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Biodegradation of dissolved jet fuel in chemostat by a mixed bacterial culture isolated from a heavily polluted site

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Abstract

A mixed bacterial culture capable of biodegrading of jet fuel was isolated from a heavily polluted site in Tapa, Estonia. Residual concentrations of pollutants in the chemostat culture were determined. The total residual concentrations of dissolved jet fuel in culture medium were 0.42 and 2.1 μg l^{-1} at the dilution rates 0.1 and 0.17 h^{-1} respectively. Benzene, toluene, ethylbenzene, and xylenes were completely degraded and thus not detected in culture broth (detection limit 0.1 μg l^{-1}) at the dilution rates 0.1 and 0.17 h^{-1}. The values of apparent substrate saturation constant (K_{Sapp}) in multisubstrate growth conditions were estimated from the experimental data. The residual concentrations satisfy the regulations in the Republic of Estonia for petroleum hydrocarbons (0.00 mg l^{-1} – 'very good'). Results obtained indicate that use of the biodegradation could be sufficient for the treatment of polluted with kerosene-type jet fuel groundwater up to the acceptable quality.

Abbreviations: BTEX – benzene, toluene, ethylbenzene, xylenes; CSTR – continuous stirred tank reactor; FID – flame ionisation detector; NAPLs – non-aqueous phase liquids; TMBs – trimethylbenzenes; WSF – water soluble fraction

Introduction

Groundwater pollution by aviation fuel at former Soviet military sites is a serious environmental problem in Estonia. The most critical situation is at the former air base in Tapa, where several thousand cubic meters of 'wide-cut' type jet fuel had been spilled for almost 40 years. Within the polluted region, dissolved components of jet fuel are spread over an area of 16 km^2, and non-aqueous phase liquids (NAPLs) have been found to cover 6 km^2 (Metsur et al. 1995). The concentration of the 'wide-cut' type jet fuel in polluted groundwater is usually in the range of 0–3 mg l^{-1}, because of its low solubility and non-equilibrium dissolution in field conditions. The biodegradation rate of dissolved jet fuel at these conditions might be limited by its low concentration as described by Monod kinetics (Monod 1942). Many reports (Alvarez et al. 1991; Grady et al. 1989; Button 1985; Robertson & Button 1987; MacQuarry et al. 1990; Jørgensen et al. 1990) describe the kinetics of biodegradation of benzene and toluene in single-substrate experiments. However, common water contaminants like gasoline, jet fuel, and crude oil, are complex mixtures of many hydrocarbons, and biodegradation kinetics might differ from the kinetics of single substrate utilisation. Although mixed-substrate utilisation (see review of Egli, 1995) and the interaction of different aromatic substances during biodegradation were studied quite intensively (Dyreborg et al. 1996a; Dyreborg et al. 1996b; Jørgensen et al. 1995; Arvin et al. 1989), the kinetics of biodegradation of mixtures of pollutants received quite little attention (Schmidt & Alexander 1985; Subba-Rao et al. 1982; Namkung & Rittmann 1987; Kovarova et al. 1997), considering into

account the importance of this topic to environmental biotechnology.

The aims of the study presented here are: 1) to assess the feasibility and efficiency of biodegradation of dissolved jet fuel by mixed bacterial culture isolated from polluted groundwater in Tapa, and 2) to investigate the kinetics of biodegradation of jet fuel components in mixed substrate culture at the different growth rates using chemostat cultivation technique.

Materials and methods

Micro-organisms and culture conditions

A mixed microbial culture isolated from groundwater contaminated with aviation fuel (Tapa region) was used in this study. Contaminated groundwater for the isolation of bacterial consortia and chemostat experiments was collected from a well into 1-litre dark glass bottles without headspace and was preserved at 4 °C prior to experiments.

Kerosene-type jet fuel, manufactured in the USSR for civil aircrafts and for which specifications and physical properties are similar to commercial civil kerosenes Jet A, Jet A1 and military JP-8 was used in the study of biodegradation. Kerosene-type jet fuel was used in the experiments instead of 'wide-cut' military jet fuel, as the latter is not used in Estonia any longer, while spilled 'wide-cut' fuel found as a NAPL in the ground is partially weathered and biodegraded.

Inoculum for the experiments was pre-grown for 3 days in enrichment culture with basal medium M9 (Adams 1959) diluted 10 fold by distilled water plus trace elements (Bauchop & Elsden 1960) and fuel (1% v/v). Culture medium for batch phase of chemostat cultivation and feeding medium was prepared as follows: basal medium M9 was diluted 10-fold by distilled water and autoclaved. Jet fuel of kerosene-type was added after autoclaving in the ratio 5:1000. Medium was stirred for 5 hours, and the insoluble part of the jet fuel (NAPL) was separated and discarded. Aqueous phase, saturated with jet fuel, was used as a medium for batch phase of chemostat and feeding. Chemostat cultivations were carried out in 2-litre fermentors. The ratio of inoculation was 1:10. The volume of culture medium in a fermentor was 1 litre, and it was kept at the constant level by an overflow device. Initially, chemostat operation was started at half of the nominal dilution rate. After 24 hours of chemostat cultivation, the dilution rate was established at the nominal value and was run for 113 hours (11.3 and 19.4 fermentor volumes for experiments at the dilution rates 0.1 and 0.17 h^{-1} respectively) in order to reach constant residual concentration of limiting substrate (dissolved jet fuel). The culture medium was maintained at 27 °C throughout the experiment. Abiotic control experiments for the determination of the air-stripping efficiency of jet fuel were carried out in batch culture at the same environmental conditions as chemostat experiments using sodium azide (2000 mg l^{-1}) for preventing of bacterial growth.

Analysis of dissolved components of aviation fuel in the ground water

The samples of groundwater were extracted with 10 mL of pentane per 1 litre of sample for 1 hour. Concentrations of hydrocarbons were measured by GC/MS. Gas chromatograph Varian 3400CX was equipped with column OV-101 (length 30 m, inner diameter 0.25 mm) that was packed with DB-1 (diameter of particles 1.0 μm). The operating conditions were the following: gas-carrier nitrogen set at a flow rate of 3 ml min^{-1}, pressured air at 350 ml min^{-1}, hydrogen at 30 ml min^{-1}, and make up gas-nitrogen at 25 ml min^{-1}. The GC was equipped with an FID detector run at a temperature of 300 °C. The oven temperature was altered according to the pre-set program: 40 °C for 2 min, then increased over 7.2 min with an acceleration of 25 °C min^{-1} until reaching 220 °C. This temperature was held for 1 min then increased to 300 °C with a slope of 15 °C min^{-1}. The detector temperature was held at 250 °C. The volume of injection was between 1 to 2 μl (depending on the concentration of jet fuel in samples). The detection limits for components of jet fuel in water were as follows: 0.1 μg l^{-1} for single components, 3 μg l^{-1} for total concentration of fuel hydrocarbons. The precision of analysis was 0.05 μg/L. The analyses were carried out in the Central Laboratory of the Ministry of Environment in the Republic of Estonia.

Results and discussion

Composition of water-soluble fraction of kerosene

Kerosene-type jet fuel, which is usually used by civil and naval military aircrafts, has a narrower distillation range (200 °C to 300 °C) than 'wide-cut' fuels (80 to 300 °C) and therefore has a lower content of light aromatics (e.g., BTEX). In spite of this, our data showed

Table 1. Analysis of feeding and culture media in chemostat cultivation

Compound	Feeding medium conc. [μg l^{-1}]	D=0.1 [h^{-1}] conc. [μg l^{-1}]	D=0.17 [h^{-1}] conc [μg l^{-1}]
benzene	41.6		
toluene	324.5		
m-xylene or ethylbenzene	117.4		
p-xylene	623.4		
o-xylene	503.8		
1-methylethylbenzene	81.1		
propylbenzene	30.5		
1,3,5 -TMB	329.8		0.24
2-ethylmethylbenzene	275.8	0.21	0.48
1,2,4 -TMB	422.4		
methylpropylbenzene	51.8	0.21	0.48
1,2,3 -TMB	365.1		0.12
1-methyl 3-propylbenzene	67.7		
1,2-Diethyl benzene	70.6		0.24
1-methyl 4-propylbenzene	98.6		
ethyldimethylbenzene	118.7		
methyldiethylbenzene	83.6		
tetramethylbenzene 1	92.4		0.6
tetramethylbenzene 2	183		
naphthalene	25.5		
2-methylnaphthalene	30		
1-methylnaphthalene	69.7		
In total	4780	0.42	2.1

that the water soluble fraction (WSF) of kerosene-type jet fuel contained a considerable amount of benzene (41.6 μg l^{-1}), toluene (324.5 μg l^{-1}), and xylenes and ethylbenzene (1244.6 μg l^{-1}) (Table 1). In total, BTEX compounds, which are known for their acute toxicity and benzene for its carcinogenic properties (Maltoni et al. 1985; Snyder & Kocsis 1975; Carpenter et al. 1975; Carpenter et al. 1976), constituted 1610.7 μg l^{-1} or 33.7% of the total concentration of dissolved kerosene-type jet fuel in water. Trimethylbenzenes (TMBs) were also present at high concentrations in WSF. All three isomers of TMB constituted 1117.3 μg l^{-1} or 23.4% of the total hydrocarbon concentration (Table 2). These data indicated a potential risk to human health of groundwater pollution caused by dissolved components of kerosene-type jet fuel.

Biodegradation of dissolved jet fuel in chemostat culture

Experimental results obtained showed that aromatic hydrocarbons were efficiently removed by the biological treatment. The residual concentrations of total non-polar hydrocarbons were 2.1 μg l^{-1} and 0.42 μg l^{-1} in experiments with dilution rates 0.17 h^{-1} and 0.1 h^{-1}, respectively. The degrees of purification obtained in the both experiments were more than 99.9%. The removal rate of dissolved fuel in chemostat culture at the dilution rate 0.17 h^{-1} was 822 μg l^{-1} h^{-1}. The treated water in both experiments meets regulatory norms of the Republic of Estonia: the allowed content of petroleum hydrocarbons in potable water has been set 0.02 mg l^{-1} for 'good' and 0.00 mg l^{-1} for 'very good' water quality.

The results obtained indicate that continuous bioremediation processes should be considered a feasible option in large scale treatment. Indeed, besides good purification effects, continuous (chemostat) treatment avoids inhibition by the high concentrations of fuel hydrocarbons, as their concentrations are maintained low in the bioreactor in comparison with those in the feeding medium. Low residual concentrations in the continuously functioning bioreactors minimise potentially dangerous volatilisation of poisonous compounds into the air.

Table 2. Changes of composition of kerosene's WSF

Compound	Concentration (μg l^{-1}) and percentage (%) in saturated solution	Residual concentration (μg l^{-1}) at D=0.17 h^{-1}
Benzene	41.6 (0.87%)	<0.1
Toluene	324.5 (6.79%)	<0.1
Xylenes	1244.6 (26.04%)	<0.3
Trimethylbenzenes	1117.3 (23.37%)	0.36
Ethylmethylbenzene	356.9 (7.47%)	0.48
Methylpropylbenzenes	218.1 (4.56%)	0.48
1,2-diethylbenzene	70.6 (1.48%)	0.24
Tetramethylbenzenes	275.4 (5.76%)	0.6
Naphthalenes	125.2 (2.62%)	<0.3

Analysis of culture medium using GC/MS showed that only two substances were present in effluent at the dilution rate 0.1 h^{-1}: ethylmethylbenzene with concentration 0.21 μg l^{-1} (0.076% of initial concentration) and methylpropylbenzene at 0.21 μg l^{-1} (0.4% of initial concentration). As mentioned before, the total residual concentration of dissolved fuel was 0.42 μg l^{-1} at D=0.1 h^{-1} (Table 1).

The residual concentration of dissolved kerosene and the number of its components increased as dilution rate increased; two components at D=0.1 h^{-1} and seven at D=0.17 h^{-1} (see Table 1). 2-ethylmethylbenzene and methylpropylbenzene were found in the both chemostat cultures. 1,3,5-TMB, 1,2,3-TMB, 1,2-Diethylbenzene, and Tetramethylbenzene 1 were detected in the effluent in the experiment with dilution rate 0.17 h^{-1}, but were not detected in the experiment with D=0.1 h^{-1}. Components of kerosene with higher molecular weight were found to have higher residual concentrations. Naphthalene and methylnaphthalenes, potentially the most recalcitrant compounds, were not detected in the effluents.

The following components of kerosene present in the untreated water were not detected in effluent medium at both dilution rates studied: BTEX, 1-methylethylbenzene, propylbenzene, 1,2,4-TMB, 1-methyl 3-propylbenzene, 1-methyl 4-propylbenzene, ethyldimethylbenzene, methyldiethylbenzene, tetramethylbenzene 2, naphthalene, and methylnaphthalenes.

Role of the volatilisation in removal of jet fuel components

Due to the relatively high vapour pressure of jet fuel components (especially benzene) (Connell et al. 1984), removal of jet fuel components during the treatment due to volatilisation can be significant, and therefore it should be discerned from biodegradation. However, design of a 'direct' control experiment where the abiotic loss by volatilisation of the fuel components could be determined in continuous bioreactor is not as obvious as it might seem to be. The peculiar feature of chemostat technique is that the residual concentration(s) of limiting substrate(s) (carbon source) are kept at very low level in comparison with the respective concentrations in influent. Volatilisation rates were estimated by us from the data obtained in abiotic batch culture run in the same experimental conditions as chemostat culture and extrapolated to the residual concentrations observed in the chemostat reactor effluent. The experiments (Figure 1) showed that the volatilisation rates of jet fuel hydrocarbons at concentration 40 μg l^{-1} was less than 0.6 μg l^{-1} h^{-1}. The total residual concentration of non-polar fuel hydrocarbons was 2.1 and 0.4 μg l^{-1} during the two chemostat cultivations. Calculation of volatilisation rates at these concentrations gives 0.16 and 0.03 μg l^{-1} h^{-1} correspondingly. There-

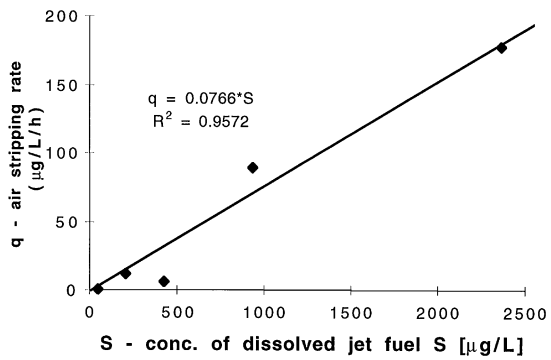

Figure 1. Dependence of air-stripping rate on concentration of dissolved fuel.

Table 3. Values of apparent substrate saturation constant for some components of kerosene's WSF

Compound	K_S [μg l^{-1}]
benzene	<0.19
toluene	<0.19
m-xylene or ethylbenzene	<0.19
p-xylene	<0.19
o-xylene	<0.19
1-methylethylbenzene	<0.19
propylbenzene	<0.19
1,3,5 -TMB	0.46
2-ethylmethylbenzene	0.61
1,2,4 -TMB	<0.19
methylpropylbenzene	0.61
1,2,3 -TMB	0.23
1-methyl 3-propylbenzene	<0.19
1,2-Diethyl benzene	0.46
1-methyl 4-propylbenzene	<0.19
ethyldimethylbenzene	<0.19
methyldiethylbenzene	<0.19
tetramethylbenzene 1	1.14
tetramethylbenzene 2	<0.19
naphthalene	<0.19
2-methylnaphthalene	<0.19
1-methylnaphthalene	<0.19
kerosene (in total)	0.46

fore, volatilisation was considered negligible, and it was assumed that biodegradation was the prevailing process for the removal of hydrocarbons in our experiments: more than 99.98% of the removal observed.

Analysis of the experimental data obtained

The calculation of apparent K_S values was carried out using the data obtained at two different growth (dilution) rates. From the Monod equation, $\mu = \mu_{max}$ (s/(s+$K_{S(app)}$)) an apparent μ_{max} was comparted: μ_{max} = μ (s+$K_{S(app)}$)/s. Assuming that the apparent maximum growth rate (for the experimental set-up used) is equal for both dilution rates, the following equation for two growth rates studied can be obtained:

$$\mu_1(s_1 + K_{S(app)})/s_1 = \mu_2(s_2 + K_{S(app)}), /s_2.$$

Apparent K_S value can be calculated easily from this equation, since S_1, S_2, μ_1, and μ_2 are known. The calculated values of $K_{S(app)}$ are given in Table 3.

Values of apparent substrate saturation constants for the mixed substrate culture studied were remarkably smaller than those reported in the literature for single substrate experiments: 10 mg l^{-1} (Grady et al. 1989) and 12.2 mg l^{-1} (Alvarez et al. 1991) for benzene; and 0.33-0.43 mg l^{-1} (Button 1985), 0.034-0.044 mg l^{-1} (Robertson & Button 1987), 0.65 mg l^{-1} (MacQuarry et al. 1990), 17.4 mg l^{-1} (Alvarez et al. 1991), and 0.15 mg l^{-1} (Jørgensen et al. 1990) for toluene.

In the case of pure-strain cultures and a single limiting substrate, biodegradation efficiency could be characterised using K_S and μ_{max}. In the case of simultaneous utilisation of several carbon sources, it is not theoretically legitimate to calculate these values as physiological constants. In the case of several carbon sources, the overall growth rate could be calculated for example in accordance with the expression (Egli 1995; Namkung & Rittman 1987):

$$\mu = Y_{XS1}*q_{S1} + Y_{XS2}*q_{S2} + ... + Y_{XSn}*q_{Sn}$$

where Y_{XSn} – yield coefficient of substrate n
q_{Sn} – specific consumption rate of substrate n
If the specific growth rate is fixed in a chemostat culture, the residual concentrations of individual substrate decrease, and the apparent K_S values calculated from these data also decrease due to the additive effect of simultaneously used individual substrates (Egli 1995; Namkung & Rittman 1987). Therefore, the K_S values determined in the literature for single limiting substrate growth conditions and $K_{S(app)}$ determined in this work cannot be compared straightforwardly. However, comparison of the quantitative values is a good starting point for the discussion of possible effects leading to the differences of the kinetics of single- and multi-substrate utilisation. The data obtained by us could be used for further systematic analysis of multi-substrate growth.

Adaptation of aquifer micro-organisms to prolonged exposure (several decades) to the low concentration of jet fuel could be another reason for the noted difference in $K_{S(app)}$ values in comparison with the K_S values given in the literature. Fuel-degrading bacteria might have adapted to the presence of the jet fuel in the environment and developed a highly efficient transport and degradation systems for dissolved fuel components. Kinetic parameters of bacteria are not constant, but are regulated by concentrations of available substrates (Egli 1995). For example, K_S values for *E. coli* growing on glucose are reported ranging from 100 mg l^{-1} (low-affinity utilisation system functioning) to 50 μg l^{-1} (high-affinity utilisation system functioning) (Senn et al. 1994).

The low values of $K_{S(app)}$ suggest that low residual concentrations observed in the field conditions (Metsur et al. 1995) could support maximum biodegradation rate, unless it is limited by mass-transfer of nutrients (including oxygen) or bioavailability of pollutant. The biodegradation rate in these conditions is not determined by pollutant concentration. To put it another way, biodegradation should take place in accordance with the zero-order kinetics in relation to pollutant concentration.

Conclusions

1. Water-soluble fraction of kerosene-type aviation jet fuel contains a considerable part of BTEX compounds (33.7% of the total concentration of non-polar hydrocarbons (4780 μg l^{-1})). Therefore, spills of kerosene-type aviation jet fuel may lead to a considerable groundwater contamination by poisonous BTEX and other aromatic compounds.

2. Biodegradation achieved a high degree of purification of the polluted water in the chemostat cultures. The residual concentration of the kerosene-type jet fuel in the reactor run at D=0.1 h^{-1} was 0.42 μg l^{-1}, whereas the residual concentration in the reactor run at D=0.17 h^{-1} was 2.1 μg l^{-1}. BTEX compounds, the most poisonous components of the fuel, were degraded completely and were not detected in the effluent culture medium. Ethylmethylbenzene and methylpropylbenzene were the most persistent components which were detected in the effluent at both dilution rates studied. Treated water with the residual concentrations determined meet the requirements of the standards for potable water in Estonia (0.00 mg l^{-1} 'very good', 0.02 mg l^{-1} 'good').

3. Apparent K_s values were low, usually substantially less than 1 mg/l. These low values probably are due to multi-substrate interactions, although adaptation also is possible.

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