Engineering Experiment Station
Reprint No. 1135

Reprinted from ENVIRONMENTAL Science & Technology

Vol. 2, July 1968, Pages 543–546

Biodegradation of Nitrilotriacetic Acid in Aerobic Systems

Bernhard H. Pfeil[1] and G. Fred Lee*

Water Chemistry Laboratory, University of Wisconsin, Madison, Wis. 53706

* G. Fred Lee & Associates, El Macero, CA gfredlee33@gmail.com www.gfredlee.com

■ The biodegradation of nitrilotriacetic acid in the biochemical oxygen demand test and laboratory activated sludge units has been investigated. Nitrilotriacetic acid was degraded with acclimatized bacterial populations in both the biochemical oxygen demand test and activated sludge. A one-week acclimatization period was needed in the activated sludge units.

The apparent accelerated rate of eutrophication of lakes and streams has been receiving increased public attention because of the obnoxious algal and higher aquatic plant blooms observed in these waters. These blooms have frequently been associated in some way with the discharge of municipal or industrial wastes. Frequently, the phosphorus in these waste waters is alleged to be one of the principal causes of many eutrophication problems. Since a significant part of the phosphorus in municipal and some industrial wastes is derived from detergents, consideration is being given to finding a compound that may be used to substitute for the condensed phosphates used in detergent formulations. One of the compounds that may be used is the trisodium salt of nitrilotriacetic acid, NTA (*Chem. Eng. News*, 1967; Pollard, 1966).

Since NTA is a strong complexing agent, questions may be raised about its possible effects on water and waste treatment processes and aquatic life. Therefore, one of the first questions to be raised is its biodegradability. Pollard (1966) states that the evidence for NTA biodegradability is inconclusive and contradictory, and that the biochemical oxygen demand, BOD, of NTA appeared to be insignificant over a 31-day period. He did not present any data to support these statements. Recently, Swisher, Crutchfield, *et al.* (1967) have reported that NTA is biodegradable in both batch and continuous activated sludge units. Pfeil (1967) has presented a literature review on the biochemical and complexing characters of NTA.

This paper reports on a study of the biodegradability of NTA by the BOD test and in laboratory activated sludge units.

Experimental Procedures and Materials

Reagents. A nitrilotriacetic acid stock solution was prepared by suspending 5.0 grams of NTA (item No. 5417; lot No. 6620, Eastman Organic Chemicals, Distillation Products Industries) in 250 ml. of distilled water. This suspension was dissolved by neutralizing with reagent grade sodium hydroxide to a pH of 7.0 ± 0.1 using a Beckman Model H-2 glass electrode pH meter. All other chemicals used were ACS reagent grade or better. Distilled water was used to prepare solutions of other reagents.

Determination of NTA. The titration method of Cihalik and Novak (1956) for the determination of Complexon I (NTA) was used. The NTA was titrated by a copper salt in the presence of sodium acetate (AcONa) without indicator or using catechol violet or murexide as an indicator. A standard copper solution (1 ml. = 1 mg. of copper), AcONa (10% w./v.), and murexide (0.1% w./v.) solutions were prepared and used to titrate the standard NTA solution (1 ml. of copper = 3 mg. of NTA). This titration procedure was used to determine the concentration of NTA in the activated sludge units.

Acclimation of Seed Organisms. Since NTA is a synthetic organic compound and would not likely be found in naturally occurring materials, an attempt was made to see if it would be necessary to acclimatize sewage organisms to this compound so that they could utilize it as a source of energy. Small, batch-type activated sludge units consisting of 2-liter separatory funnels were set up and seeded with activated sludge (1 liter of activated sludge plus 1 liter of raw sewage). These units were kept at room temperature and were supplied with unfiltered compressed air from the laboratory compressed air system. Once or twice a day the air supply to the units was shut off and the activated sludge floc was allowed to settle for 10 to 15 minutes. Five hundred milliliters of the supernatant liquid was removed and replaced with 500 ml. of raw sewage and the air supply turned on again. Unit A was used as a control. In Unit B, increasing amounts of NTA solution were added to the 500-ml. portion of raw sewage before being added to this activated sludge unit. The concentration of NTA in the raw sewage was increased on successive days from 10 mg. per liter to a maximum of 100 mg. per liter and the dosage was maintained at this level. Acclimated seed organisms for biochemical oxygen demand and oxygen uptake determinations were obtained from this source. No change in settling characteristics of the sludge was observed as a result of adding NTA.

The raw sewage and activated sludge were obtained either from Nine Springs Treatment Works, Madison, Metropolitan Sewerage District, Madison, Wis., or from the small experimental activated sludge pilot plant located in the Civil Engineering Building, Randall Ave., University of Wisconsin, Madison, Wis.

Biochemical Oxygen Demand. The general procedure for the biochemical oxygen demand (BOD) determination was followed as outlined in Standard Methods for the Examination of Water and Wastewater (A.P.H.A., 1965). The concentration of NTA was varied in the BOD bottles and ranged from 0 to 320 mg. per liter. Either raw sewage that had been

[1] Present address, New York State Department of Health, Division of Laboratories and Research, Albany, N. Y.

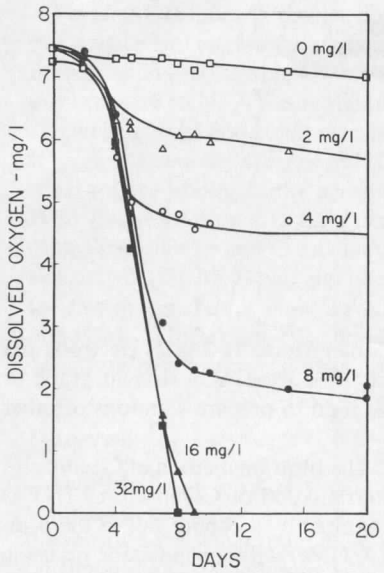

Figure 1. Dissolved oxygen remaining in BOD bottles containing NTA in standard dilution water with acclimated seed

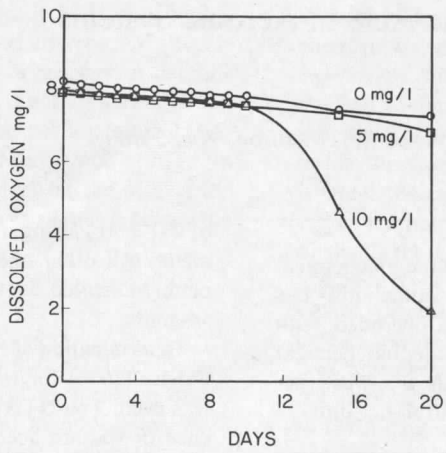

Figure 2. Dissolved oxygen remaining in BOD bottles containing NTA in standard dilution water with nonacclimated seed

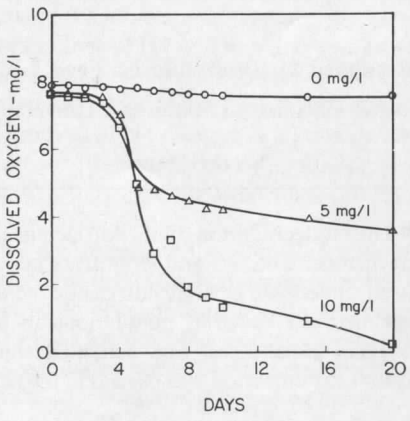

Figure 3. Dissolved oxygen remaining in BOD bottles containing NTA in standard dilution water with acclimated seed

kept at room temperature for 24 hours in an open container (aged seed), or activated sludge (unit A) was used as the source of the nonacclimated seed organisms. The activated sludge exposed to NTA (unit B) was used as the source of the acclimated seed organisms. This sewage was filtered through several layers of cheesecloth to remove the large particles and obtain a fairly homogeneous seed culture prior to adding it to the standard BOD dilution water. The BOD bottles were stored in the dark in a 20° C. thermostatically controlled air incubator until removed for titration of the residual oxygen.

Total Suspended Solids. The total suspended solids or mixed liquor suspended solids (MLSS) of the activated sludge units were determined by filtering, with the aid of a water aspirator, 25 ml. of the sample through a tared fiber-glass mat in a Gooch crucible. The difference between the tare weight and weight of filtered sample after drying for one hour at 103–105° C. was used to determine the total suspended solids in the aeration chamber.

Results

BOD Experiments. Since the purpose of this study was to determine the biodegradability of NTA, emphasis in the experimental work was first placed on the BOD of this compound.

An exploratory set of BOD experiments were set up to determine the 5-day BOD of NTA. Increasing concentrations of NTA in standard synthetic BOD dilution water containing aged sewage as source of seed organisms were prepared and placed in standard 300-ml. BOD bottles. The concentration of NTA ranged from 0 to 320 mg. per liter. The 5-day BOD value of NTA, average of three results, was negligible under conditions of this experiment.

The BOD determination was then repeated at concentrations of 20 and 100 mg. per liter of NTA to compare the aged sewage seed with seed obtained from unit B which had been exposed to increasing amounts of NTA during the previous week. The data for this experiment (Table I) show that by the 13th day of incubation, the BOD bottles that contained the seed organisms previously exposed to NTA had completely depleted the available oxygen. The aged sewage seed organism had not been able to utilize the NTA in the sample and had not significantly depleted the available oxygen in 30 days of incubation.

The BOD determination was again utilized to determine the oxygen requirement for the degradation of NTA by acclimatized seed organisms. The concentration of NTA varied from 0 to 32 mg. per liter. The results are presented in Figure 1. There appears to be a lag period of a few days before the degradation of NTA occurs. At the two higher concentrations of NTA, the available oxygen in the BOD bottle was depleted by the eighth and ninth day of incubation. The 20-day value for the 4 mg. per liter concentration appears out of line with the rest of the results and cannot be explained.

To confirm the results of the experiments discussed above, additional BOD experiments were conducted. The source of nonacclimated seed organisms was activated sludge unit A,

Table I. Degradation of NTA by Seed Organisms from Two Different Sources

Dissolved oxygen, mg./l., remaining in BOD bottles[a]

No. of Days Incubation	Aged Sewage Seed Organisms, Mg./L. NTA			Prior Exposure of Seed Organisms to NTA, Mg./L. NTA		
	0	20	100	0	20	100
0	7.55	7.55	7.1	7.6	7.35	6.85
5	7.2	7.4	6.85	7.35	7.1	6.75
11	7.0	7.25	6.8	7.05	0.05	3.5
13	7.1	7.2	6.55	7.05	<0.05	<0.05
15	7.1	7.3	6.6			
20	7.0	6.95	6.4			
24	7.1	7.10	6.5			
30	6.2	5.5	5.7			

[a] Results from single determinations.

and the acclimated seed organisms were obtained from activated sludge unit B. The concentration of NTA was 5 mg. per liter and 10 mg. per liter and a control containing 0 mg. per liter. The results of this experiment are presented in Figures 2 and 3. No obvious degradation of NTA by the nonacclimated seed organisms was observed after 20 days' incubation at 5 mg. per liter. The acclimatized seed organisms were able to degrade the NTA following a lag period of three days, confirming the results of the previous experiment. The apparent oxygen utilization by the nonacclimated seed organisms in the 10 mg. per liter of NTA concentration on the 15th and 20th day of incubation may possibly be due to the development of some acclimatized organisms to NTA.

Acclimation of Seed Organisms. An activated sludge aeration cylinder unit C, similar to the other two units, was set up with 1 liter of activated sludge obtained from the activated sludge aeration tank at the Civil Engineering Building and 1 liter of raw sewage. At approximately 12-hour intervals thereafter, the air supply was shut off for 10 to 15 minutes to allow the activated sludge solids to settle, 500 ml. of supernatant liquid was removed and replaced with 500 ml. of raw sewage containing NTA. The concentration of NTA in the raw sewage was increased daily, and after the first day, the concentration of NTA in the aeration cylinder was determined by the titration procedure before and after the addition of the raw sewage containing the NTA. The mixed liquor suspended solids (MLSS) was about 1760 mg. per liter for this experiment.

The results are plotted in Figure 4. The vertical line represents the amount of NTA that was added with the raw sewage. The slanted line represents the degradation of NTA accomplished by the acclimatized organisms over the 12-hour period between additions of NTA. The results show that up to the fifth day very little degradation of NTA occurred. From the fifth to the seventh day organisms had developed that were able to degrade rapidly the added NTA. They were able to handle loadings as high as 110 mg. per liter without any adverse effect.

To ascertain if the absence of NTA in the raw sewage had any effect on the ability of the acclimated organisms to degrade NTA, raw sewage that was fed to the activated sludge units from two to five days contained no NTA. The results of the degradation of NTA in these units is presented in Figures 5, 6, and 7.

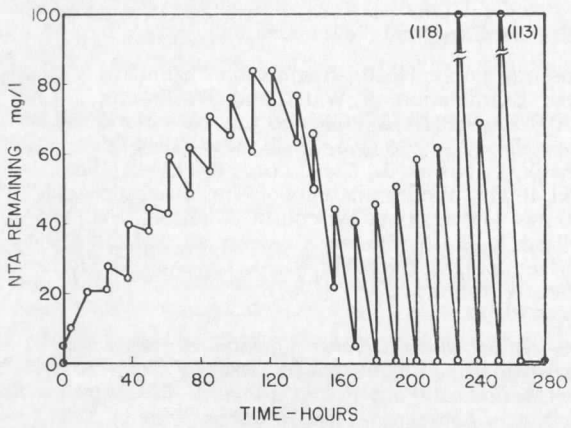

Figure 4. Activated sludge unit C, development of acclimated organisms to NTA

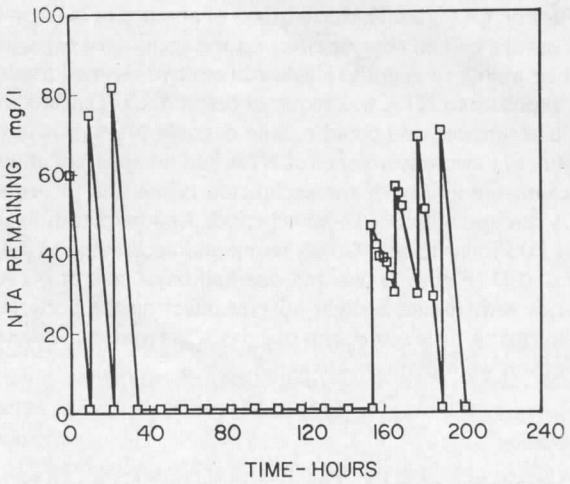

Figure 5. Activated sludge unit C, containing organisms, acclimated to NTA

NTA degradation before and after 5 days' absence of NTA raw sewage feed

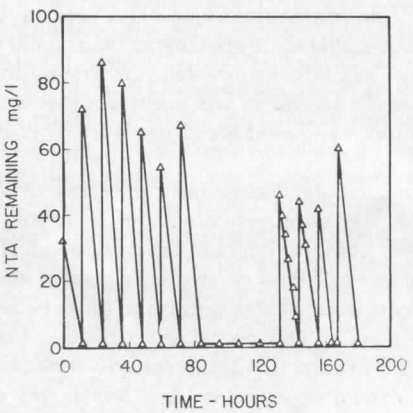

Figure 6. Activated sludge unit B, containing organisms acclimated to NTA

NTA degradation before and after 2 days' absence of NTA in raw sewage feed

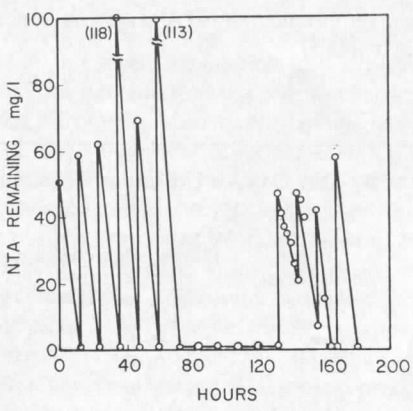

Figure 7. Activated sludge unit D, containing organisms acclimated to NTA

NTA degradation before and after 2.5 days' absence of NTA in raw sewage feed

For unit C (Figure 5), the five days of absence of NTA in the raw sewage had an adverse effect on the acclimated organisms in their ability to degrade NTA. Almost two days of continuous exposure to NTA was required before the organisms were again acclimated and could readily degrade NTA. For unit B (Figure 6) a two-day absence of NTA had no apparent adverse effect on the ability of the acclimated organisms to degrade NTA dosages within a 12-hour period. Another aeration unit (unit D) similar to unit C was set up and acclimated to NTA. For unit D (Figure 7) two and one-half days' lack of NTA in the raw sewage had a slight adverse effect on the acclimated organisms. A little more than one day was required before the organisms were again acclimated.

Discussion

Degradation of NTA. The data show that NTA can be biologically degraded following a period of acclimation of the organisms to this compound. A period of about one week under laboratory conditions is needed for the organisms to become acclimatized to accept this compound as a source of energy after initial and continuous exposure to it. After acclimatization this compound can be readily degraded.

Although the concentrations of NTA used in these experiments were probably somewhat higher than would be expected in municipal sanitary wastewater, the results indicate that organisms in the secondary treatment facilities would be able to degrade this compound about one week after initial exposure.

The acclimated organisms appear to be able to withstand up to a two-day starvation period without NTA with no adverse effect on their ability to degrade this compound. If this period is two and one-half days or greater, a certain length of time would be required for the organisms to again become adapted to degrading NTA. This period of acclimation would depend on the length of time that the organisms were without NTA and could extend up to seven days which was the original length of time for the acclimatization of the organisms.

Oxygen Requirement of NTA. The results of the BOD determinations also indicate that NTA is biodegradable as shown by the oxygen utilization by the acclimated organisms in the process of degrading this compound. In the BOD determination there appears to be a lag period of about three days before there is a significant increase of oxygen utilization. A summary of the oxygen requirement of NTA at various concentrations is presented in Table II.

Table II. Twenty-Day Oxygen Utilization Requirement of NTA

Concentration of NTA, Mg./L.	Oxygen Utilized in 20 Days, Mg./L.[a]	Oxygen Required, Mg./L. per Mg./L. of NTA	Ratio of 5-Day to 20-Day Oxygen Utilization
2	1.35	0.68	0.82
4	2.45[b]	0.61[b]	0.96[b]
5	3.75	0.72	0.73
8	5.20	0.65	0.49
10	7.10	0.71	0.42

[a] Corrected for blank.
[b] Based on 15-day value.

The results show that the oxygen requirement of NTA varied from 0.61 to 0.72 mg. per liter of oxygen per milligram per liter of NTA at the end of 20 days. The average value was 0.67 mg. per liter of oxygen per milligram per liter of NTA. This value is slightly higher than the experimental value obtained by the chemical oxygen demand (COD) value of 0.65 mg. per liter of oxygen per milligram per liter of NTA. The value obtained by the BOD determination could be expected to be slightly higher than the COD determination since organisms are better able to utilize the acetic acid radical in NTA in the oxidation process, whereas the carbonaceous portion of this compound appears to be incompletely oxidized by the acid-dichromate procedure of the COD determination.

The ratio of the five-day oxygen utilization value to the 20-day oxygen utilization value decreases as the concentration increases. At an NTA concentration of 2 mg. per liter this ratio is 0.82 and decreases to 0.42 for the NTA concentration of 10 mg. per liter. Under these experimental conditions, the amount of NTA degraded in the BOD determination after five days of incubation is approximately the same regardless of its initial concentration; or as the concentration of NTA increases a small percentage of the total amount of NTA would be degraded in the five days of incubation. Since these ratios vary considerably, a five-day BOD value of this compound would not give any indication of the total concentration of NTA nor a percentage figure for the total BOD of the compound.

These results are similar to those reported by Swisher, Crutchfield, *et al.* (1967), in which they found that NTA was biodegradable in laboratory activated sludge units. They noted that a period of acclimatization was necessary and that removal of NTA was by degradation rather than sorption. They also found that NTA did not affect performance of laboratory activated sludge units.

Summary

NTA can be biologically degraded by organisms in activated sludge following a period of one week for acclimatization of the organisms to this compound. The five-day BOD determination of this compound is zero unless the seed organisms, used in this determination, have been previously acclimatized to this compound. Periods of up to two days without NTA will not adversely effect ability of acclimated organisms to degrade again NTA without time for acclimation.

Literature Cited

American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," 12th ed., AWWA, APHA, WPCF, 769 pp., New York, 1965.
Chem. Eng. News **45** (10), 18–20 (May 1, 1967).
Cihalik, J., Novak, J., *Chem. Listy* **50,** 1193–5 (1956).
Pfeil, B. H., "Biodegradability of Nitrilotriacetic Acid," M. S. thesis, University of Wisconsin, Madison, Wis., 1967.
Pollard, R. R., *Hydrocarbon Process.* **45,** 197–204 (1966).
Swisher, R. D., Crutchfield, M. M., Caldwell, D. W., Environ Sci. & Tech. **1,** 820–7 (1967).

Received for review October 30, 1967. Accepted May 8, 1968. Investigation was supported by Training Grant 5T1WP-22-05 from the Federal Water Pollution Control Administration and the Division of Laboratories and Research, State of New York. In addition, support was given this study by the University of Wisconsin Department of Civil Engineering and the Engineering Experiment Station.