Effect of Surfactants on Soil Bacteria

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Waste water reclamation is becoming the method of choice for solving water problems in arid climates. An example of this procedure is the Whittier Narrows Project (1), in Southern California, which was established to study all the technical, biological and biochemical problems connected with recharging sewage plant effluent into the groundwater by way of infiltration beds.

In Central Europe, the agricultural use of raw and treated sewage results in similar problems. Among these are the effects of residues of synthetic substances, for example surfactants, on agricultural crops and also on the organisms living in the soil. Recharge of sewage into the groundwater by means of infiltration beds and the agricultural use of sewage in crop production can only be practiced if the top soil and its biota are relatively undisturbed.

Studies on the influence of surfactants on plants (2) and bioadsorption phenomena associated with newer detergents (3) have already been reported.

The results reported in this paper are from experiments designed to reveal whether or not, and to what degree, the natural bacterial flora of soils is affected by surfactants.

Bulletin of Environmental Contamination & Toxicology,
Vol. 1, No. 6, 1966, published by Springer-Verlag New York Inc.

Methodology

The experiments were performed using the replica plating method of Lederberg and Lederberg (4,5). A dilution of the soil material to be tested was used to inoculate a Petri-dish containing 10% Trytic Soy Broth and 2% agar (pH: 7.0). After colonies had developed (48 hrs. at 20 C) they were transferred by the Lederberg method to other Petri plates containing the same medium augmented with the following concentrations of detergents: 0, 10, 20, 40, 75 and 100 ppm. After two days of incubation, the growth on the new plates was compared with that of the original culture and the missing colonies counted.

The soils used were from an oak forest and from a grassy field. Both soils belong to the para-brown-soil-type. In addition, two samples of surface water were tested.

Results and Discussion

The results summarized in the Table indicate that soil bacteria are more sensitive to surfactants than water-bacteria. In almost every case the losses of bacterial colonies from the soil samples are considerably higher then those of the water samples. This may be due to the fact that a greater part of the soil bacteria belong to the gram-positive type, while water-borne bacteria are in most cases gram-negative. The higher sensitivity of gram-positive bacteria to anionactive detergents has been reported previously (5).

The second striking result is that with increasing soil

Table. Effects of Surfactants on Bacteria

Type	conc. ppm	Percentage Loss of Bacterial Colonies with Depth (cm.) in forest soil 0	forest soil 4	forest soil 8	forest soil 15	grass field 0	grass field 2	grass field 4	in water brook	in water well
Alkylbenzenesulfonate (old, hard type) M.W. 348	10	10	0	55	20	0	3	25	0	0
	20	25	38	67	39	10	6	25	0	0
	40	55	81	67	53	14	12	42	0	0
	75	60	94	72	72	17	15	42	0	0
	100	60	94	72	75	17	18	58	0	0
Alkylbenzenesulfonate (new, soft type) M.W. 348	10	18	10	12	29	0	3	9	0	0
	20	35	30	12	47	14	3	9	0	19
	40	51	65	12	80	14	9	18	0	19
	75	51	75	24	83	22	9	37	3	19
	100	51	75	41	85	22	25	37	3	19
Ethersulfate M.W. 439	10	0	0	8	11	0	0	0	0	5
	20	0	0	15	21	7	3	5	0	5
	40	4	0	23	37	7	3	5	0	5
	75	4	11	23	40	7	3	5	5	5
	100	4	17	23	42	7	3	5	5	5
Alkylacrylsulfonate	10	0	8	54	45	4	4	0	0	0
	20	0	23	54	48	7	7	4	0	0
	40	10	39	54	68	11	7	8	0	0
	75	16	46	85	90	18	18	17	0	0
	100	27	62	85	90	18	18	25	0	0
Oil-acid hexa-methyleneimid	10	23	28	55	90	4	4	0	0	0
	20	46	46	82	100	8	10	0	0	0
	40	51	82	82	100	8	10	18	0	0
	75	51	82	82	100	12	14	18	0	0
	100	51	82	82	100	12	18	23	0	0
Oxethylized octylphenol	10	2	25	9	39	0	0	4	4	0
	20	50	69	73	87	0	4	17	11	6
	40	58	87	91	96	0	4	25	11	6
	75	58	87	91	96	6	4	29	11	6
	100	58	87	100	100	6	8	29	22	6
Fatty alcohol sulfate M.W. 334	10	4	0	9	0	0	-	0	0	0
	20	4	0	18	4	0	-	0	0	0
	40	7	8	27	43	0	-	0	0	7
	75	11	15	27	67	0	-	5	0	7
	100	14	23	46	67	0	-	11	11	7

depths the percentage of surfactant-sensitive bacteria also increases, while the biocoenosis of the soil surface is in most cases only slightly affected. This can be seen, for example, in the experiment with forest soil and the detergent "oil-acid-hexamethyleneimide". In this case the loss of bacterial colonies by adding 10 ppm of surfactant to the surface sample is only 22 percent. However, 90 percent of the colonies are lost in the sample taken at 15 centimeters depth. With higher concentrations of surfactants, the loss is even more evident. The application of 100 ppm of the surfactant reduces the number of bacterial colonies in the surface sample by 51 percent. In the 15 centimeter sample, no colonies developed.

There are not only differences between soil and water samples but also differences between different soil samples. With forest soil the previously mentioned substance, "oil-acid-hexamethyleneimide," shows the greatest toxicity, but in the soil samples from the grassy field, this toxicity is surpassed by the two alkyl-benzene-sulfonate-types.

It is also interesting to compare the new so-called "soft" alkylbenzenesulfonate with the old so-called "hard" type. The toxicity of the new type seems to be slightly higher than the old for both water and soil borne bacteria.

These results do not permit conclusions on the biochemical mechanism of the observed toxicities. Since the detergents are rather large molecules, they probably do not enter the living bacterial cell. By their very nature, these substances tend to accumulate at the borderline between two different phases, and it is probable, that the observed toxicity is due to reactions at the cell surface. Depolarization of the cell membrane by the absorption of detergents could result in decreased absorption of essential nutrients and decreased release of toxic metabolic products resulting ultimately in the death of the organism. This was also indicated in previously published results (6).

The adsorption of ABS leads to a reduction of the food uptake and oxygen consumption of aerobic bacteria. This occurs with both gram-positive and gram-negative bacteria. The gram-negative bacteria absorb detergent at a slower rate and, since this phenomenon is dependent on the free and adsorbed phases being in equilibrium, higher concentrations of ABS must be used for gram-negative organisms.

These results show that the presence of detergents in sewage-derived irrigation water is apt to destroy the natural composition of the bacterial population in the soil. Certain sensitive species will vanish, while others may be stimulated, but the original biological equilibrium will be disturbed to a degree which will have adverse effects on the purification capability of the top soil.

In natural soils with high concentrations of substances capable of adsorbing detergents this danger will be reduced, but will not be totally avoided. Experiments in progress have shown that while the carbon dioxide content in the atmosphere of undisturbed soil usually amounts to 3 to 4 percent, the

CO_2-content in soil columns (15 cm high) irrigated with soft type ABS enriched tap water dropped to values as low as 0.5 percent. These results indicate that even the "soft" - partly degradable types of detergents cause a disturbance in the soil biota.

Acknowledgment

This work was supported in part by a grant from the Wirtschaftsministerium Baden-Wurttemberg.

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