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Microbial Enhancement of Hydrazine Degradation in Soil and Water

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In an early study (Ou and Street 1987), we reported that hydrazine was rapidly degraded in Arredondo fine sand. Even at 500 µg/g hydrazine disappeared completely in eight days. By comparing the degradation results in sterile and nonsterile soils, it was concluded that biological degradation was responsible for about 20% of hydrazine disappearance from soil. Ou (1987) isolated a heterotrophic bacterium, Achromobacter sp., from the Arredondo soil and found that the organism had a high capacity to degrade hydrazine to the nontoxic product dinitrogen gas. Solonim and Gisclard (1976) reported that the stability of hydrazine at low concentrations (0.25 to 5.0 µg/mL) in surface waters (lake, pond, and tap water) varied according to source. Degradation rates were not related to dissolved oxygen content. Degradation of hydrazine is much more rapid in soil than in water. Hydrazine at 10 µg/g in Arredondo soil disappeared completely in less than two hours (Ou and Street 1987).

In the present study, we attempted to enhance hydrazine degradation in water and soil samples by inoculating with a hydrazine-degrading bacterium, Achromobacter sp. Factors that influence hydrazine degradation in water and soil are discussed.

MATERIALS AND METHODS

Two river-water samples, two lake-water samples, tap water, and distilled water were used in this study. Selected properties of the waters samples are shown in Table 1. Arredondo fine sand was used, with key properties as reported previously (Ou and Street 1987).

Achromobacter sp. was maintained in a basal mineral medium containing hydrazine sulfate and glucose as described by Ou (1987). 30 mL of 18 hour-old bacterial culture was centrifuged at 20,000xg for 20 minutes. The cell pellets were suspended in 5 mL of water. After adding an appropriate amount of sterile aqueous hydrazine (0.04 - 0.2 mL), the samples were incubated at 25°C for two hours.

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Table 1. Selected properties of the six waters used in this study.

Water	Cu (µg/mL)	Fe (µg/mL)	Bacteria (cfu/mL) X10^{-3}	Fungi (cfu/mL)	pH	Suspended solid (mg/mL)
Santa Fe River	0.04	0.04	206	4	7.7	3
Prairie Creek	0.01	0.24	1	3	6.6	3
Lake Alice	0.20	0	25	22	7.4	3
Newmans Lake	0	0.28	9	9	7.7	3
Tap water	0	0	0	0	8.5	3
Dist. Water	0	0	0	0	6.4	0

The aqueous hydrazine (250 or 2500 µg/mL) was sterilized by filtration through a 0.22 µm filter and then stored in the dark at 4°C. Under this condition hydrazine solution was stable for more than a month. In addition, autoclaved bacterial cells were tested for their capacity to degrade hydrazine in autoclaved and filtered water samples. Hydrazine sulfate was used for all experiments unless otherwise specified.

Water samples (5 mL) were fortified with phosphates (0.024 g of K_2HPO_4 and 0.024 g of KH_2PO_4) in order to increase their buffer capacity. Eighteen hour-old bacterial cells were suspended in the buffered waters. After hydrazine had been added, the samples were incubated at 25°C for two hours, following which the cell suspensions were centrifuged at 20,000xg for 20 minutes. 0.01 to 0.1 mL of the supernatant solutions was used for determination of hydrazine concentration using a colorimetric method (Watt and Chrisp 1952).

One mL of the 18 hour-old cell suspension of Achromobacter sp. (12±1 mg/mL) was also added to 10 g of air-dry Arredondo soil in a plastic centrifuge tube, and an appropriate amount of sterile hydrazine was immediately added to give hydrazine concentrations of 25, 50, or 100 µg/g of soil. An identical experiment was set up for autoclaved soil. In addition, the bacterial cells (2.2±0.2 mg/mL) were suspended in 5 mL of either distilled water or buffered distilled water in plastic centrifuge tubes which contained 0.5g of autoclaved or nonautoclaved Arredondo soil. Appropriate amounts of sterile hydrazine (25 and 50 µg/mL) were added to each suspension. All samples were incubated at 25°C for one hour. After incubation, the soil suspensions were immediately centrifuged in the cold (4°C) at 20,000xg for 15 minutes. Corresponding soil samples were immediately suspended in 30 mL of cold 0.1 M NaCl, and centrifuged in the cold. Hydrazine in each supernatant

was determined colorimetrically as described above.

RESULTS AND DISCUSSION

Hydrazine was not degraded in any of the six waters during two hours of incubation. Hydrazine was not stable, however, in these waters when suspended with 18 hour-old cells of Achromobacter sp. The degree of stability in the cell suspensions depended upon hydrazine concentration and type of water. Table 2 shows % reduction of hydrazine in six waters suspended with the Achromobacter sp. after 2 hours of incubation. The initial hydrazine concentrations ranged from 11 to 75 µg/mL. Bacterial cells suspended in Santa Fe River water were the most active in degrading hydrazine. Bacterial cells suspended in Lake Alice water also had a high capacity to degrade the chemical. It appeared that the Achromobacter sp. in Santa Fe River water and Lake Alice water declined in their capacity to degrade hydrazine at 50 µg/mL. The same bacterium in other waters started to decline in degradation capacity at 25 µg/mL. No degradation was observed in the six waters when suspended with autoclaved bacterial cells during two hours of incubation. Similar degradation capacity was observed whether the bacterial cells were suspended in autoclaved or filtered water samples.

When the water samples were buffered with phosphates, the degradation capacity of the Achromobacter sp. in Santa Fe River

Table 2. Hydrazine degradation in waters suspended with a soil bacterium Achromobacter sp.*

Water	Initial N_2H_4 (µg/mL)	% Reduction	Initial N_2H_4 (µg/mL)	% Reduction
Santa Fe River	11	90	25	92
	50	96	75	51
Lake Alice	11	91	25	96
	50	78	75	46
Newmans Lake	11	90	25	72
	50	30		
Prairie Creek	11	90	25	76
	50	28		
Tap Water	11	91	25	80
	50	52	75	9
Distilled water	11	90	25	32
	50	22	75	5

* Bacterial cells were added at 2.2 ± 0.2 mg/mL.

water, Lake Alice water, tap water, and distilled water was enhanced (Table 3). Even in distilled water 57 and 53% of the hydrazine was degraded when initial hydrazine concentrations were 75 and 100 µg/mL, respectively. pH values for the buffered waters were at least 3.6 units higher than for unbuffered waters, with the exception of the Santa Fe River water. This water appeared to have a high buffer capacity against hydrazine sulfate (Table 4). The critical pH value was approximately 3.5. Below this range the capacity for the organism to degrade hydrazine decreased drastically.

Table 3. Hydrazine degradation in buffered waters suspended with cells of an Achromobacter sp.*

Water	Initial N_2H_4 (µg/mL)	% Reduction	Initial N_2H_4 (µg/mL)	% Reduction
Santa Fe River	75	63	100	49
Lake Alice	75	63	100	52
Tap water	75	65	100	53
Distilled water	75	57	100	53

* Bacterial cells were added at 2.2±0.2 mg/mL.

Table 4. pH values of waters amended with various concentrations of hydrazine

Water	pH				
	Hydrazine concentration (µg/mL)				
	25	50	75	50	75
	Unbuffered			Buffered	
Santa Fe River	6.8	6.4	5.9	6.7	6.7
Prairie Creek	3.4	3.2	3.0	6.7	6.7
Lake Alice	6.4	5.5	3.4	6.7	6.7
Newmans Lake	3.5	3.2	3.0	6.7	6.7
Tap water	5.8	3.5	3.1	6.7	6.7
Distilled water	3.3	3.1	2.9	6.7	6.7

Table 5 shows the stability of hydrazine in autoclaved and non-autoclaved waters. Among the six unsterile waters, hydrazine in Santa Fe River water was the most unstable. Hydrazine in this water disappeared completely in 14 days. Even in autoclaved water, 66% of the hydrazine was degraded in 14 days. On the other hand, hydrazine in Newmans Lake water and in Prairie Creek water was very stable, even more so than in distilled water. No degradation occurred in the two waters during 14 days of incubation. By comparing the degradation results in sterile and nonsterile waters, it appeared that biological degradation was more important initially for the Santa Fe River water, whereas autooxidation was more important initially for the Lake Alice water. It was not clear why Newmans Lake water and Prairie Creek water did not degrade hydrazine, and why hydrazine in these waters was more stable than in distilled water. Bacterial and fungal populations in these two waters were very low (see Table 1). No bacteria or fungi were detected in the distilled water sample.

Table 5. Hydrazine degradation in nonsterile and sterile waters.*

Time (days)	% Reduction					
	Santa Fe River	Lake Alice	Newmans Lake	Prairie Creek	Tap water	Dist. water
1	28**(5)***	0(8)	0(0)	0(0)	2(0)	0(0)
2	40(9)	9(9)	0(0)	0(0)	2(0)	0(0)
5	85(33)	42(34)	0(0)	0(0)	12(0)	0(0)
7	96(45)	56(48)	0(0)	0(0)	19(2)	2(0)
14	100(66)	86(71)	0(0)	0(0)	28(8)	2(0)

* Initial hydrazine concentration was 25 µg/mL
** Unsterile water
*** Sterile water

Although cupric ions have a capacity to catalyze the autooxidation of hydrazine in waters (Schmidt 1984), no relationship between copper content and rate of hydrazine degradation was found for the waters used in this study. Likewise, ferric ions may also catalize oxidation of the chemical in waters. Again no correlation was found. However, the levels of copper and iron in the waters were either very low or negligible (Table 1). At these low levels cupric ions or ferric ions may not exert any catalytic effect on hydrazine oxidation. Since all samples were incubated under the same conditions, it would be expected that dissolved oxygen levels in the waters would be about the same. In addition, no correlation was found between dissolved oxygen content and hydrazine degradation in the waters (Solonim and Gisclard 1976). Our results indicate that microbial activity and buffer capacity of

the waters play a key role in the degradation of hydrazine sulfate. In light of the rapid hydrazine degradation and high bacterial populations of the Santa Fe River water, a large number of specific degraders may be present in this water. In addition, the high buffer capacity of this water may reduce hydrazine toxicity toward bacteria.

Table 6. Degradation of hydrazine monohydrate in waters suspended with cells of Achromobacter sp.*

Water	Initial N_2H_4 (µg/mL)	% Reduction	Initial N_2H_4 (µg/mL)	% Reduction
Santa Fe River	17	94	34	94
Lake Alice	17	94	34	94
Tap water	17	95	34	93
Distilled water	17	94	34	92
Santa Fe River	68	51		
Lake Alice	68	54		
Tap water	68	54		
Distilled water	68	49		

* Bacterial cells were added at 2.2 ± 0.2 mg/mL.

The Achromobacter sp. in the waters of Santa Fe River and Lake Alice, as well as in tap water and distilled water, also had a high capacity to degrade nonsalt forms of hydrazine (the monohydrate in this case) (Table 6). In fact, the bacterium in these waters degraded the nonsalt form better than the salt form. The average pH values of the four waters supplied with hydrazine monohydrate at 17, 34, and 68 µg/mL were 8.42 ± 0.24, 8.58 ± 0.20, and 8.73 ± 0.17, respectively. At concentrations of 17 and 34 µg/mL, hydrazine monohydrate exerted no adverse effect on degradative activity of the Achromobacter sp. At these concentrations 92 to 95% of the nonsalt form was degraded. At a concentration of 68 µg/mL similar degradation activity was observed for all four waters. No degradation was observed, however, in the waters without adding the bacterium during two hours of incubation. Also, no degradation was observed after adding autoclaved cells of Achromobacter sp. to the waters.

The Achromobacter sp. enhanced hydrazine degradation in all water samples but did not do so in Arredondo soil during one hour of incubation. Degradation of hydrazine in soil treated and untreated with Achromobacter sp. was not significantly different (1% level) at hydrazine concentration of 100 µg/g to 500 µg/g.

Hydrazine in soil at concentrations below 100 µg/g was rapidly degraded by autooxidation, even during one hour of incubation. Thus, the effect of adding the bacterium to enhance hydrazine degradation could not be observed. However, addition of the bacterium to soil suspensions (10% soil) did enhance hydrazine degradation (Table 7). The degree of enhancement by the bacterium in the soil suspensions was not as great, however, as for the water samples.

Table 7. Hydrazine degradation in soil suspensions with and without added Achromobacter sp.*

Soil Suspension	Initial N_2H_4 (µg/mL)	% Reduction	Initial N_2H_4 (µg/mL)	% Reduction
		Unbuffered		
Unsterile + cells**	25	56	50	45
Sterile + cells**	25	57	50	45
Unsterile	25	45	50	30
Sterile	25	41	50	30
		Buffered		
Unsterile + cells**	25	92	50	81
Sterile + cells**	25	91	50	83
Unsterile	25	85	50	59
Sterile	25	85	50	64

* 0.5 g of Arredondo soil in 5 mL of unbuffered or buffered distilled water.
** Weight of cells 2.2 ± 0.2 mg/mL.

Inability of the Achromobacter sp. to enhance hydrazine degradation in soil may be in part due to hydrazine toxicity to the bacterium and in part due to a soil surface effect. The soil-water content used in this study was 0.1 mL/g of soil. Since hydrazine is highly water-soluble, at a concentration of 100 µg/g of soil the concentration of hydrazine in the aqueous phase would be 1000 µg/mL (assuming no adsorption to soil surfaces). At this concentration the activity of Achromobacter sp. would be inhibited (Ou 1987). Soil surfaces may hinder the activity of the organism by adsorbing the organism. This could result in loss of its enzyme activity, or in physical separation of the organism from the target chemical. In short, Achromobacter sp. may have potential as an agent for the detoxification of hydrazine in contaminated waters and liquid wastes.

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