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Biological Degradation of Isoproturon, Chlortoluron and Fenitrothion

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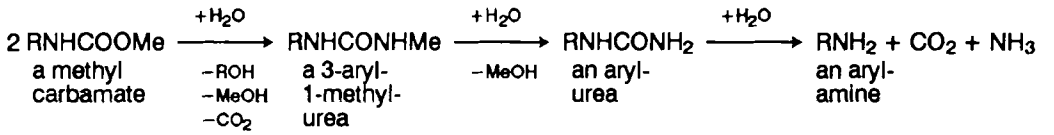
ABSTRACT. For the purpose of biodegradation studies several microorganisms were isolated from soil and adapted under laboratory conditions in the presence of pesticides, with the following degradation results: isoproturon after 72 h – 86 %, chlortoluron after 72 h – 93 %, after 65 h – 88 % and fenitrothion after 72 h – 66 %. The cultures can be used for the biodegradation of pesticides in the lyophilized state with or without an organic carrier.

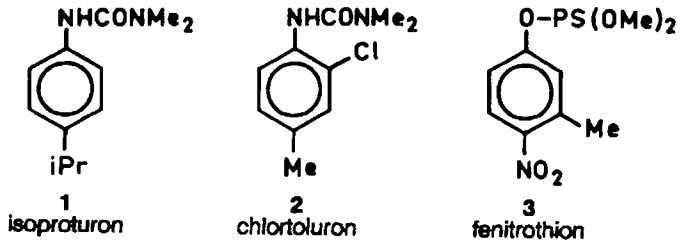
All of the urea derivatives used here are strongly absorbed by soil and subsequently by the plant root system (Ware 1983). They are applied as herbicides manufactured under brand names Monuron, Diuron, Siduron, Limuron, Isoproturon and many others.

Degradation of urea-based herbicides in nature is of a relatively long duration. Active compounds in soil or water are destroyed by UV irradiation, by acids or by alkaline compounds in the environment. Compounds adsorbed by plants are metabolically degraded like those adsorbed in soil by its microflora and microfauna. Microbial degradation has been found to be most effective, gradually transforming the urea derivatives to 3-arylureas which are then decomposed to arylamines, CO_2 and NH_3 (Engelhard *et al.* 1972) by cultures of *Bacillus sphæricus* and *Rhizopus japonicus*:



Skryabin and Golovleva (1976) applied other microorganisms capable of destroying the above herbicides, *viz.* the genera *Xanthomonas*, *Sarcina*, *Bacillus*, *Penicillium* and *Aspergillus*, all of which are able to utilize urea derivatives as a sole carbon source. At the same time, the cleavage of methoxy groups and their oxidation proceed in a way similar to deamination and decarboxylation. As intermediate products the following compounds can be formed: RNH–CO–NHMe, RNH–$CONH_2$ and RNH_2. The basis of decomposition is N-demethylation, followed by oxidation of the aromatic ring. Due to this reaction a cleavage of the aromatic ring and generation of muconic acid, acetate and succinate take place. Microorganisms participating in the detoxication of chlorinated pesticides are found in soil and water. Enzymes liberating halogens in an ionic form are called dehalogenases. A number of experiments were done with the enzymic preparations of bacteria to release chlorine in the presence of NAD. It is assumed that the indicated process is a cometabolism in which the enzyme synthesis is induced. Sporulating bacteria with vitamin B_{12} in the medium are able to release chlorine from a pesticide and the same property is displayed by *Trichoderma viride*. In the detoxication of chloro-sulfur compounds an optimization of N, P and C factors as well as the presence of cysteine and glutathione in the cultivation medium are very important. During the experiments with *Arthrobacter* sp. it was shown that halogen was liberated into the medium already before the beginning of the exponential growth phase of the culture (Mogilevitch 1982). The decomposition of chlorinated phenols depends on the number and position of chlorine atoms (Alexander 1969; Čerňáková 1994).

The present study concerns the isolation of bacteria capable of degrading isoproturon [N,N-dimethyl-N′-(4-isopropylphenyl)]urea (1) in the form of Arelon 75 WP containing 33 % of active ingredient (AI), chlortoluron [N,N-dimethyl-N′-(3-chloro-4-tolyl)]urea (2) in the form of Dicuran containing 88 % of AI and fenitrothion [O,O′-dimethyl-O″-3-methyl-4-nitrophenyl)]thiophosphate (3) in the form of Metathion E-50 containing 98 % of AI.



Fenitrothion (3) is used as an insecticide to protect plants against chewing and sucking insects attacking a broad spectrum of cereals.

The present study was intended to prepare an adapted culture that would be usable in an intensified process of purification of organically polluted water and soil.

MATERIALS AND METHODS

The pesticides 1–3 were supplied by the *Central Institute of Agriculture for Control and Testing* (Bratislava) as dimethyl sulfoxide solutions. They involved isoproturon in the form of Arelon 75 WP containing 33 % of AI, chlortoluron in the form of Dicuran containing 88 % of AI and fenitrothion in the form of metathion E-50 containing 98 % of AI.

Isolation of microorganisms. For inoculation a nonagricultural soil was used which was in a long-lasting contact with the material tested at a relatively high concentration. For the isolation of microorganisms the soil from the area of *Istrochem*, Bratislava, was used. The isolated bacteria have not been so far identified. It can be reliably claimed that for each pesticide tested the biodegradation was performed by a different kind of bacterium.

The isolation and adaptation of microorganisms was carried out in a test-tube with MPB (meat peptone broth 2) in the presence of isoproturon (1) 1000, 100, and 10 μg/L, chlortoluron (2) 40 mL/L and fenitrothion (3) 20 mL/L. Cultivation took place at room temperature for 21 d. Bacteria were isolated in a Petri dish on solid soil MPA (meat peptone agar 2) in the presence of the above isoproturon concentrations. At 1 g/L the largest colonies were used for isolation. In the case of chlortoluron, 400, 800 and 1200 μg/L of the pesticide were added to MPA. The concentrations of fenitrothion were 200, 400 and 800 μg/L. Higher concentrations were difficult to achieve because of the low solubility of fenitrothion. The rest of the sample was used for the analysis of pesticide degradation. The most overgrown colonies at higher concentrations were inoculated for a long-time adaptation back to MPB in the presence of the pesticide. The respective cultures were transferred to a fresh medium with the pesticide.

Preservation of cultures. The isolated bacteria were kept in a test-tube on MPA in the presence of the pesticides, sealed with paraffin oil and maintained at 4 °C.

Propagation of biomass. For the purpose of biodegradation a propagation of biomass was necessary. The propagation was implemented in MPB in the presence of the pesticide in a reciprocal shaker at laboratory temperature.

Biological degradation. Degradation of biological pesticides caused by a propagated culture proceeded in the following solution (g/L): NH_4Cl 59.34, KH_2PO_4 8.79, K_2HPO_4 11.25, $Na_2HPO_4 \cdot 12H_2O$ 23.13, $NaH_2PO_4 \cdot 2H_2O$ 10.07, tap water 1 L; pH 7.4.

Testing of the pesticide effect on isolated bacteria. The effect of agricultural concentrations on bacteria was assessed by a diffusion method, using as before four variants with 1 g of the lyophilized culture I/L and the carrier NX 29 g/L as follows: (*1*) carrier unsoaked, (*2*) culture I soaked in water for 1 d prior to the onset of experiment, (*3*) culture I soaked in advance and the carrier NX unsoaked, (*4*) culture I and the carrier NX, both soaked in advance.

Culture I was isolated from soil for the purpose of isoproturon biodegradation. Carrier NX is a highly porous, organic material from crushed wood bark.

The isoproturon (1) concentration was 260 mg/L. Sampling was done after 0.5, 1 and 24 h.

Conditions for analytical determinations: Liquid chromatograph Varian VISA 5560; Integrator Varian 4270, Column-Micro Pak MB-2 C 10-5 150 × 2 mm i.d.; detector UV 220 nm.

RESULTS AND DISCUSSION

The biodegradation results are shown in Table I and Fig. 1. It is assumed that by applying the higher biomass concentration the time of degradation is reduced. With a multiple repetition of experiments the reproducibility of the indicated results was achieved. It may be seen that the anticipated positive effect of sludge (column 2 of Table I) could not be confirmed, apparently because the bacteria were devoured by protozoas.

Table I. Content of herbicides (mg/L) and efficiency of their biodegradation (%); experiments no. 1–5 [a]

Time h	Isoproturon (1)				Chlortoluron (2)				Fenitrothion (3)	
	1		2[b]		3		4		5	
	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%
0	201	0	190	0	130	0	255	0	48.5	0
6	131	35	190	0	77.0	41	–	–	46.0[c]	5.2[c]
12	103	49	177	7	61.8	53	–	–	–	–
18.5	–	–	–	–	–	–	–	–	39.2	19
24	48.1	76	122	36	58.6	55	206	19	33.2[d]	31[d]
41	–	–	–	–	–	–	56.4	78	–	–
48	43.9	78	122	36	29.4	78	–	–	16.6	66
65	–	–	–	–	–	–	31.8	88	–	–
72	27.9	86	122	36	8.8	93	–	–	16.6[e]	66[e]
96	–	–	–	–	4.7	96	–	–	15.6[f]	68[f]
164	–	–	–	–	–	–	–	–	4.9	90
186	–	–	–	–	–	–	–	–	4.1	92

[a]Dry mass (g/L) in experiments no.: 1 – 0.76; 3 – 0.35; 4 – 9.8; 5 – 0.45.
[b]With bacteria and sludge.
[c]At 7 h. [d]At 22 h. [e]At 46 h. [f]At 94 h.

The culture can be applied for purification either alone without a carrier, or it can be used with it. The carrier should have a highly porous surface and some other advantageous properties. The highly porous, organic carrier NX, which can be easily liquidated, was selected. It is prepared from sludge retained in mechanical effluent-treatment plants of pulp and paper industry. It consists of short pulp fibers which, after being dehydrated, represent a compact material that cannot be aerated. For this reason crushed wood bark is added (Bezúch *et al.* 1989). The wood bark is capable of bonding the biocidic materials, including pesticides (Blažej *et al.* 1981). The advantage of the carrier is derived from the fact that it does not need to be liquidated and its surface is covered by a great number of bacteria, actinomycetes and micromycetes (Figs 2–3) which can also join in the degradation process. These are microorganisms adapted to the phenolic substrate.

Metabolism of organophosphoric pesticides. The metabolism of organophosphoric pesticides includes several reaction types, the majority of which have an oxidative character and can give rise to the product of higher acute toxicity, corresponding to that of the initial compound (Matsumura 1975; Scheme I).

(*1*) Oxidation of thiophosphates and dithiophosphates to the respective phosphates and thiophosphates is performed by nonspecific oxidases and is also called desulfuration (P=S → P=O), the products of which are the so-called oxones; *e.g.* fenitrothion (**3**) is changed to fenitrooxone (**4**).

(*2*) Oxidation cleavage of the P–O aryl bond; fenitrothion is decomposed to dimethylphosphoric acid (**5**) and 4-nitro-3-cresol (**6**).

(*3*) Hydroxylation of side chains.

(*4*) Isomerization of thiophosphates. The $(RO)_2P(S)-O-$ group is usually changed into RS–(RO–)P(O)–O-. In this way fenitrothion is isomerized to its S-methyl isomer (**7**).

(*5*) Dealkylation or dearylation caused by glutathione S-transferases (*e.g.* demethylfenitrothion, **8**).

(*6*) Reduction of the nitrophenyl group to the aminophenyl one and the formation of aminofenitrothion (**9**).

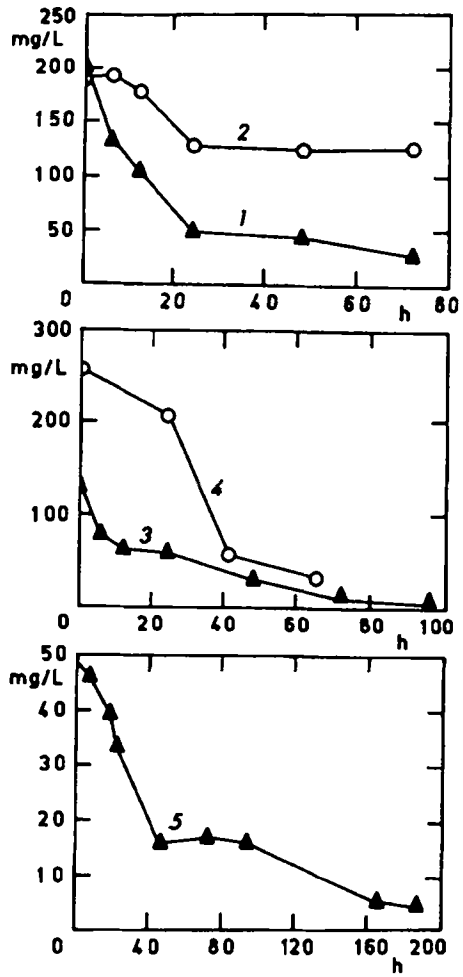


Fig. 1. Degradation (concentration, mg/L) of isoproturon (*top*), chlortoluron (*middle*) and fenitrothion (*bottom*); numbers of experiments are given (see the text and Table I).

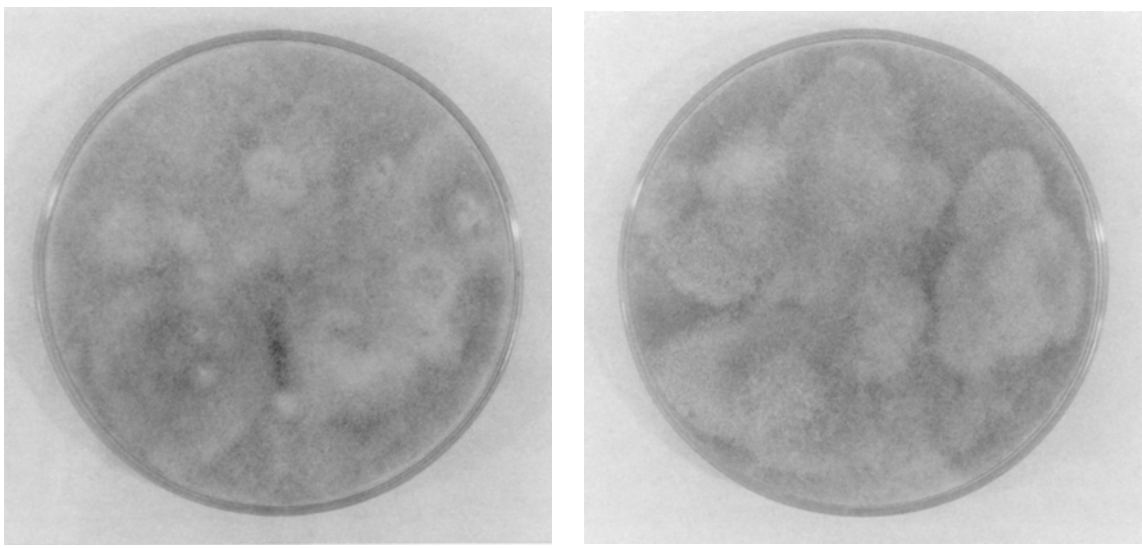


Fig. 2. Malt extract agar, 0.1 g of the carrier unused before the biodegradation (*left*) and used after the biodegradation (*right*).

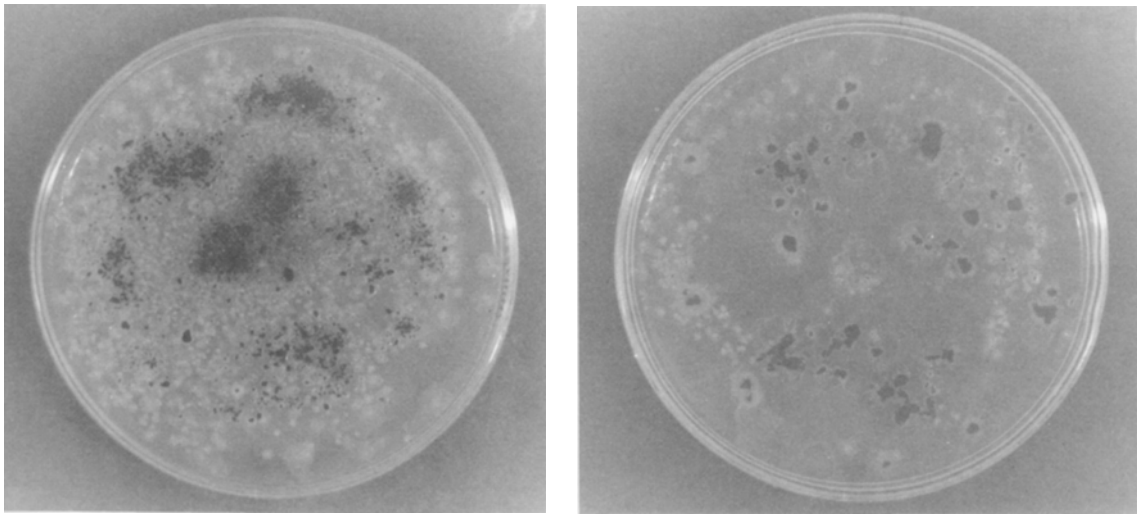
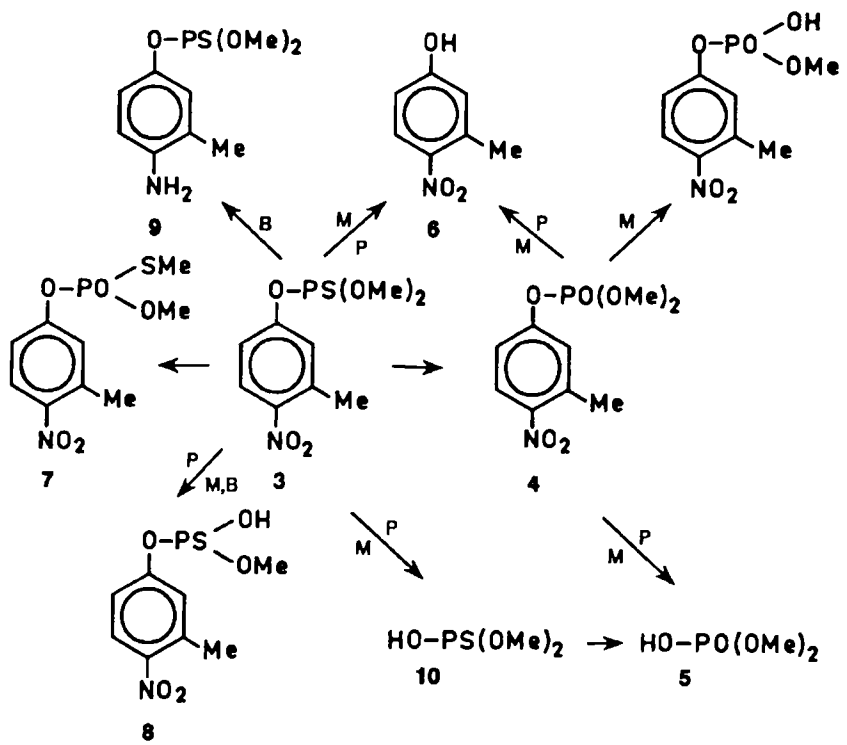


Fig. 3. Nutrient broth agar, 0.1 g of the carrier unused before the biodegradation (*left*) and used after the biodegradation (*right*).



Scheme I. Metabolism of fenitrothion (**3**); **4** – fenitrooxone, **8** – demethylfenitrothion, **9** – aminofenitrothion; for further compounds see the text; B – bacteria, M – mammals, P – plants.

Biotransformation of fenitrothion (**3**) is the result of the overall interaction with various enzyme systems, particularly with nonspecific microsomal oxidases, phosphatases and glutathione S-transferases. By oxidizing the thiophosphoryl group (P=S bond) to phosphoryl (P=O bond) fenitrothion is changed from the compound of low anticholinesterase efficiency to a strong inhibitor of cholinesterase, fenitrooxone (**4**). Simultaneously, oxidative cleavage of fenitrothion to dimethylthiophosphoric acid (**10**) and to free 4-nitro-3-cresol (**6**) takes place. This process is one of the principal modes of removing the toxically dangerous residua from biological environment. The conjugation reaction brought about by glutathione S-transferases is another mode of elimination where the dealkylated derivative of demethylfenitrothion (**8**; Fukunaga *et al.* 1969) is formed.

As demonstrated in Table II, the carrier itself displays high adsorption properties. It is able to adsorb isoproturon (1) with the efficiency of 89 % already after 1 h. When the carrier is applied to soil, it represents a suitable compost.

Table II. Biodegradation of isoproturon (1) with culture I and carrier NX

Conditions	Concentration of 1 (mg/L) after[a]		
	30 min	1 h	1 d
Carrier unsoaked	28.6	29.2	29.4
Bacteria soaked			
alone	31.0	24.4	23.6
with carrier unsoaked	33.6	28.8	25.8
with carrier soaked	23.5	17.6	15.3

[a]Initial concentration 260 mg/L.

From among four variants of the experiment performed with a culture and carrier the best one was the fourth variant in which the culture and carrier were soaked in water for 1 d before the start of the experiment (Table II).

The pesticide degradation occurring in nature takes a long time because of the lower number of nonadapted microorganisms participating in it. By applying a higher amount of adapted biomass in the lyophilized state to the contaminated locality, soil, waste water or rinsing water generated in the plant for the preparation and use of pesticides in agriculture the biodegradation process will be substantially accelerated. The lag phase will become shorter and the culture will be without stress.

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