Reports

Biodegradation of Nitrilotriacetic Acid and Related Imino and Amino Acids in River Water

Abstract. *With a gas chromatography procedure capable of quantitatively detecting nitrilotriacetic acid, N-methyliminodiacetic acid, iminodiacetic acid, sarcosine, and glycine at concentrations of 0.025 milligram per liter, it was shown that these compounds were biodegraded by river water. In particular, nitrilotriacetic acid was degraded without the accumulation of any of the amino acids mentioned above or the appearance of any new peaks in the gas chromatography analysis.*

Nitrilotriacetic acid (NTA), $N(CH_2CO_2H)_3$, is a relatively simple, symmetrical molecule commercially produced from ammonia, formaldehyde, and hydrogen cyanide. It forms water-soluble complexes with calcium and magnesium that are stable at *p*H 10 (washing *p*H) but not at *p*H 7 (*1*) (biological *p*H), is substantially nontoxic (*2, 3*), and is readily degraded by microorganisms found in sewage facilities of all types (*4*), in rivers and streams (*4*), and in soils (*5*). This combination of inexpensive starting materials and properties has prompted the commercial development of NTA as a partial replacement for phosphate in detergent formulations.

As an ingredient in detergents, NTA becomes a component of waste water and thus finds ubiquitous entry into the environment. Therefore, environmental safety—not only of NTA itself but also of all chelates of NTA (*6*), all possible secondary reaction products of NTA, and all possible intermediates of NTA biodegradation and their secondary reaction products—should be considered.

We have examined some aspects of the last-mentioned topic, NTA biodegradation intermediates and their secondary reaction products. Epstein (*3*) pointed out a possible environmental hazard in this area. He suggested that NTA could degrade by successive loss of one or two carbon fragments and create as intermediates a family of amino acids: *N*-methyliminodiacetic acid (NMIDA), iminodiacetic acid (IDA), sarcosine (SARC), glycine (GLY), and *N,N*-dimethylglycine. Of these, IDA and SARC are secondary amines; secondary amines in general react with nitrous acid to form stable *N*-nitrosamines, many of which are carcinogens (*7*). Like other secondary amines, IDA and SARC react with nitrous acid; they form *N*-nitrosoiminodiacetic acid (*N*-nitrosoIDA) (*8*) and *N*-nitrososarcosine (*N*-nitrosoSARC) (*9*), respectively. Although no data are available on the carcinogenicity of *N*-nitrosoIDA, *N*-nitrosoSARC is carcinogenic to rats (*9*). The question posed by Epstein was whether biodegradation of NTA could lead to the formation of IDA and SARC and thence to *N*-nitroso derivatives by reaction of the secondary amines with nitrite ions before or after ingestion.

To answer this question we developed a gas chromatography procedure capable of quantitative detection of NTA, NMIDA, IDA, SARC, and GLY and qualitative detection of *N,N*-dimethylGLY and *N*-nitrosoIDA in river water, primary and secondary sewage effluent, and water containing nitrite ions (*10*). We used this procedure to search for intermediates during and after the degradation of NTA and to determine the biodegradability of NTA, NMIDA, IDA, SARC, GLY, and *N*-nitrosoIDA.

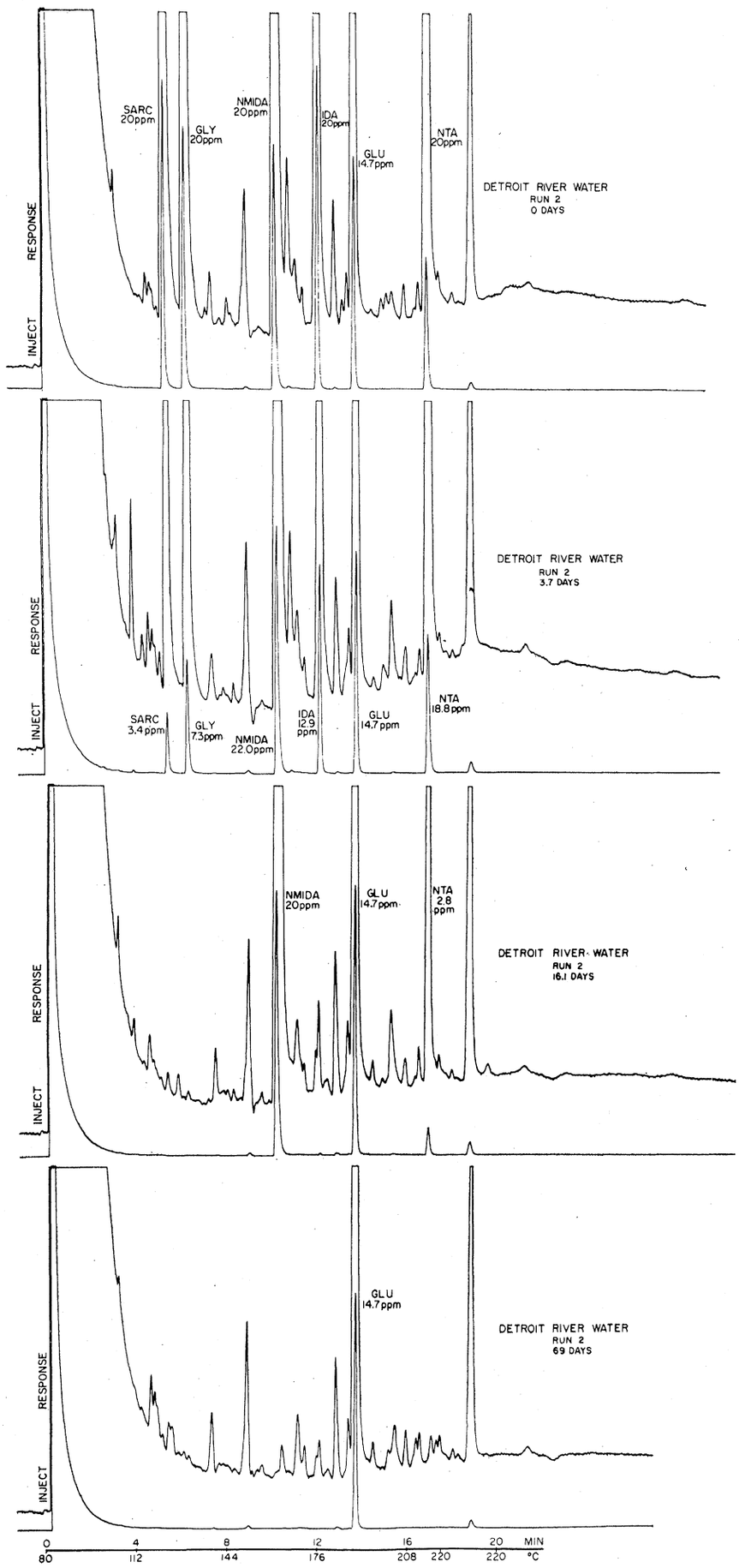
The Detroit and Meramec rivers (*11*) were the sources of biodegradation media. To portions of the water were added amino acids, singly or in combination (Table 1), and the portions were placed in 480- or 960-ml widemouth jars. The jars were stored in the dark to suppress algal growth.

Samples (10 ml) were withdrawn periodically from the jars and placed into 20 by 125 mm screw-capped test tubes. An internal standard, 1.0 μmole of glutamic acid (GLU), was added to each sample, and the water was removed by lyophilization. The residue was treated with 2.0 ml of 3.0*N* HCl in 1-butanol. The resulting mixture was heated to 65°C for 45 minutes, and the volatile reagents were removed by rotary evaporation. The oily residue was shaken with 2 ml of 0.5*M* $KHCO_3$ and extracted with three 2-ml portions of methylene chloride. Trifluoroacetic anhydride (1.0 ml) was added to the methylene chloride extract. The resulting solution was allowed to stand at room temperature for 30 minutes and then was stripped to dryness with a stream of nitrogen. The residue was dissolved in 0.5 ml of 1,1,2-trichlorotrifluoroethane (Freon 113). A sample

Table 1. Biodegradation of NTA and other amino acids in river water. Nitrilotriacetic acid (20 ppm, then 10 ppm) was added to Detroit River water and allowed to degrade; this acclimated water was used in runs 4, 5, and 7. Concentrations are expressed in terms of the free acids (molecular weights: NTA, 191.1; NMIDA, 147.1; IDA, 133.1; SARC, 89.1; GLY, 75.1).

Run	Initial concentration in parts per million (disappearance time in days)					
	NTA	NMIDA	IDA	SARC	GLY	*N*-NitrosoIDA
			Detroit River water			
1	20 (14.0)	20 (34)	20 (8.1)	20 (5.2)		
2	20 (16.8)	20 (69)	20 (7.7)	20 (4.5)	20 (4.7)	
6			20 (3.7)			
			Detroit River water acclimated to NTA			
4	5 (2.9)			5 (2.9)		
5	5 (3.0)		5 (3.0)			
7	5 (3.2)					5*
			Meramec River water			
3	15 (10)	20 (16.2)	14 (7)	14 (7)		
8						20†
9						5†
10	15 (9.3)					

* No degradation within 42 days. † No degradation within 52 days.



of this solution (1 to 3 μl) was injected onto a 1.9 m by 2 mm (inner diameter) glass U column which had been packed with 0.65 percent ethylene glycol adipate on acid-washed Chromosorb W (*12*). The gas chromatograms were obtained with a Varian Aerograph 2100 gas chromatograph operated under the following conditions: column oven, 80° to 220°C at 8° min^{-1}; detector temperature, 250°C; and injector temperature, 230°C.

This derivatization procedure converted the tertiary amines—NTA, NMIDA, and *N,N*-dimethylGLY—to *n*-butyl ester derivatives. The primary and secondary amines—IDA, SARC, GLY, and GLU—were converted to *N*-trifluoroacetyl *n*-butyl ester derivatives. In the procedure, *N*-nitrosoIDA was cleaved to IDA (*13*) and was subsequently detected as the *N*-trifluoroacetyl *n*-butyl ester derivative of IDA. The procedure was quantitative for samples ranging from 200 to 0.25 μg (concentrations in river water of 20 to 0.025 mg/liter) each of NTA, NMIDA, IDA, SARC, and GLY; the relative standard deviations (as percentages) were NTA, 8.7; NMIDA, 9.9; IDA, 6.2; SARC, 10.9; and GLY, 13.6. The procedure was qualitative for *N*-nitrosoIDA and *N,N*-dimethylGLY; the lower limit of detectability for these compounds was 0.05 mg/liter.

Older analytical methods for NTA (*14*) detected only that compound; our gas chromatography procedure specifically separates and detects not only NTA but also many other volatile organic compounds (*15*). This feature enabled us to follow the degradation of mixtures of NTA, NMIDA, IDA, SARC, and GLY in river water. A set of chromatograms for one such experiment is shown in Fig. 1; disappearance times and initial concentrations of amino acids are given in Table 1.

In runs 1 to 3 all five amino acids were completely degraded at approximately equal rates and with no accumulation of intermediates. The ac-

Fig. 1. Chromatograms obtained in run 2 for degradation of SARC, GLY, NMIDA, IDA, and NTA in Detroit River water; GLU was added as an internal standard. Numbers are concentrations in parts per million (*ppm*). Attenuation values are 1×10^{-11} amp/mv for the upper pen and 50×10^{-11} amp/mv for the lower pen. Peaks other than those for the amino acids mentioned above are due to organic material initially present in the river water.

climation times for the series—that is, the delay times before onset of degradation—always fit the following order:

$$GLY = SARC \leqslant IDA < NTA \ll NMIDA$$

Of these substances, IDA, SARC, and GLY were readily used by microorganisms in the river water and began to degrade within 2 days. The length of the acclimation period for NTA, 7 to 14 days, was unaffected by the presence of IDA, SARC, and GLY and indicated that enzyme induction was required for degradation of NTA. In water acclimated to NTA (runs 4 and 5), IDA, SARC, and NTA were degraded within 3 days. We therefore conclude that any buildup of IDA and SARC as a result of NTA breakdown is impossible.

In runs 1 to 3, in which mixtures of all five amino acids were used, NTA was completely degraded long before the system had acclimated to NMIDA. On the other hand, NTA alone (run 10) was degraded with no accumulation of NMIDA. Therefore, NMIDA appears not to be an intermediate in the biodegradation of NTA. The question of whether IDA, SARC, and GLY are such intermediates is not settled (*16*).

Unlike our analytical procedure, the microorganisms present in river water were able to differentiate IDA from *N*-nitrosoIDA; the former compound was always rapidly and completely degraded (runs 1, 2, 3, 5, and 6), while the latter was not degraded in our samples of river water (runs 7 to 9). The presence of *N*-nitrosoIDA, however, did not hinder the degradation of NTA (compare runs 4 and 5 with run 7). If nitrosation of IDA had occurred during any of our biodegradation runs, we would have observed an apparent accumulation of IDA. Such an accumulation was never observed (*17*).

When NTA was added to Meramec River water (run 10) or sequentially added to Detroit River water (Table 1) it was always completely degraded. There was no accumulation of NMIDA, IDA, SARC, GLY, or *N,N*-dimethyl-GLY, and there were no new peaks in the chromatograms. So efficient was NTA degradation by microorganisms present in our river water samples that no clues were obtained about its biodegradation pathway.

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11. All biodegradation studies described in this report were done at room temperature (23° ±2°C). Detroit River water (*p*H 8.0) was collected downstream from Detroit at the water intake of the Detroit Edison plant. Meramec River water (*p*H 8.3) was collected at Valley Park, Mo., 16 km southwest of St. Louis.
12. C. W. Gehrke, K. Kuo, R. W. Zumwalt, *J. Chromatogr.* **57**, 209 (1971), and references cited therein.
13. Nitrosamines are cleaved to amines by alcoholic HCl [P. A. S. Smith, *The Chemistry of Open Chain Organic Nitrogen Compounds* (Benjamin, New York, 1966), vol. 2, p. 473].
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15. Another advantage of our gas chromatoggraphy procedure is that heavy metal ions do not cause a negative interference. We were able to analyze for all five amino acids in distilled water that contained Cu^{2+}, 3.5 mg/liter, and in distilled water and secondary sewage effluent that contained Pb^{2+}, 0.2 mg/liter; Zn^{2+}, 0.6 mg/liter; Ni^{2+}, 0.2 mg/liter; Cd^{2+}, 0.1 mg/liter; and Fe^{2+}, 5.0 mg/liter. The presence of a large amount of dissolved salts (for example, phosphate buffer) causes the formation of a residue insoluble in a solution of HCl in butanol. The result is incomplete esterification of the amino acids. We recommend the following procedure: dissolve the residue in 1.0 ml of undiluted trifluoroacetic acid, strip off the volatile acid with a stream of nitrogen, and then proceed with the esterification step.
16. J. M. Tiedje and C. B. Warren, unpublished data.
17. No *N*-nitrosoIDA was observed when NTA was degraded in systems containing nitrite ions (R. D. Swisher and C. B. Warren, unpublished data).
18. We thank R. D. Swisher for advice and for the Meramec River samples and W. A. Feiler for a sample of highly purified NTA.

20 September 1971; revised 7 February 1972 ■

Mars: The Lineament Systems

Abstract. *Analysis of the Mariner 4, Mariner 6, and Mariner 7 photographs shows that Mars has at least two distinct types of lineament systems. The most prominent is a well-developed global-type system. The second consists of radial and concentric lineaments associated with the Hellas and south polar basins.*

An investigation of the Mariner 6 and Mariner 7 photographs has established the existence of a well-defined set of lineament systems on Mars. Prior to these missions the presence of a martian lineament system on the scale of 100 km was suggested by Katterfel'd (*1*), who based his conclusions on a study of the Martian canals. Using the Mariner 4 imagery, Binder (*2*) showed that, on the scale of 1 to 10 km, the cratered terrain in Mare Sirenum and Amazonis contains a lineament system whose characteristics are similar to those of a plane-wide system as theoretically investigated by Vening Meinesz (*3*) and to those of the lunar lineament system, known as the lunar grid system, empirically defined by Strom (*4*). The more extensive coverage and better definition provided by the Mariner 6 and Mariner 7 imagery has allowed a more complete and definitive study of the martian lineaments than could be made with the Mariner 4 data.

The photographs used in this study include the final Mariner 4 photographs as well as the new Mariner 6 and Mariner 7 photographs. The former were rectified photographically and the coordinates and orientation of each image were obtained from the Mariner 4 final progress report (*5*). The latter are the computer rectified versions of the maximum discriminability imagery provided by Jet Propulsion Laboratory. The coordinates and orientation of each of the 1969 images were obtained from the Pegasis (*6*).

Because of the comparatively low quality of the Mariner 4 imagery only about 160 lineaments could be positively identified on the eight frames studied. These lineaments consist of linear wall segments of polygonalized craters found on most frames, graben-like features on frame 11, and linear albedo boundaries such as those found on frames 13 and 14.

In contrast, the Mariner 6 and Mariner 7 imagery contains a great number