Biodegradation **14:** 173–182, 2003.

Anaerobic desulphurisation of thiophenes by mixed microbial communities from oilfields

C.L.M. Marcelis[1], A.E. Ivanova[2], A.J.H. Janssen[1,3] & A.J.M. Stams[2,*]
[1]*Paques B.V., T. de Boerstraat 24, P.O. Box 52, 8560 AB, Balk, The Netherlands;* [2]*Laboratory for Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT, Wageningen, The Netherlands;* [3]*Sub-department of Environmental Technology, Wageningen University, Bomenweg 2, 6700 EV, Wageningen, The Netherlands (*author for correspondence: e-mail: Fons.Stams@wur.nl)*

Accepted 3 December 2002

Key words: anaerobic biodesulphurisation, sulphate reduction, thiophenes

Abstract

Anaerobic enrichment cultures obtained from oil fields degraded various thiophenic compounds i.e. thiophene, benzothiophene and dibenzothiophene, with the concomitant formation of sulphide using hydrogen, lactate and ethanol as possible electron donors. It was demonstrated that dibenzothiophene was converted to biphenyl. However, hydrocarbon products from benzothiophene and thiophene desulphurisation could not be detected. After further enrichment on thiophenic compounds as the sole electron acceptor, the conversion activity disappeared while homo-acetogenic bacteria became abundantly present. In order to gain stable conversions of thiophenic compounds, attempts were made to isolate the sulphide-producing bacteria. Two highly enriched cultures were obtained, which degraded thiophenic compounds, but the activity remained low and homo-acetogenesis remained dominant.

Abbreviations: BT – benzothiophene; DBT – dibenzothiophene; MSD – mass selective detection; SRB – sulphate reducing bacteria; T – thiophene

Introduction

Depending on its origin, crude oils may contain high quantities of organic sulphur compounds. When the organically bound sulphur is not sufficiently removed during the refining process, SO_2 will be formed during combustion. To minimize this environmental concern, stringent regulations on the sulphur content of fuels will come in place worldwide. In addition, low-sulphur crude oils are less available nowadays. Consequently, proper processes for the effective elimination of organic sulphur compounds are needed. The current physico-chemical methods to desulphurise hydrocarbon fractions rely on hydrodesulphurisation using metal catalysts in the presence of hydrogen gas under high pressure and temperature. Total sulphur levels below 50 ppm (Anabtawi et al. 1996) are difficult to reach, because of the steric hindrance of alkyl substitutions adjacent to the S-atom on especially the dibenzothiophene molecules (Kabe et al. 1992; Shafi & Hutchings 2000). As thiophene converting enzymes may be very specific, biodesulphurisation of fuels might be an attractive, complementary process to reach sulphur levels below 50 ppm.

Aerobic microbiological conversion of thiophenes has been studied extensively (Kobayashi et al. 2001; Folsom et al. 1999; Grossman et al. 1999; Hirasawa et al. 2001). However, only limited data are available in the literature concerning the sulphur specific anaerobic conversion of thiophenes. Furthermore, clear evidence for significant anaerobic desulphurisation is scarce. It is proposed that thiophene molecules can be used as alternative electron acceptor leading to the formation of the remaining hydrocarbon molecule and H_2S (Kim et al. 1990a). The main advantage of this reaction is the selective removal of the sulphur

atom, thus retaining the caloric value of the hydrocarbon. The sulphate reducing bacterium *Desulfovibrio desulfuricans* M6 was reported to desulphurise various sulphur-containing organic compounds present in crude oils and distillates (Kim et al. 1995). In an assay with methyl viologen as the artificial electron donor using a concentrated cell suspension of *D. desulfuricans* M6, biphenyl was found to be the major reaction product from dibenzothiophene desulphurisation, suggesting specific cleavage of the C–S bond (Kim et al. 1990a, b). This work suggested that sulphate reducing bacteria (SRB) have the potential of reducing organosulphur compounds. However, no conclusions could be drawn about the desulphurisation capacity by these bacteria at growing conditions. Armstrong et al. (1997) tested several pure cultures of SRB and a sulphate reducing community on their ability to convert thiophenes using the method of Kim et al. (1995) and by using growing conditions in the absence of methyl viologen. None of these methods led to significant reductions in the sulphur content of dibenzothiophene or in total sulphur content of vacuum gas oil, deasphalted oil or bitumen.

In this study, the anaerobic conversion of thiophenes was examined under the conditions where growth can be expected. This strategy was chosen because in practical applications anaerobic biomass should grow in a continuously operated biodesulphurisation reactor. In our experiments sulphate reducing enrichment cultures obtained from oilfields were used.

Materials and methods

Source of micro organisms and screening approach

Aqueous samples were collected from three Russian oilfields where sulphide formation occurred. The first inoculum was obtained from the Romashkinskoe oilfield (Tatarstan republic). The second biomass source was sampled at seven spots at the Binagady oilfields (Baky region, Sabunchi). The third inoculum type was from the Talinskoe oilfield (Western Siberia). After activation of the initial samples with 3 mM sulphate, the sulphate reducing cultures were further cultivated on a combination of 3 mM sulphate and various thiophenic compounds in the primary enrichment . In parallel with the primary enrichment four highly enriched sulphate reducing cultures (designated Ap1 up to Ap4) were obtained from the Romashkinskoe oilfield sample using serial dilution. The occurrence of desulphurisation was tested by the capability of the biomass to produce sulphide from organically bound sulphur in addition to sulphide formed from sulphate reduction. Only samples where more than 0.3 mM extra sulphide was formed were judged positive and were transferred to the secondary enrichment. This corresponds to 10% conversion of the thiophenic compounds. In the secondary enrichment the most promising enrichments were cultivated further in the presence of various combinations of thiophene (T), benzothiophene (BT), or dibenzothiophene (DBT) as the sole electron acceptor, while no sulphate was present. Furthermore, an attempt was made to obtain pure cultures from biomass of the secondary enrichments.

Media and cultivation

A bicarbonate-buffered medium was prepared as described by Stams et al. (1993). Bacteria were cultured at 30 °C in 120-ml serum vials closed with Viton stoppers and aluminium crimp seals. The vials contained 50 ml medium and 2.5 ml organic phase. The desulphurisation reactions of complex alkylated derivates from T, BT and DBT present in fuels are simplified by investigating the degradation of the parent molecules as model compounds. Due to the limited aqueous solubility of the thiophenes, these compounds were added to the cultures in an organic overlay of *n*-dodecane. This solvent has physical properties (boiling point 215 °C and a viscosity of 1.27 mPas at 30 °C) that are representative of diesel fuel distillates. Different concentrations T, BT or DBT were applied ranging from 20 up to 160 mM in n-dodecane. The gas phase consisted of 200 kPa of N_2–CO_2 (80:20% v/v) when 10 mM lactate or 10 mM ethanol was applied as carbon- and energy source. In the experiments with H_2 as the main electron donor, a H_2–CO_2 mixture (200 kPa, 80:20% v/v) supplemented with 0.7 mM acetate as carbon source was used.

Additional experiments under bicarbonate-limiting conditions were buffered using 15 mM HEPES (N-[2-hydroxyethyl]piperazine-N′-[ethane-sulfonic acid]). A 100% (v/v) 200 kPa H_2 atmosphere, 1 mM HCO_3^- and 1 mM acetate were applied as the sole carbon and energy sources.

The electron donors (lactate or ethanol or acetate) and acceptors (sulphate or thiosulphate or organic sulphur) were added separately by syringe from sterile anoxic stock solutions. The inoculum size was 5% (v/v). Uninoculated controls and controls inoculated with autoclaved-killed biomass were included to ascertain the biological nature of the desulphurisa-

tion reaction. Strict anaerobic techniques were used throughout all steps of the culture preparation. For isolation experiments using biomass from the secondary enrichments bicarbonate-buffered medium was solidified using 1.5% (w/v) agar (Bacto difco). Combinations of thiosulphate or sulphate with thiophene mixtures or thiophene mixtures solely were applied as potential electron acceptors.

Chemicals

All chemicals were of analytical grade and commercially available.

Analytical methods

The concentrations of organic sulphur compounds were determined using a HP 6890 gas chromatograph (GC) equipped with a flame ionisation detector (FID) and a CP-Sil 5 CB (25 m × 0.25 mm × 0.25 μm) column. The column temperature was programmed from 80 °C (held 2.1 min) with an increasing rate of 25 °C/min up to 260 °C (hold 3 min). The injector and FID temperature were 280 and 300 °C, respectively. The flow of the helium carrier gas was 1.0 ml/min.

Identification of desulphurisation products present in the organic phase was carried out using mass selective detection (MSD). A HP 5890 series II GC was used, equipped with a HP 5971 Series MS detector. The *n*-dodecane sample was diluted with *n*-hexane and one microliter of sample was subjected to analyses. A HP-5MS capillary column (30 m × 0.25 mm) and a helium carrier gas flow of 0.7 ml/min was applied. The column temperature was programmed from 40 °C (held 3.5 min) with an increasing rate of 20 °C/min to a final temperature of 250 °C. The injector temperature was 250 °C and the detector temperature was 280 °C.

Volatile alkanes and alkenes were analysed using a HP 5890 GC equipped with FID and a Chrompack Al_2O_3/KCl PLOT column (50 m × 0.32 mm × 5.2 μm) at a helium carrier flow of 1.6 ml/min. The column temperature was 80 °C (isotherm), the injector and detector temperature were 105 °C and 250 °C, respectively.

Possible water-soluble metabolites were monitored using high performance liquid chromatography (HPLC) equipped with a reversed-phase column as previously described by Van de Pas et al. (2001). The mobile phase consisted of acetonitril – 0.01 M H_3PO_4 (20:80 v/v) at a flow rate of 1 ml/min. Substrates were measured by HPLC as described by Stams et al. (1993) and for detection of sulphide a modified colorimetric method as described by Trüper & Schlegel (1964) was used.

Results

Utilization of organic sulphur compounds in the presence of sulphate

To enrich for micro-organisms capable of utilizing thiophenes, anaerobic cultures with lactate, ethanol or hydrogen as substrates, were cultivated in the presence of sulphate and different (combinations of) thiophenes (primary enrichment). The results of the capability to produce sulphide from thiophenes in the presence of sulphate are presented in Table 1. Several enrichments from each site showed a clear extra sulphide formation, indicating the conversion of thiophenes (Table 1). When the highly enriched sulphate reducing cultures (viz. Ap1 up to Ap4) were used as the inoculum, no additional sulphide formation could be observed. The biomass with positive results was used as inoculum for the secondary enrichment.

Secondary enrichments

The main difference between the primary and the secondary enrichments is the absence of sulphate for the expression of sulphate reducing enzymes in the secondary enrichments. Thiophenes are the sole electron acceptor and sulphide formation must be the result of thiophene conversion. Enrichments A and B2, which showed a clear extra sulphide formation, were selected for further enrichment. Sulphide formation started after a lag phase of 10 up to 15 days (Figure 1). The utilization of organic sulphur compounds is accompanied by growth and proceeds up to 45 days of incubation time. The conversion rate was relatively slow compared to the sulphate reducing control experiments (see Figure 1). Further enrichment of samples B3, B4 and B5 were less successful (data not shown), while enrichments C1 and C2 showed no growth at all.

The conversion efficiencies calculated on basis of thiophene depletion and sulphide formation for the incubations in Figure 1 are presented in Table 2. The sulphide formation was compared to the maximum theoretical values that could be obtained from complete conversion. The efficiency on the basis of thiophene depletion was calculated by comparison with a matching control experiment containing autoclaved biomass. The efficiencies obtained from

Table 1. Sulphide production from thiophenes by primary enrichments and pre-cultures from different oil fields

Inoculum	Code	Substrate	Thiophene* (mM)	Sulphide formation**
Binagady oil field	A	EtOH	T 4 (160)	−
			T 2 (40) + BT 2 (40)	++
			DBT 4 (160)	++
Romashkinskoe oil field	B1	H_2/CO_2	T 4 (160)	−
			T 2 (40) + BT 2 (40)	+
			DBT 4 (160)	+
			DBT 2 (80)	+
Romashkinskoe oil field	B2	Lactate	T 4 (160)	−
			T 2 (40) + BT 2 (40)	++
			DBT 4 (160)	++
			DBT 2 (80)	++
Romashkinskoe oil field	B3	H_2/CO_2	T 4 (160)	−
			T 2 (40) + BT 2 (40)	++
			DBT 4 (160)	+
Romashkinskoe oil field	B4	Lactate	T 4 (160)	−
			T 2 (40) + BT 2 (40)	++
			DBT 2 (80)	+
Romashkinskoe oil field	B5	Lactate	T 4 (160)	−−
			T 2 (40) + BT 2 (40)	+
			DBT 2 (80)	+
Romashkinskoe oil field	B6	H_2/CO_2	T 4 (160)	+
			T 2 (40) + BT 2 (40)	++
			DBT 2 (80)	+
Romashkinskoe oil field	B7	Lactate	T 4 (160)	+
			T 2 (40) + BT 2 (40)	+
			DBT 4 (160)	+
Talinskoe oil field	C1	Lactate	T 4 (160)	+
			T 2 (40) + BT 2 (40)	++
			DBT 4 (160)	+
			DBT 2 (80)	++
Talinskoe oil field	C2	H_2/CO_2	T 4 (160)	+
			T 2 (40) + BT 2 (40)	++
			DBT 4 (160)	+
Binagady oil field	Ap1	Lactate	T 4 (160)	−−
			T 2 (40) + BT 2 (40)	−−
			DBT 4 (160)	−−
			DBT 2 (80)	−−
Binagady oil field	Ap2	EtOH	T 4 (160)	−−
			T 2 (40) + BT 2 (40)	+
			DBT 4 (160)	+
Binagady oil field	Ap3	Lactate	T 4 (160)	−
			T 2 (40) + BT 2 (40)	−
			DBT 4 (160)	+
			DBT 2 (80)	+
Binagady oil field	Ap4	H_2/CO_2	T 4 (160)	−
			T 2 (40) + BT 2 (40)	+
			DBT 4 (160)	+
			DBT 2 (80)	+

*T = thiophene, BT = benzothiophene, DBT = dibenzothiophene. Since bacteria thrive in the aqueous phase, the thiophene concentration is expressed in the medium phase using the applied oil-water phase ratio of 1:20. Consequently, e.g. 20 mM organically bound sulphur will give 1 mM sulphur present in the water phase (abbreviated as 1(20) mM), thus 1 mM of sulphide can be formed upon complete conversion.

**Sulphide formation: (+) complete 3 mM SO_4^{2-} reduction, (++) 0.3 mM extra sulphide in addition of sulphide from SO_4^{2-} reduction, (−) delayed incomplete SO_4^{2-} reduction, (−−) no SO_4^{2-} reduction observed.

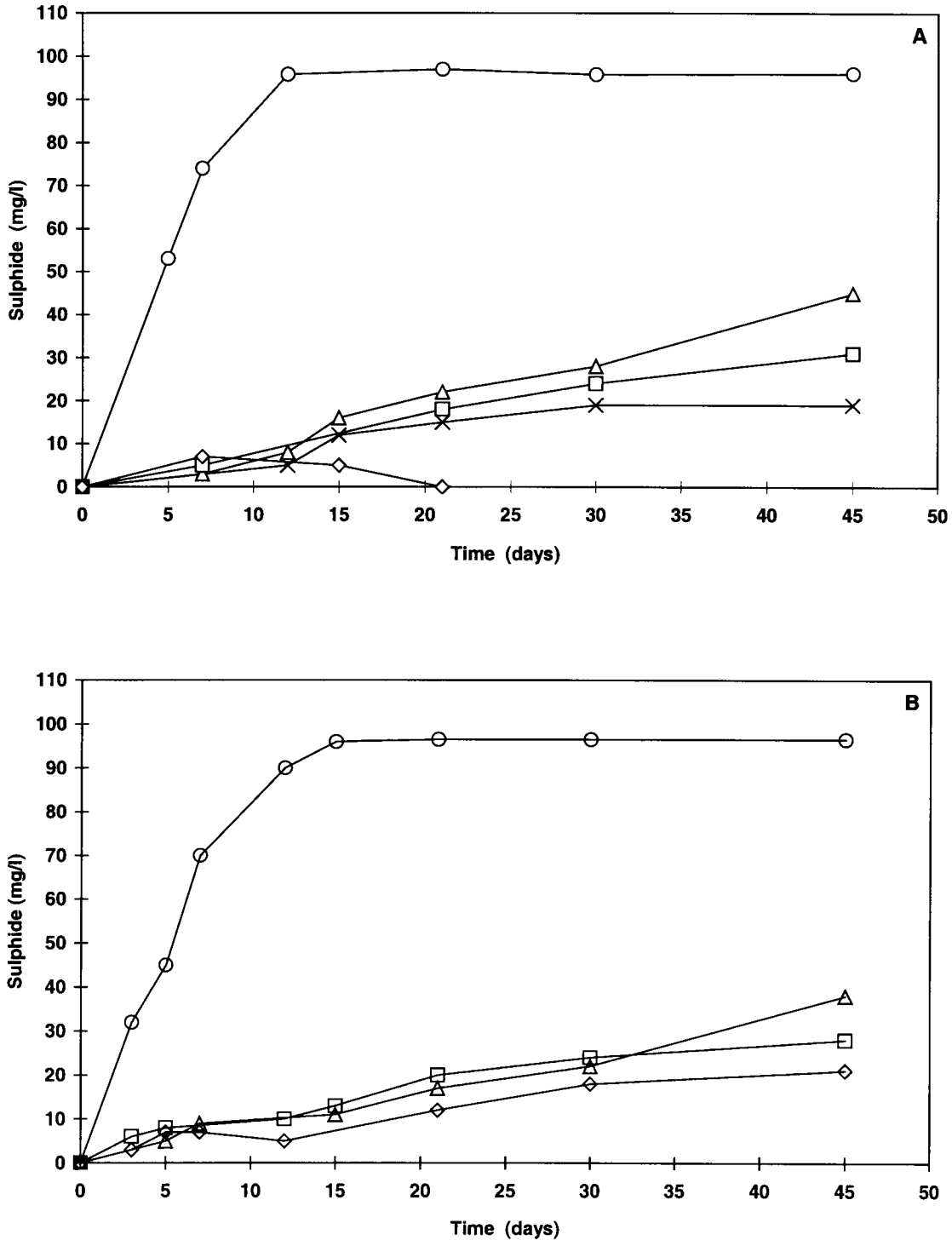

Figure 1. Profile of sulphide formation versus incubation time for Binagady oilfield enrichment (enrichment A) on ethanol and Romashkinskoe oilfield enrichment (enrichment B2) on lactate. Key Figure 1A (enrichment A): ○, 3 mM SO_4^{2-}; △, 2(40) mM BT; □, 2(40) mM DBT; ×, 1(20) mM DBT; ◊, 1(20) mM T. Key Figure 1B (enrichment B2): ○, 3 mM SO_4^{2-}; △, 2(40) mM BT; □, 2(40) mM DBT; ◊, 1(20) mM T.

Table 2. Conversion of thiophenes by enrichments A and B2

Enrichment A	Concentration water (organic) phase	Conversion based on	
		Thiophene conversion	Sulphide formation
DBT	2 (40) mM	12%	13%
DBT	1 (20) mM	37%	48%
BT	2 (40) mM	55%	59%
T	1 (20) mM	–	–
Enrichment B2			
DBT	4 (80) mM	–	–
DBT	2 (40) mM	29%	44%
BT	2 (40) mM	48%	59%
T	1 (20) mM	49%	65%

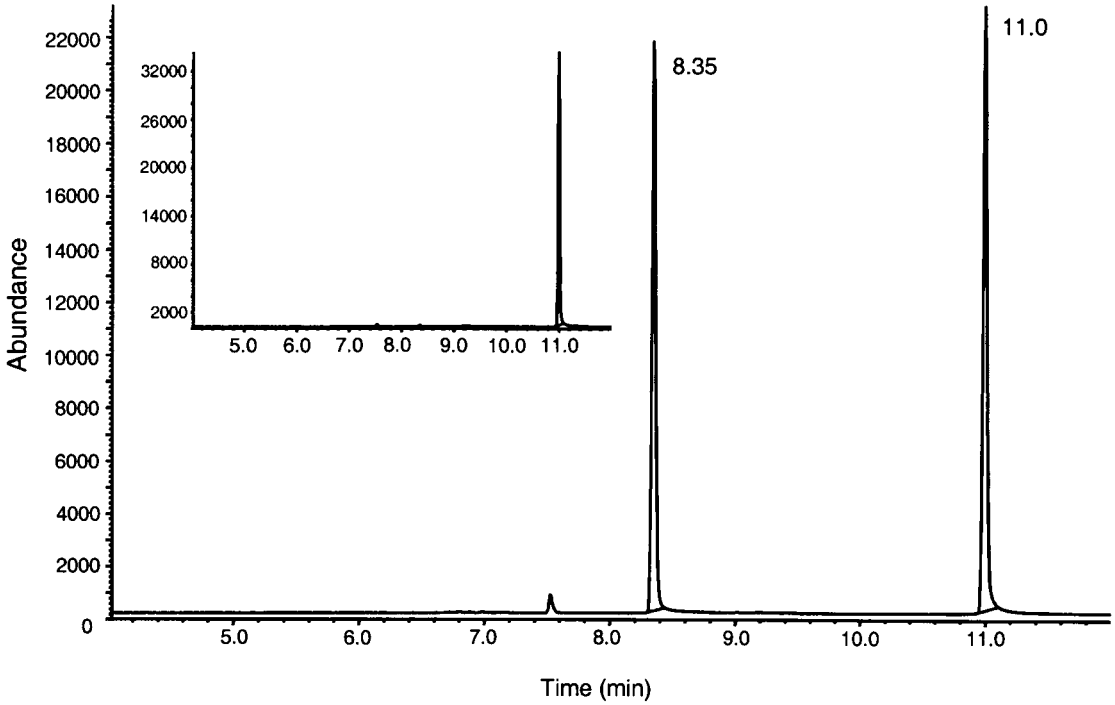

Figure 2. Total ion chromatogram of the organic layer after incubation with 20 mM DBT, see Figure 1A. DBT has a retention time of 11.00 min and BiPh has a retention time of 8.35 min. The inserted total ion chromatogram is obtained from the matching control vial. GC-MSD analysis was as described in Materials and Methods.

thiophene depletion and sulphide formation are in relatively good agreement. On the basis of sulphide formation the desulphurisation efficiency was somewhat overestimated (Table 2).

Conclusive evidence for the sulphur selective anaerobic conversion of DBT should be based on DBT depletion and the demonstration of product formation during the incubations. Besides sulphide the expected hydrocarbon product from DBT conversion is biphenyl. An example of a GC-MSD chromatogram showing biphenyl formation from DBT is presented in Figure 2. The matching mass spectra for biphenyl

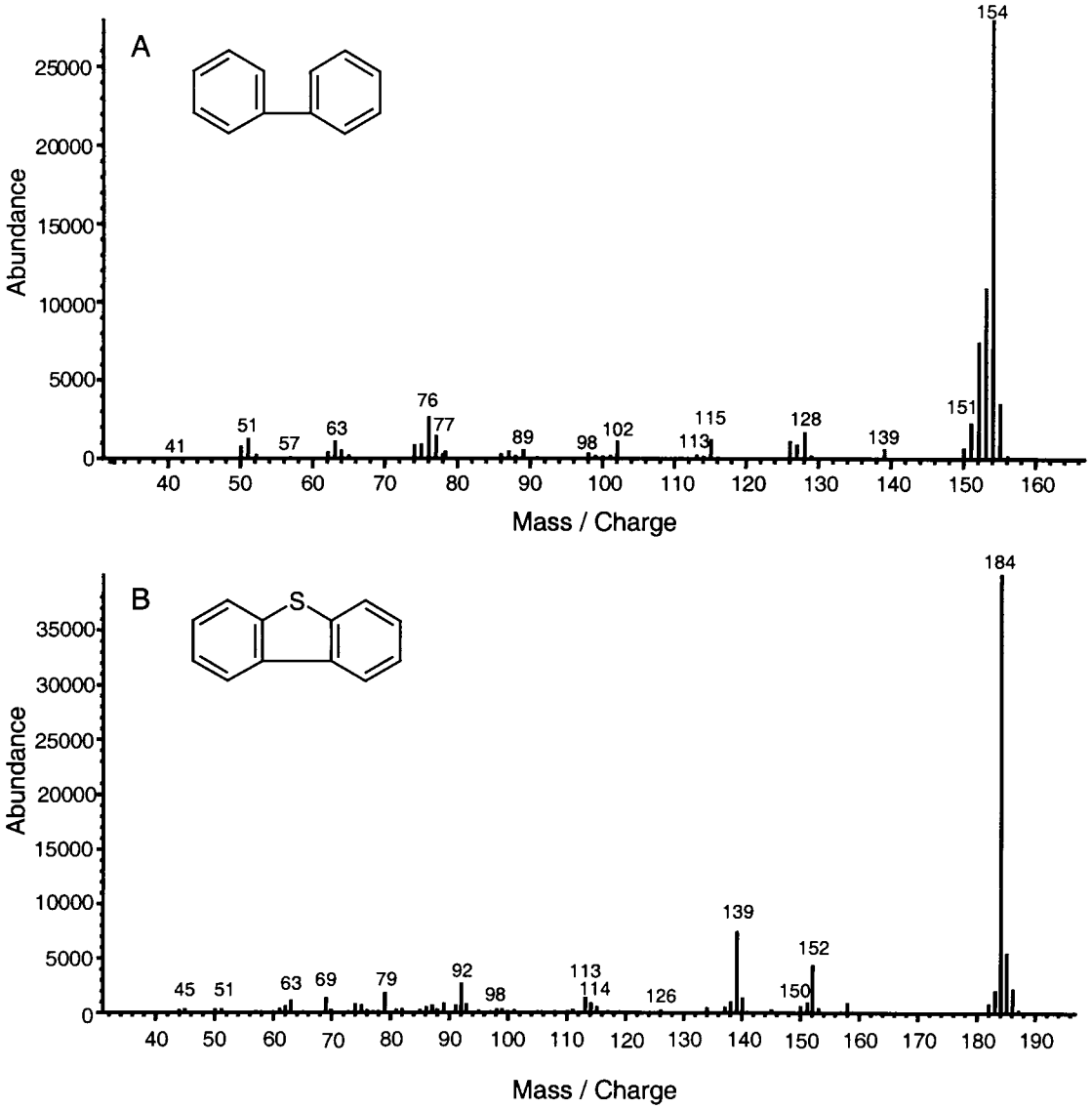

Figure 3. Mass spectra of metabolite biphenyl (A, molecular weight of 154) and dibenzothiophene (B, molecular weight of 184) from Figure 2.

and DBT are depicted in Figure 3. The presence of biphenyl was demonstrated in every incubation where DBT was converted (Table 2).

Apart from the product identification of DBT desulphurisation considerable effort was put in the identification of possible BT desulphurisation products. Mass selective single ion monitoring could not reveal the presence of ethyl benzene or styrene as the most likely desulphurisation products. Furthermore, MSD scans have excluded the presence of C_6 up to C_8 hydrocarbon fragments after desulphurisation. To check if any water-soluble products were formed, HPLC measurements were applied. Also in the water phase no hydroxylated, carboxylated or oxygenated products were detected. For the incubation where T conversion was observed (Figure 1B) an attempt was made to identify the desulphurisation product of T by analysing the gas phase. Assuming that the mechanism of T conversion is similar to that of DBT conversion (Kim et al. 1990a), the most likely product would be a volatile C4 molecule; e.g. butane, butene or butadiene. However, none of these compounds were detected.

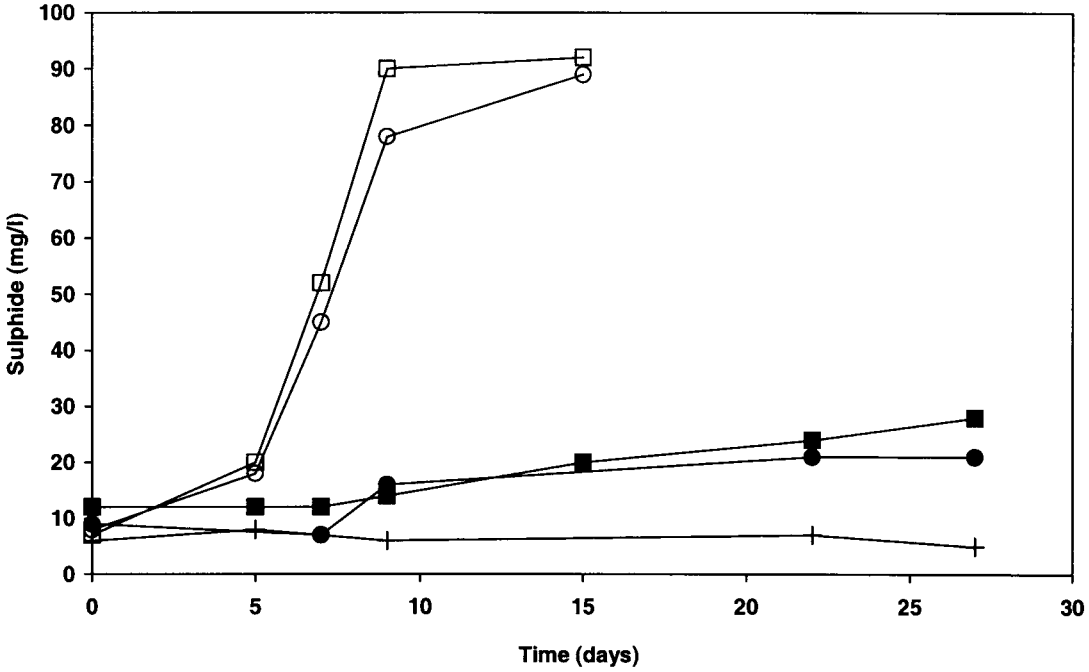

Figure 4. Profile of sulphide formation versus incubation time for the highly enriched cultures OSR1 and OSR2 cultivated in the presence of H_2CO_2. Key: □, OSR1, 3 mM SO_4^{2-}; ○, OSR2, 3 mM SO_4^{2-}; □, OSR1, 2(40) mM T; ●, OSR2, 2(40) mM BT and 2(40) mM DBT; +, Control experiment).

Follow up experiments

It was expected that micro organisms grown in the secondary enrichment would represent the best inocula for new batch experiments, leading to enhanced conversion efficiencies. Unfortunately, this was not the case. The biological desulphurisation activity was lost due to growth of homo-acetogenic bacteria present in the biomass population. These bacteria apparently have better kinetic growth properties compared to desulphurising bacteria. Therefore, an attempt was made to isolate the bacteria responsible for desulphurisation. Various Romashkinskoe enrichment samples were diluted in agar roll tubes and incubated with different combinations of electron acceptors. In these isolation experiments the focus was on the utilization of H_2 as electron donor.

In the presence of sulphate or thiosulphate, mainly colonies of homo-acetogenic biomass were obtained. Only in the case of thiosulphate in combination with thiophene (T) as potential electron acceptors, suitable colonies developed after three months of incubation. Two colonies designated OSR1 and OSR2 were cultivated further. The sulphide formation as a function of time of OSR1 on thiophene [2(40) mM] and OSR2 on benzothiophene + dibenzothiophene [2(40) + 2(40) mM] is depicted in Figure 4. To demonstrate the sulfate reducing capacity the bacteria were also cultivated in the presence of 3 mM sulphate (see Figure 4). Cultures OSR1 and OSR2 were indeed sulphate reducing cultures and able to produce sulphide when thiophenes were present as the sole electron acceptor. However, the rate of sulphide formation was low (0.4 up to 0.8 mg/L.day). Because of the small scale of the experiment appropriate analysis of the organic layer was not practicable. Upon consecutive transfers in fresh medium acetate formation was observed again, indicating that homo-acetogens were still present.

To limit the growth of homo-acetogens, a medium low in bicarbonate (1 mM) was used. Growth of homo-acetogens was prevented effectively, a pH increase due to the consumption of protons was absent and no acetate formation occurred (data not shown). However, this approach did not result in a higher conversion efficiency of thiophenes. Addition of a sulphate pulse (2.5 mM) during growth on thiophenes did not result in a stimulation of the thiophene conversion (data not shown). This indicates that thiophene conversion occurs independent from sulphate reduction.

Discussion

The objective of our research was to obtain a suitable biomass population that is able to use thiophenes as the terminal electron acceptor for growth. From the secondary enrichments several lines of evidence for anaerobic conversion of DBT were obtained. Apart from the depletion of DBT also the products sulphide and biphenyl were demonstrated conclusively. Recently, Bahrami et al. (2001) reported a 98% degradation of DBT using a thermophilic anaerobic consortium. That study demonstrated that there was no correlation of DBT conversion with biphenyl and sulphide formation. Biphenyl or other possible metabolic products were not detected, indicating that a still unknown reaction occurred.

Results of this study revealed that measurable amounts of sulphide were formed from thiophene and benzothiophene. In addition, thiophene and benzothiophene depletion was observed, but no hydrocarbon products could be demonstrated. Anweiler et al. (2001) reported that a sulphate-reducing enrichment culture growing with naphthalene as the sole source of carbon and energy was not able to grow with benzothiophene as the primary substrate. In that study, selective removal of organically bound sulphur could not be demonstrated, but carboxybenzothiophenes were formed cometabolically. In the present study, significant amounts of sulfide were formed from benzothiophene in the absence of sulphate. No polar derivatives (like carboxybenzothiophenes) could be revealed, thus carboxylation of benzothiophene is not an initial activating process. Rueter et al. (1994) reported that alkylbenzenes from crude oil can serve as electron donors by sulphate reducing enrichments. In another previous study (Harms et al. 1999) oxidation of *o*-xylene and *p*-xylene by sulphate reducing bacteria was observed. In our experiments, complete oxidation to CO_2 by bacteria of the secondary enrichments is not likely, since sulphate was not present as electron acceptor. Consequently, it was expected that the metabolites should be excreted.

From the results of the secondary enrichment it is difficult to draw unambiguous conclusions about the concentration effects of the thiophenes. Results obtained by Londry and Suflita (1998) indicated that the inhibitory effects of thiophene and benzothiophene on sulphate reduction at the levels used in this study are not very pronounced. Their study was conducted with oily sludge as inoculum and lactate as carbon and energy source. A concentration effect caused by a change in the solvent due to the action of bacteria is not likely. The anaerobic oxidation of *n*-dodecane has been reported (Kropp et al. 2000; Aeckersberg et al. 1991, 1998), but this reaction cannot occur in the absence of the electron acceptor sulphate.

In the primary enrichment experiments thiophene had a large effect on growth, which may be explained by the water solubility of thiophene. An inhibiting effect on the growth of biomass could explain the low activities. The solubility of benzothiophene and dibenzothiophene in the water phase can be neglected under the applied experimental conditions and a direct influence on the biomass is not likely. Highly enriched sulphate-reducing cultures (Ap1 up to Ap4) obtained from the Romashkinskoe oilfield were not capable of desulphurising thiophenes in the primary enrichment. Probably, the bacteria capable of converting thiophenes were lost during the consecutive transfers.

We did not succeed to obtain pure cultures of the desulphurising bacteria pre-grown in secondary enrichment experiments, but two highly enriched cultures were obtained. The main problem was that homo-acetogens remained present. Omission of bicarbonate buffer from the medium using an alternative HEPES prevented homo-acetogenesis. However, the desulphurisation efficiency compared to secondary enrichment cultures was still low.

From the different experiments the relation between sulphate reduction and the reduction of organic sulphur remains unclear. The expression of sulphate reducing enzymes has no direct link with the conversion of thiophenes and therefore the expression of another enzyme system is necessary.

Sulphide can have large effects on the performance of sulphate reducers converting aromatic hydrocarbons. Edwards et al. (1992) have demonstrated a severe inhibition of sulphate reduction at a concentration of only 1 mM Na_2S, when aromatic compounds (e.g. toluene, xylene) were used as the carbon- and energy source. This phenomenon indicates that even in the presence of a favourable electron acceptor the conversion of aromatic compounds is not an ubiquitous capacity of sulphate reducing bacteria.

In conclusion, this study shows that thiophenes can be anaerobically converted. They are however poor electron acceptors to stimulate growth. The use of enrichments resulted in a proof of principle, but the activity could not be enhanced. Isolation experiments yielded highly enriched cultures. Additional studies are necessary to get a better understanding of the conversion of thiophenic compounds.

Acknowledgements

This study was financially supported by Technology Foundation STW (grant WPR.4064). The authors wish to thank Josina Koning, Hans Jongsma and Roy Geerts for their (technical) assistance on the analytical equipment.

References

Aeckersberg F, Bak F & Widdel F (1991) Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulphate reducing bacterium. Arch. Microbiol. 156: 5–14

Aeckersberg F, Rainey FA & Widdel F (1998) Growth, natural relationships, cellular fatty acids and metabolic adaptation of sulphate reducing bacteria that utilize long-chain alkanes under anoxic conditions. Arch. Microbiol. 170: 361–369

Anabtawi JA, Ali SA & Ali MA (1996) Impact of gasoline and diesel specifications on the refining industry. Energy Source 18: 203–214

Annweiler E, Michaelis W & Meckenstock RU (2001) Anaerobic cometabolic conversion of benzothiophene by a sulphate reducing enrichment culture and in a tar-oil-contaminated aquifer. Appl. Environ. Microbiol. 67: 5077–5083

Armstrong SM, Sankey BM & Verdouw G (1997) Evaluation of sulphate reducing bacteria for desulphurising bitumen or its fractions. Fuel Process. Technol. 76: 223–227

Bahrami A, Shojaosadati SA & Mohebali G (2001) Biodegradation of dibenzothiophenes by thermophilic bacteria. Biotechnol. Lett. 23: 899–901

Edwards EA, Wills LE, Reinhard M, Grbic-Galic D (1992) Anaerobic degradation of toluene and xylene by aquifer micro organisms under sulfate reducing conditions. Appl. Environ. Microbiol. 58: 794–800

Folsom BR, Schieche DR, DiGrazia PM, Werner J & Palmer S (1999) Microbial desulphurisation of alkylated dibenzothiophenes from a hydrodesulphurised middle distillate by *Rhodococcus erythropolis* I19. Appl. Environ. Microbiol. 65: 4967–4972

Grossman MJ, Lee MK, Prince RC, Garrett KK, George GN & Pickering IJ (1999) Microbial desulphurisation of a crude oil middle-distillate fraction: analysis of the extent of sulphur removal and the effect of removal on remaining sulphur. Appl. Environ. Microbiol. 65: 181–188

Harms G, Zengler K, Rabus R, Aeckersberg F, Minz D, Rossell>-Mora R & Widdel F (1999) Anaerobic oxidation of *o*-xylene, *m*-xylene, and homologous alkylbenzenes by new types of sulphate reducing bacteria. Appl. Environ. Microbiol. 65: 999–1004

Hirasawa K, Ishii Y, Kobayashi M, Koizumi K & Maruhashi K (2001) Improvement of desulphurisation activity in Rhodococcus erythropolis KA2-5-1 by genetic engineering. Biosci. Biotechnol. Biochem. 65: 239–246

Kabe T, Ishihara A & Tajima H (1992) Hydrodesulphurisation of sulphur-containing polyaromatic compounds in light oil. Ind. Eng. Chem. Res. 31: 1577–1580

Kim HY, Kim TS & Kim BH (1990a) Degradation of organic sulphur compounds and the reduction of dibenzothiophene to biphenyl and hydrogen sulphide by *Desulfovibrio desulfuricans* M6. Biotechnol. Lett. 12: 761–764

Kim TS, Kim HY & Kim BH (1990b) Petroleum desulphurisation by *Desulfovibrio desulfuricans* M6 using electrochemically supplied reducing equivalent. Biotechnol. Lett. 12: 757–760

Kim BY, Kim HY, Kim TS & Park DH (1995) Selectivity of desulphurisation activity of *Desulfovibrio desulfuricans* M6 on different petroleum products. Fuel Process. Technol. 43: 87–94

Kobayashi M, Horiuchi K, Yoshikawa O, Hirasawa K, Ishii Y, Fujino K, Sugiyama H & Maruhashi K (2001) Kinetic analysis of microbial desulphurisation of model and light gas oil containing multiple alkyl dibenzothiophenes. Biosci. Biotechnol. Biochem. 65: 298–304

Kropp KG, Davidova IA & Suflita JM (2000) Anaerobic oxidation of *n*-dodecane by an addition reaction in a sulphate-reducing bacterial enrichment culture, Appl. Environ. Microbiol. 66: 5393–5398

Londry KL & Sulfita JM (1998) Toxicity effects of organosulphur compounds on anaerobic microbial metabolism. Environ. Toxicol. Chem. 17: 1199–1206

Rueter P, Rabus R, Wilkes H, Aeckersberg F, Rainey FA, Jannasch HW & Widdel F (1994) Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate reducing bacteria. Nature 372: 455–458

Shafi R & Hutchings G J (2000) Hydrodesulphurisation of hindered dibenzothiophenes: an overview. Catal. Today 59: 423–442

Stams AJM, Van Dijk JB, Dijkema C & Plugge CM (1993) Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. Appl. Environ. Microbiol. 59: 1114–1119

Trüper HG & Schlegel HG (1964) Sulphur metabolism in *Thiorhodaceae*. Quantitative measurements on growing cells of *Chromatium okenii.* Antonie van Leeuwenhoek J. Microbiol. Ser. 30: 225–238

Van de Pas BA, Jansen S, Dijkema C, Schraa G, De Vos WM & Stams AJM (2001) Energy yield of respiration on chloroaromatic compounds in *Desulfitobacterium dehalogenans.* Appl. Environ. Microbiol. 67: 3958–3963