figure 4. It can be seen that the specific utilization rate increases as the initial F/M increases. It can also be seen that in general as the pH increases, the specific utilization rate also increases. The maximum specific utilization rates occurred at a pH of 8.5. It can also be observed that the rate of change in U versus F/M is a function of pH.

The change in the specific utilization rate with the initial F/M for various temperatures is shown in Figure 5. Again, it can be seen that the specific utilization rate increases as the initial F/M increases. It can also be seen that the specific utilization rate increases as the temperature increases from $10^{o}C$ to $30^{o}C$, but at a

TABLE I

VARIABLE pH TEST RESULTS

pH	Initial NH_3 Conc (mg/l)	Initial Suspended Solids Conc (mg/l)	Initial F/M	k mg NH_3/min $X10^{-3}$	U mg NH_3/min mg Solids
6.5	31.0	54.1	0.573	7.50	0.139
6.5	28.0	533.9	0.052	8.96	0.017
6.5	26.0	298.6	0.087	3.94	0.013
7.0	31.0	37.6	0.825	5.71	0.152
7.0	30.0	47.0	0.638	7.14	0.152
7.0	27.5	163.1	0.169	17.86	0.11
7.4	33.5	43.8	0.765	17.5	0.4
7.4	27.5	239.9	0.115	20.0	0.083
7.4	26.0	69.0	0.377	16.67	0.242
8.0	22.3	77.1	0.289	24.52	0.318
8.0	20.3	47.7	0.426	19.38	0.406
8.5	25.5	20.8	1.226	20.83	1.002
8.5	37.3	70.0	0.533	49.43	0.706

TABLE II

VARIABLE TEMPERATURE TEST RESULTS

Temperature °C	Initial NH_3 Conc (mg/l)	Initial Suspended Solids Conc (mg/l)	Initial F/M	k mg NH_3/min $X10^3$	U mg NH_3/min mg Solids
10	23.5	17.5	1.343	5.95	0.340
10	20.3	31.0	0.655	4.79	0.155
10	21.0	35.0	0.600	5.88	0.168
10	20.6	56.3	0.366	4.44	0.079
20	22.3	77.1	0.289	24.5	0.318
20	20.3	47.7	0.426	19.4	0.406
30	17.5	44.2	0.396	28.7	0.649
30	27.4	38.4	0.714	28.1	0.732
40	24.4	117.4	0.208	6.15	0.052
40	24.1	101.5	0.237	6.67	0.066
40	23.9	85.5	0.28	5.43	0.063

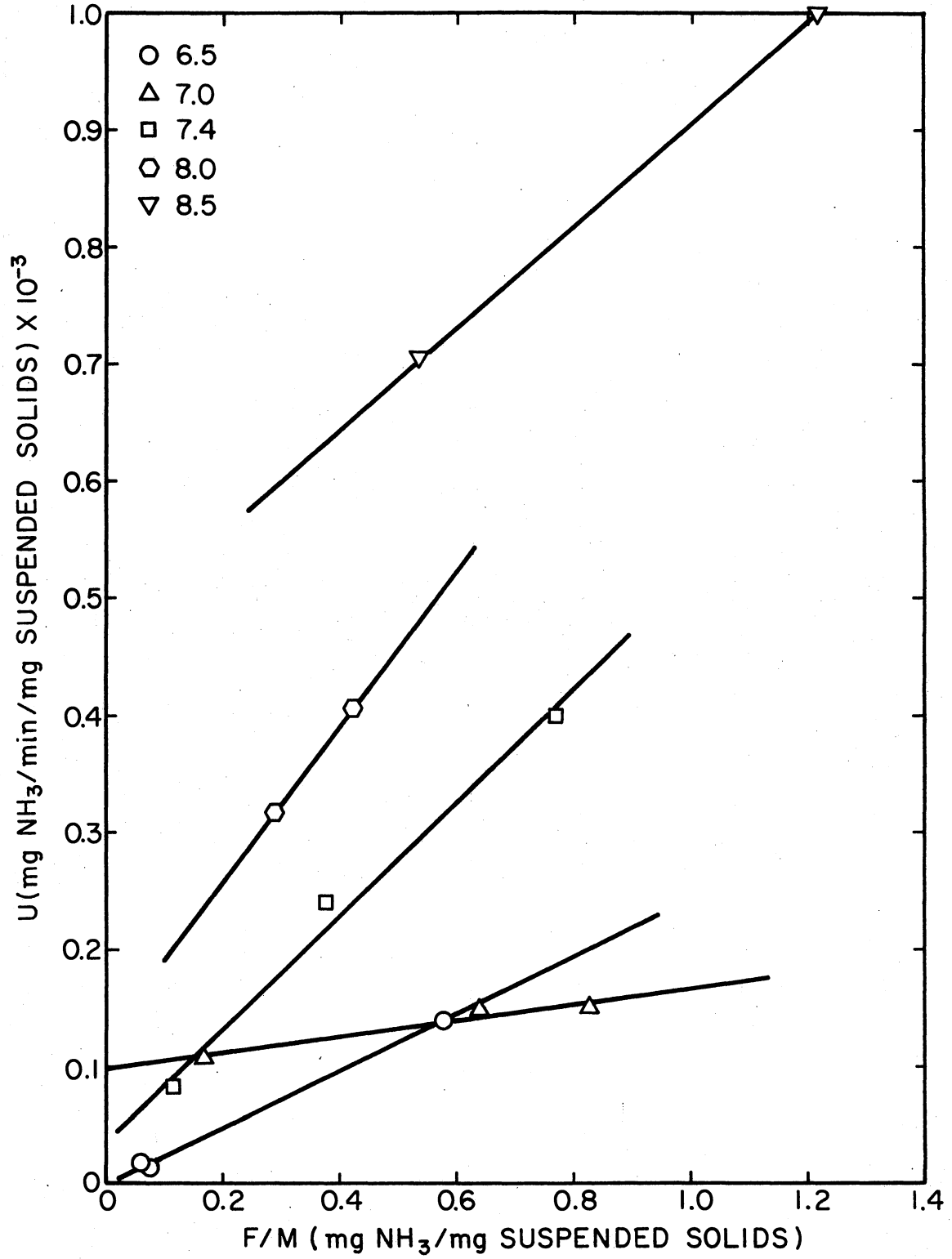

Figure 4. U versus F/M for Various pHs

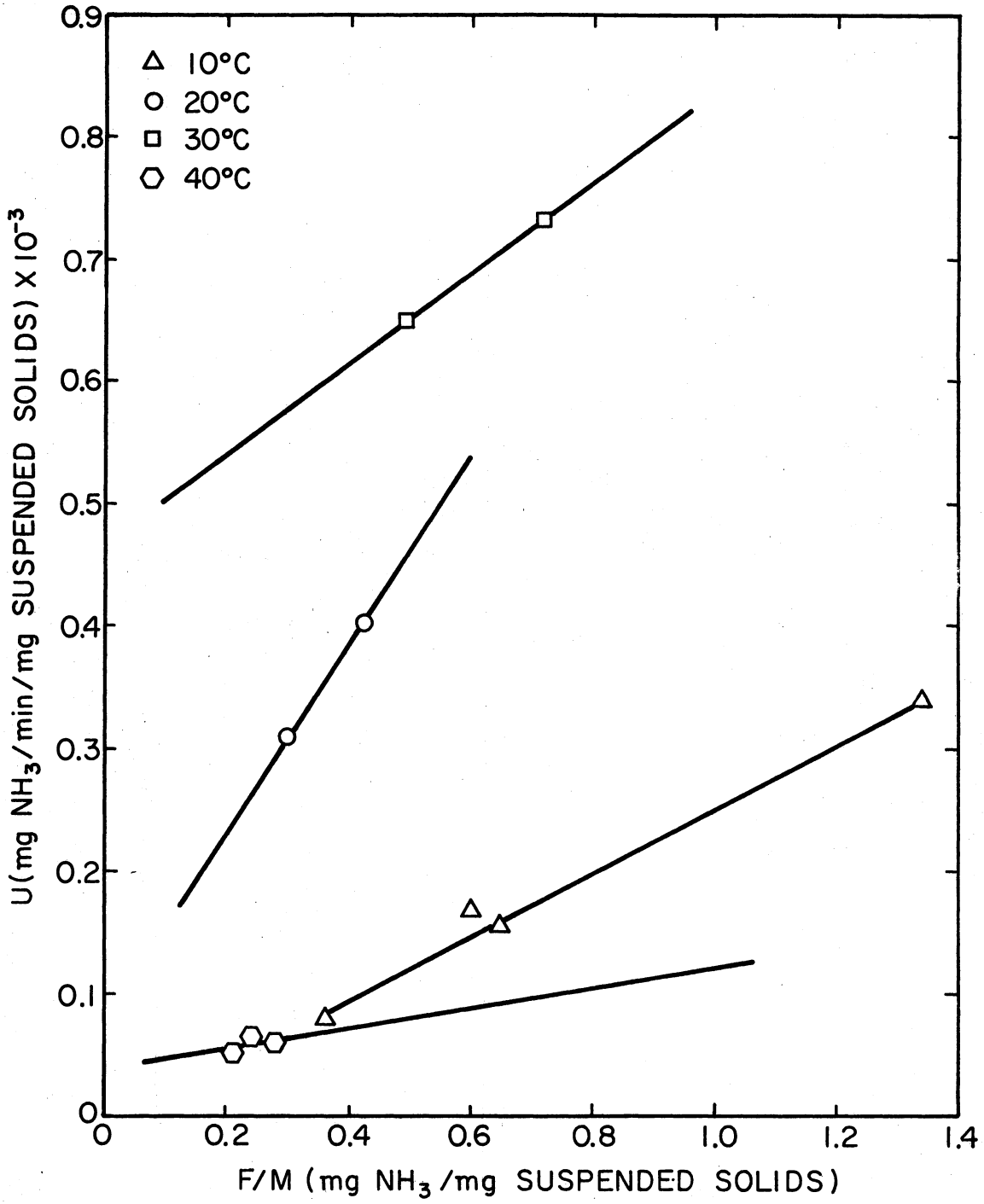

Figure 5. U versus F/M for Various Temperatures

temperature of 40°C, the specific utilization rates are less than those for 10°C. Also, it can be seen that rate of change in the specific utilization rates with F/M is different for the various temperatures.

Figure 6 shows the change in the specific utilization rate for the various pH values studied. It can be seen that the specific utilization rate increases from a low value at pH = 6.5 to a high at pH = 8.5 if constant F/M values are maintained.

In Figure 7 the specific utilization rate is plotted against the various temperatures studied. It can be seen that the specific utilization rate varies with the temperature. The maximum specific utilization rates resulted from a temperature of 30°C.

It was observed that the slopes of the plots of U versus F/M varied for the pH values and temperatures studied. This variation is shown in Figures 8 and 9. Figure 8 shows that the maximum rate of change of U in regard to F/M occurs at a pH of 8.0, and Figure 9 shows the maximum rate of change also occurs at a temperature of 20°C.

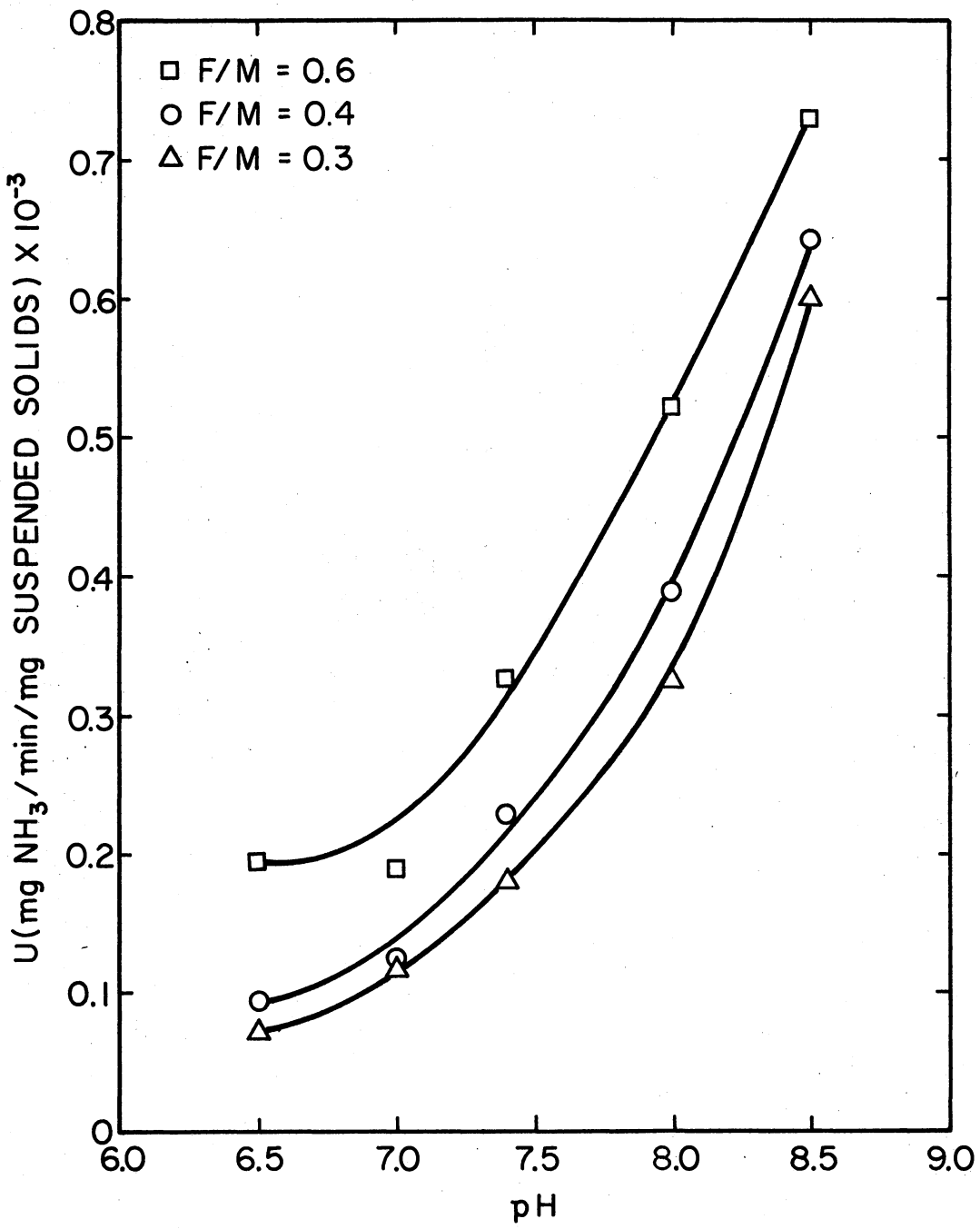

Figure 6. U versus pH for Specific F/Ms

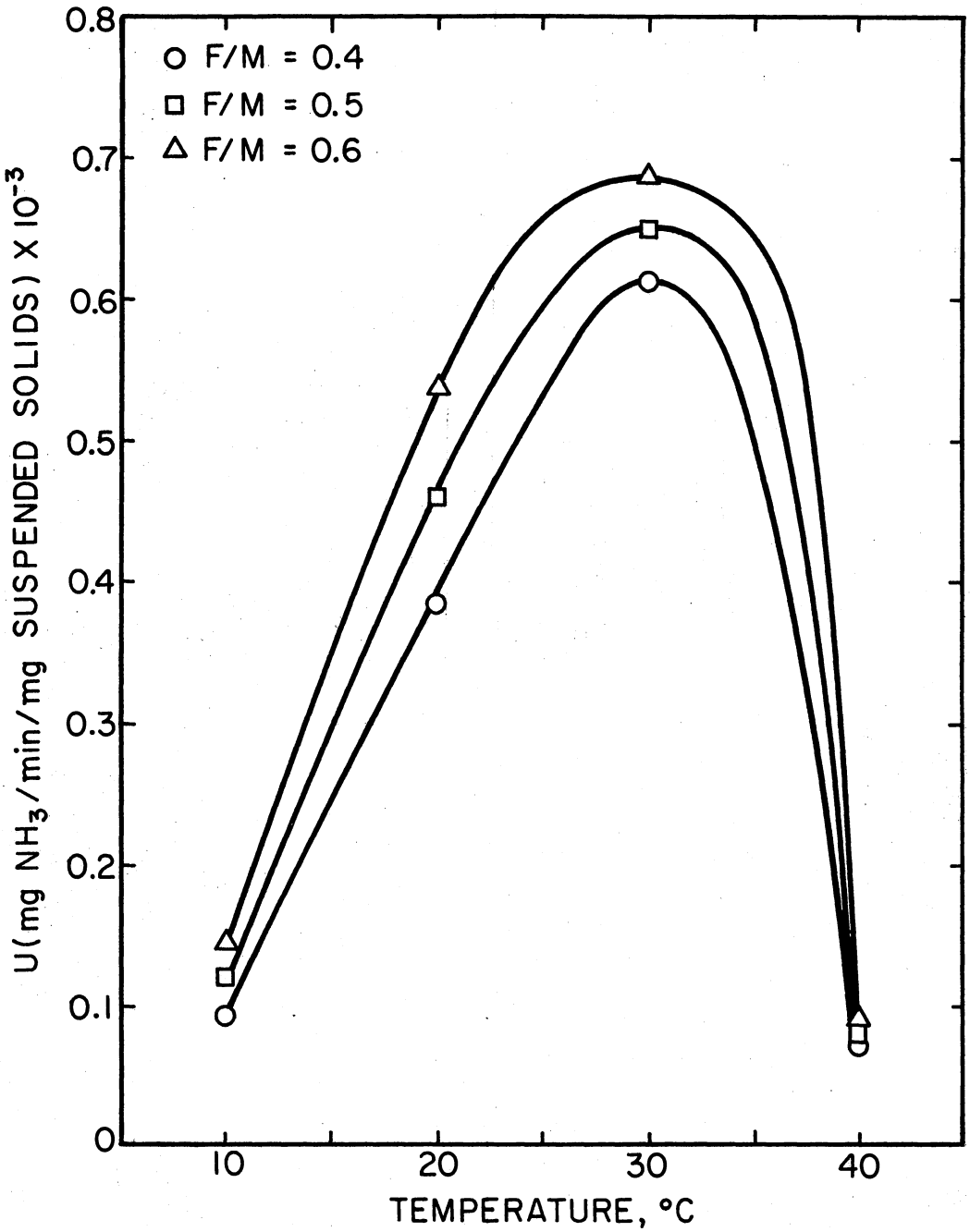

Figure 7. U versus Temperature for Specific F/Ms

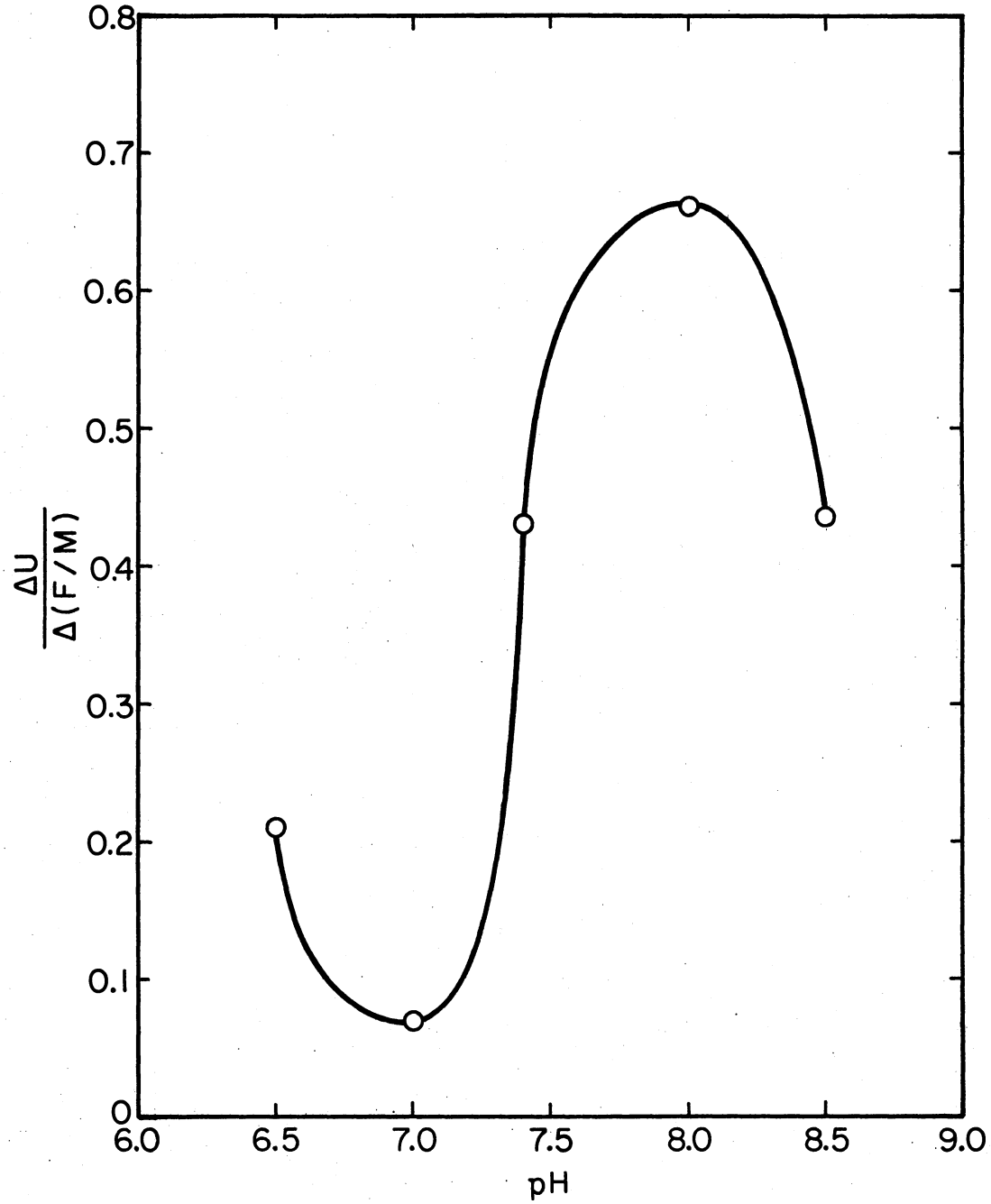

Figure 8. $\Delta U/\Delta(F/M)$ versus pH

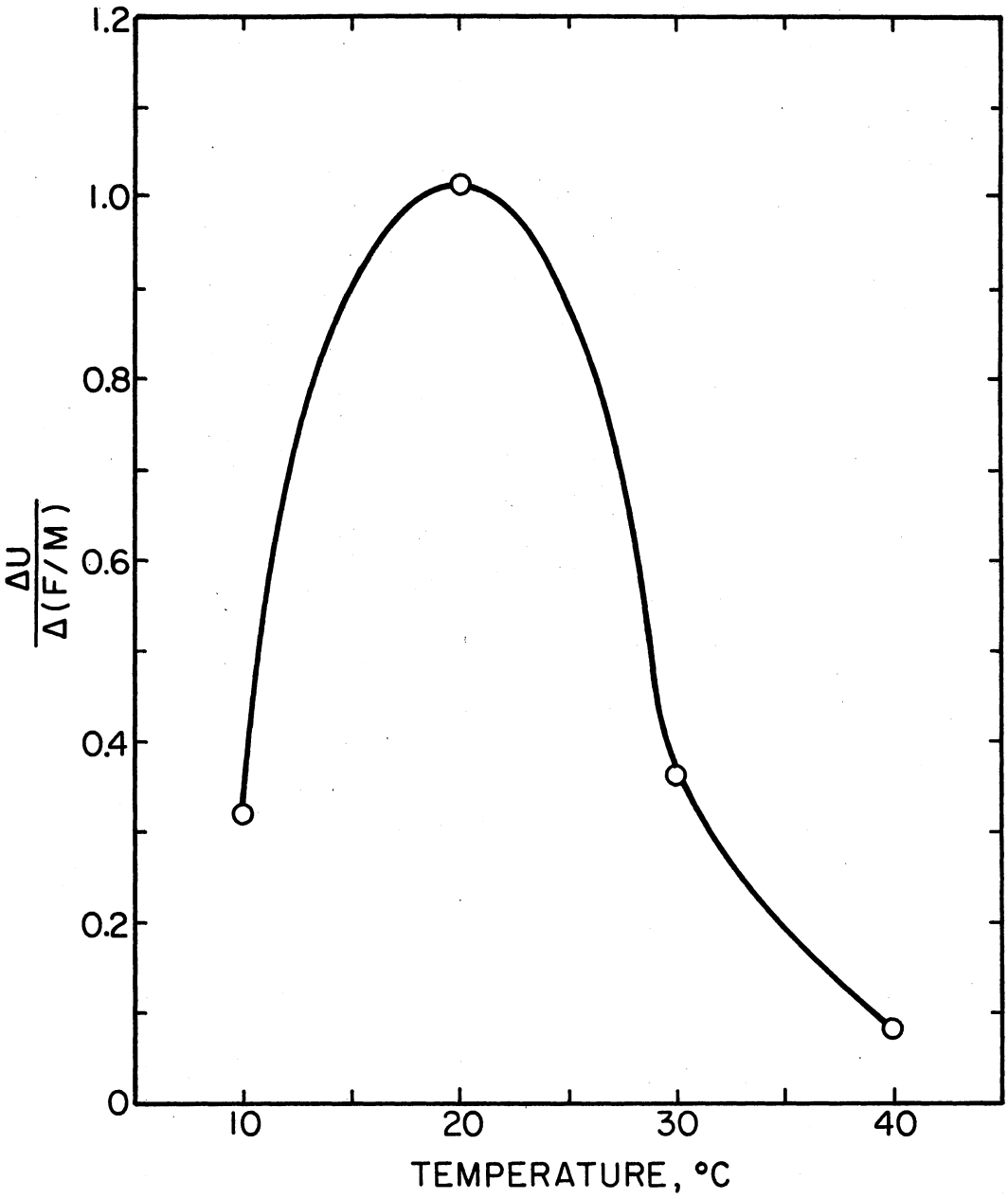

Figure 9. ΔU/Δ(F/M) versus Temperature

CHAPTER V

DISCUSSION

In the tests to establish a workable feed ammonia concentration, removal to less than 1.0 mg/l residual ammonia was achieved in the 25 mg/l flask in less than 24 hours, and removal to 6.5 mg/l residual ammonia was achieved in the 50 mg/l flask in 100 hours. No measurable reduction of ammonia concentration was found in the 100 mg/l test. Prakasam and Loehr (11) noted that the rate of nitrification decreased with an increased ammonia nitrogen concentration. They suggest the possibility of the inhibition of oxidation of ammonia by repression of the enzyme synthesis by the nitrites and nitrates formed in the oxidation itself. The limited data from the 50 and 25 mg/l tests discussed above is supportive of those statements. The lack of measurable ammonia removal in the 100 mg/l test also supports Prakasam and Loehr, although there is a wide variety of other possible causes for what appears to be the death of the culture.

In the plot of U versus F/M for various pHs (Figure 4), pH 8.5 exhibits the greatest specific utilization in the range of the test; that is, of the pHs tested, pH = 8.5 exhibits the greatest ammonia removal per minute per mg of suspended solids. The slopes of the lines plotted in Figure 4 are an indication of the dependence of the culture on the F/M for specific utilization, the change in specific utilization divided by the change in initial food to microorganism ratio. The plot

of these slopes versus pH is shown in Figure 8. Obviously, the most F/M-dependent culture is pH = 8.0, while the least dependent is pH = 7.0. This is shown once again in Figure 7, where the vertical distance between the curves is an indication of the magnitude of the effect of F/M change on specific utilization rate in the pH range studied.

In Figure 5, specific utilization is plotted versus initial food to microorganism ratio for the temperatures tested. The maximum specific utilization rates in the test range were 30°C. It is entirely possible that at an F/M greater than 1.0, a system operating at 20°C would achieve a greater specific utilization rate, but there is no data from this study to support that argument. In the plot of slopes (change in U per change in F/M) versus temperature, Figure 9, a maximum dependence of the specific utilization rate on F/M is seen at 20°C. This is shown again in the plot of U versus temperature for three F/Ms, Figure 7.

Since these studies were all done in batch reactors on a small scale and at very low suspended solids concentrations, it would be difficult to maintain that the results presented here could be used in any way to scale up specific utilization rates to apply to a large-scale waste water treatment system, particularly since essentially no carbonaceous oxygen demand was present in the reactor and would most probably be present in an activated sludge system (even a two-stage activated sludge system). The results do indicate, however, that the specific utilization rate is a function of the initial food to microorganism ratio, and that the specific utilization rate generally increases with increasing F/M. There most probably exists a maximum F/M beyond which the system fails, but none was found in the course of this study. It should be pointed out that the F/Ms in this study were not out of the

range accepted as "typical;" consequently one should not be surprised that a maximum F/M was not discovered. It is highly probable that a maximum ammonia level, beyond which the ammonia or its nitrification by-products is inhibitory if not completely toxic to the organisms, would be reached before the maximum F/M in a system with greater suspended solids concentrations.

In any case, F/M does have a recognizable effect on the specific utilization rate of the nitrifiers, as does temperature and pH. Temperature appears to be the more critical of the environmental parameters; a clearly-defined optimum can be observed, although the author would hesitate to name that temperature without several more temperatures added to the four in this study. The specific utilization rate is a function of pH, but the lower pHs are less affected by the F/M than the higher pHs, which have generally higher Us.

CHAPTER VI

CONCLUSIONS

The results of this study support the following conclusions:

1. Specific utilization rate is a function of the initial food to microorganism ratio in a batch study, as well as of pH and temperature.

2. Specific utilization rate will be the greatest at near $30^{o}C$, and will be most dependent on the initial food to microorganism ratio at $20^{o}C$.

3. Specific utilization rate is affected by pH. At lower pHs, the specific utilization rate is low and not as F/M-dependent as at higher pHs where specific utilization rate is high and very F/M-dependent.

CHAPTER VII

SUGGESTIONS FOR FUTURE RESEARCH

It is suggested that to more accurately define the temperature for optimum specific utilization, a study be conducted in the 15-to-25^{o}C temperature range. Investigations of higher pH values should also be made.

Nitrite and nitrate concentrations have a better opportunity to build up in a batch system than they would in a continuous-flow system. Since the extent of inhibition due to the presence of nitrites and nitrates is not yet established, it is suggested that following studies be conducted in a continuous-flow system.

BIBLIOGRAPHY

1. Sawyer, C. N., and McCarty, P. L., Chemistry for Sanitary Engineers, McGraw-Hill (1967).

2. Wild, H. E., Sawyer, C. N., and McMahon, T. C., "Factors Affecting Nitrification Kinetics." Journal Water Pollution Control Federation, 43, 1845-1854 (1971).

3. Balakrishnan, S., and Eckenfelder, W. W., "Nitrogen Removal by Modified Activated Sludge." Journal of Sanitary Engineering Division, Proceedings ASCE, 96, SA2, 501-512 (1970).

4. Stanier, R. Y., "Nitrification." General Microbiology, 428-429 (1958).

5. Aleem, M. I. H., and Alexander, M., "Nutrition and Physiology of Nitrobacter agilis." Applied Microbiology, 8, 80-84 (1970).

6. Lyles, S. J., "Metabolism and Energy." Biology of Microorganisms, 285-286 (1969).

7. Stankewich, M. J. Jr., "Biological Nitrification with the High Purity Oxygenation Process." Presented at Purdue Industrial Waste Conference, Lafayette, Indiana (1972).

8. Downing, A. L., Painter, H. A., and Knowles, G., "Nitrification in the Activated Sludge Process." Journal Institute Sew. Purif., 130-151 (1964).

9. Johnson, W. K., and Schroepfer, G. J., "Nitrogen Removal by Nitrification and Denitrification." Journal Water Pollution Control Federation, 36, 1015-1035 (1964).

10. Jenkins, D., and Garrison, W. E., "Control of Activated Sludge by Mean Cell Residence Time." Journal Water Pollution Control Federation, 40, 1905-1919 (1968).

11. Prakasam, T. B. S., and Loehr, R. C., "Microbial Nitrification and Denitrification in Concentrated Wastes." Water Research, 6, 859-868 (1972).

12. Beckman, W. J., Avendt, R. J., Mulligan, T. J., and Kehrberger, G. J., "Combined Carbon Oxidation-Nitrification." Journal Water Pollution Control Federation, 44, 1916-1931 (1972).

13. Ludzack, F. J., and Ettinger, M. B., "Controlling Operation to Minimize Activated Sludge Effluent Nitrogen." Journal Water Pollution Control Federation, 34, 922-931 (1962).

14. Barth, E. F., Mulbarger, M. C., Salotto, B. V., and Ettinger, M. B., "Removal of Nitrogen by Municipal Wastewater Treatment Plants." Journal Water Pollution Control Federation, 38, 1208-1219 (1966).

15. Mulbarger, M. C., "Nitrification and Denitrification in Activated Sludge Systems." Journal Water Pollution Control Federation, 43, 2059-2070 (1971).

16. Ecker, R. E., and Lockhart, W. R., "Specific Effect of Limiting Nutrient and Physiological Events During Culture Growth." Journal of Bacteriology, 82, 511-516 (1961).

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