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DEGRADATION OF TRICHLOROETHYLENE BY BACTERIA ISOLATED FROM ACTIVATED SLUDGE

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ABSTRACT
Trichloroethylene (TCE) is one of the most serious chlorinated pollutants of some groundwater of many industrial countries so that a possibility of its microbial degradation is an aim of many research and engineer teams. Natural strain C. testosteroni RF2 and its derivative strain VM obtained by mutagenesis were tested for their capability to trichloroethylene (TCE) degradation. Both strains were able to grow on lactate and phenol in the presence of TCE at its concentrations of 0.8 to 50 mg/l and in the state of resting cells were able to degrade TCE for approx. 22 – 24 hours. TCE degradation by both strains led to more than 95 % release of inorganic chlorides, but VM culture produced a significantly higher quantity of trichloroacetic acid (3.4 mol %) than maternal strain RF2 (0.15 mol %). Using lactate and phenol as the growth and inductive substrates respectively, it was possible to carry out three consecutive stages of TCE degradation by the strain RF2 under growing conditions, with substantial regeneration of its transformation capacity.
Keywords: Trichloroethylene, TCE, degradation

1. INTRODUCTION
Trichloroethylene (TCE) is a recalcitrant solvent occurring in many groundwaters or soils of industrial countries and its microbial degradation is a promising way to environment decontamination. Phenol-, toluene- or methane-utilizing bacteria belong to the best known degraders of the compound; however, their abilities to trichloroethylene transformation are usually time-limited due to inducible nature of appropriate enzyme(s). An action of above mentioned bacteria consists in their abilities to produce certain type(s) of oxygenases, cometabolically transforming trichloroethylene to TCE-epoxide or similar unstable intermediate yielding organic acids, carbon monoxide and chlorides as the end products of transformation. Only in some type of methanothrophs certain amounts of di- and tri-chloroacetic acids were after TCE degradation found (1).

The trouble linked with relatively short time of degradation done by inducible character of the oxygenases may be principally solved either by a preparation of genetically modified microorganisms with constitutive enzyme production or by repeating inducing and degrading phases in natural strains.

Several years ago we isolated phenol utilizing strain of *Comamonas testosteroni* showing high degrading activity against trichloroethylene. In this work we studied some aspects of trichloroethylene degradation by two strains of *C. testosteroni* aimed to their potential use for bioremediation of groundwater contaminated.

2. EXPERIMENTAL

Bacterial culture.
Comamonas testosteroni RF2 (Cat. No. CCM 7350) was used throughout the study. It is able to grow on phenol at its concentrations up to 300 mg/l; it can utilise some organic salts (acetate, citrate, lactate, glyoxylate) as carbon and energy source. *Comamonas testosteroni* VM was derived from RF2 culture by mutagenesis with the help of sodium azide (1 mg/l, 3 hours) and selection on agar plates with 500 mg/l phenol as the only carbon source.

TCE degradation under growing condition.
Portions of 10 ml mineral medium were placed into 40-ml sample vials with lactate (100 mg/l), phenol (100 mg/l) and yeast extract (20 mg/l) added. Each sample vial was inoculated with 10 μl of cell suspension and immediately after adding TCE (concentration range 0.8 – 50 mg/l) the vials were closed. TCE stock water solution or methanol solution (60 g/l) were used. Cultivation took place statically in darkness, at 25°C for 5 days, followed by determination of final TCE concentrations by gas chromatography.

Repeated TCE degradation under growing condition.
Six sets of the same sample vials were prepared. All the vials were inoculated with 10 μl of *C. testosteroni* cell suspension. TCE at a concentration of approx. 11 mg/l was into the vials of three ("test") sets added. After 5-day cultivations at 25°C on rotary shaker the final TCE concentrations in the vials of first set were determined. Rest TCE in the second and third test sets was subsequently stripped by air and lactate, phenol and TCE were repeatedly added at the same concentrations. Cells surviving first degradation were used as inoculums. Cultivation was carried out in identical manner and the whole procedure was even repeated for a third time. Further three sets of sample vials were applied in parallel manner, during which TCE was not added during the first or first and second stages of growth respectively, but was added only in the second or third stage of degradation respectively ("comparative" sets).

TCE determinations.
Samples of water phase (0,5 ml) were analysed by gas chromatography (Hewlett Packard 5890) after sample concentrating by Tekmar 2000. The volatile agents were separated on capillary column Quadrex (30 m x 0,53 mm, 3 μm film) and detected by FID.

Determination of chloride ions released during degradation.
Enriched and inducted cells were washed and suspended in a chlorides-free medium and TCE degradation (4.3 mg/l) by resting cells was performed. The quantity of chloride ions was determined by the spectrophotometric method according to Iwasaki and corrected for the blank tests (cells without TCE and TCE in distilled water).

Determination of trichloroacetic acid (TCA).
Validated methods of LABTECH Brno Company were used for TCA determination after TCE degradation. The cells were removed by centrifugation.

3. RESULTS AND DISCUSSION

Determination of the strain's ability to TCE degradation under growing condition
The growth of *C. testosteroni* RF2 and VM on a mixture of lactate and phenol in the presence of different TCE concentration including estimations of TCE removal are in the Table 1 presented. The tests showed the ability of the culture to grow in the presence of TCE at different concentrations indicating applicability of the strain in contaminated environments. Applying of phenol, lactate and yeast extract led to full TCE removals at its concentrations 0.8 and 1.5 mg/l and to partial TCE removals at 5 and 10 mg/l, respectively.

Table 1: TCE degradation by RF2 and VM strains after their 5-days growth in a presence of TCE (means and SD, for n = 3)

Initial TCE concentration (mg/l)	Culture growth		TCE removal (%)	
	RF2	VM	RF2	VM
0.76 ± 0.02	++	++	100	100
1.51 ± 0.03	++	++	100	100
5.05 ± 0.14	++	++	54.6 ± 2.2	73.8 ± 1.6
10.0 ± 0.26	++	++	28.7 ± 1.4	39.7 ± 2.1
25.2 ± 1.1	++	++	N	10.0 ± 0.8
49.8 ± 1.7	+	++	N	0

\+ culture growth on lactate and phenol in 72 hours
++ culture growth on lactate and phenol in 24 hours
N not tested

Determination of the level of TCE mineralization

Owing to great importance of end products formed by bacterial TCE degradation the levels of trichloroacetic acid (as the most hazardous potential end product) and of inorganic chlorides were estimated. Tests were performed separately and TCE was applied at a concentration of 4.3 mg/l; both strains were used in state of resting cells and final concentrations of inorganic chlorides and trichloroacetic acid were determined. TCE degradation by both strains led to more than 95 % release of inorganic chlorides, but VM culture produced a significantly higher quantity of trichloroacetic acid (3.4 mol %) than maternal strain RF2 (0.15 mol %). TCA productions in strain RF2 may be considered as nearly negligible; in addition to this, in the last years TCA was even proved as naturally originating compound in some soils (2,3,4). Generally, TCE degradation performed by *C. testosteroni* RF2 may be considered as a process not producing environmentally hazardous compounds.

Testing of the strain RF2 to perform three repeating degradation steps.

We tested the possibility of the strain to perform three consecutive TCE degradations out, each at initial concentration of 11 mg/l. Besides to determination of TCE removals the cell numbers at the end of each stage of degradation were estimated. Results of the test and comparative cells (not undergoing TCE degradation in the first or first and second stage respectively) are given in the Table 2, including blanks subtraction.

The test essentially showed the possibility of repeating growth of the culture in the presence of TCE followed by pollutant degradation. As generally expected, the most significant level of TCE removal was found in the first stage; in the following ones a mild reductions in cells degradation capability already occurred (degradation 80.8 – 70.0 – 60.3%), nevertheless removal of approx. two thirds of added trichloroethylene in the second and third stages may be regarded as a promising result.

Table 2: Repeated degradation of TCE by culture RF2 under growing condition (means and SD, for n = 4)

Stage No.	Set of vials	Cell number (CFU / ml)	TCE concentration (mg/l)		TCE removal (%)
			Initial	Final	
1	Test	$2x\ 10^6$	11.03 ± 0.09	2.11 ± 0.20	80.8
	Comparative	$2x\ 10^7$	0.0	0.0	-
2	Test	$6x\ 10^6$	11.03 ± 0.09	3.30 ± 0.19	70.0
	Comparative	$8x\ 10^7$	11.03 ± 0.09	1.28 ± 0.16	88.3
3	Test	$6x\ 10^6$	11.03 ± 0.09	4.37 ± 0.34	60.3
	Comparative	$9x\ 10^7$	11.03 ± 0.09	1.16 ± 0.22	89.5

4. CONCLUSION

Experiments showed that *Comamonas testosteroni* RF2 is able to grow at TCE concentrations up to min. 25 mg/l. TCE degradation by the strain leads to high level of TCE mineralization. Degrading ability of the cell suspension may be regenerated by repeating addition of the growth and inductive substrates. Furthermore, owing to the properties of *Comamonas testosteroni* it appears to be a suitable species for remediation of groundwater contaminated by trichloroethylene.

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5. REFERENCES

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