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DEGRADATION OF ORGANIC SULFUR COMPOUND IN HEAVY OIL BY A PSEUDOMONAS SPECIES

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ABSTRACT

The microbial degradation of organic sulfur compounds was examined in aerobic conditions employing a pure culture of a Pseudomonas sp., isolated from the soil. The effect of n-alkanes on the degradation of dibenzothiophene (DBT) showed that the assimilation of the sulfur compound by the microorganism is favoured by n-dodecane. Moreover, the saturated fraction was seen to enhance the degradation of sulfur compounds to be found in a deasphaltenated heavy oil.

INTRODUCTION

During recent years, the public and authorities have paid more attention to the ever-increasing problem of water pollution by crude oil and oil mud resulting from refining and heavy maritime transport of crude oil. The oil industry has therefore been showing growing interest towards the reduction of organic sulfur in heavy oil, as this compound has a toxic effect on marine life and also responsible for the heavy emission of sulfurous gas in the atmosphere during combustion causing both water and air pollution.

Various attempts have been made to develop alternative biotechnological processes based on microbiological desulphurization employing aerobic (Verbeek et.al 1993, Fedorak and Peakman,1992) or anaerobic (Mac Kinnon and Boerger, 1991, Tiehm et.al 1995) bacteria.

The sulfur found in crude oil is either inorganic (metal sulfides, sulfates, thiosulfates) or organic (thioles, thiophenes, benzo- and dibenzothiophenes). Inorganic sulfur can be removed at a lower cost and in a shorter time by chemical-physical processes than by microbial ones (Verbeek et.al 1993). The removal of organic sulfur, however, is a problem which remains to be solved. Because dibenzothiophene (DBT) derivatives are the most frequently encountered organic sulfur compounds, DBT is

generally employed as a model molecule in microbiological studies of crude oil desulfurization (Mac Kinnon and Sethi, 1993).

The present study gives the results of a degradation process of DBT and the organic sulfur compounds in heavy oil by means of CP_{80} a pure isolated aerobic culture and taxonomically referred to Pseudomonas sP. The study also examines the effect of the several dispersants, viz. dimethylformamide (DMFA), *n*-dodecane and *n*-hexadecane on the amount of degradated DBT, which was then compared with the degradation of organic sulfur present in heavy oil and in its aromatic fraction.

MATERIALS AND METHODS

Materials: all chemical used were reagent grade and were purchased from Cario Erba and Merk. Heavy oil (residue of an industrial treatment in which the inorganic sulfur was removed) was obtained from Santafee Oil Company (Egypt).

Methods:-

Enrichment, Isolation and Identification of Microorganisms:

Enrichment cultures were obtained from fuel-contaminated oil material of refineries of Santafee Oil Company. The following medium was employed:

(g/L). $MgSO_47H_2O$ 0.2, Na- benzoate 2.5, K_2HPO_4 10.0, $NaNH_4HPO_4$ 3.5, pH 7, deasphaltenated crude oil was added at the concentration of 10. The cultures were incubated at 28°C, with continuous shaking at 200 rpm for 20 days and transferred four times to fresh medium in the same conditions. DBT degradation was then performed by adding to the culture medium 0.1% (w/v) DBT, which, as the case required, was dissolved in the following amounts of dispersant (ml): 0.5mL DMFA, 2.5 *n*-dodecane and 2.5 *n*-hexadecane. In the heavy oil degradation trials 0.6% (w/v) deasphaltenated heavy oil or 0.2% (w/v) of only its relative aromatic fraction were added to the culture medium.

In order to isolate pure strains enrichment cultures showing maximum growth rates on DBT medium were plated on nutrient agar (Difco). The different colonies were retrieved into selective medium and then assayed for their capability for DBT degradation. The bacterial strains obtained were identified by Gram-staining and selected physiological and biochemical tests according to the criteria outlined by Stolp and Godkari (1991).

Deasphaltenated heavy oil and relative aromatic fraction: Deasphaltenation was performed by precipitating the asphaltenes with *n*-hexane in a crude oil: organic solvent ratio of 1:30 (w/v). The aromatic fraction was separated from the deasphaltanated crude oil liquid chromatography on a silica gel 60 (70-230 mesh ASTM) column (60 x 180 mm) eluted with *n*-hexane.

Analysis: The residues of the microbial degradation of DBT, of the heavy oil and its relative aromatic fraction, were recovered from the culture medium liquid-to-liquid extraction using *n*-hexane after acidification of the aqueous phase to pH 2.0. Controls were carried out before each trial without inoculation of the microorganism in the culture medium.

The amount of DBT was determined by spectrophotometric analysis using Uvikon 860 spectrophotometer (Kontron Instr.) on the basis of the two relative maximum values of 312 and 325.5nm, which are characteristic of this compound.

The amount of organic sulfur contained in the deasphaltenated heavy oil and in its relative aromatic fraction was determined by elementary analysis using an element analyzer(Carlo Erba).

Gas-chromatographic analyses' were performed using a Hewlett Packard 5890 G.C. fitted with a flame photometric detector and a methylsilicone capillary column (0.25mm x 25m). Hydrogen was employed as a carrier gas (4mL/min). The temperatures at which trials were carried out were of 300°C for the injector, 250°C for the detector and a programmed temperature of 40°C for 5 minutes, 50°C/min up to 90°C and 40°C/min up to 300°C. One microliter of sample was injected into the capillary column.

RESULTS

Isolation and Characterization of Microorganism

From the enrichment culture growing fastest on DBT medium dilutions were plated onto Nutrient agar, and on the basis of colonial morphology a total of 15 bacterial isolates were obtained. Each isolate was screened for its ability to degrade DBT. Among them strain CP80 showed the highest degradative capability and was used for further studies. This strain on the basis of its morphological, physiological and biochemical characters was assigned *Pseudomonas* sp.

DBT Degradation

The pure culture CP80 is capable of degrading 4 to 19 mg DBT in 50 mL culture medium (Table I). Degradation values were found to be highest in the trials in which *n*-dodecane and *n*-hexadecane were used as dispersants and lowest in the trials in which DBT was dispersed in DMFA. Of the two hydrocarbons tested, *n*-dodecane was the one which allowed the removal of the greatest amount of sulfur compound by the microorganism.

Table 1: *Amount of DBT removed (mg) by the culture CP80 as a function of the type of dispersant*

Dispersant	DBT added (mg)	DBT removed (mg)*
DMFA	48.5	3.6 ± 0.9
n-Dodecane	55.8	18.9 ± 7.8
n-Hexadecane	62.6	5.6 ± 0.5

* All data reported represent the average values calculated over five trails

Degradation of the Sulfur Compounds Present in Heavy Oil and in the Relative Aromatic Fraction

Experiments showed that the drop in organic sulfur in heavy oil (3.82 mg) is greater than that obtained in its aromatic fraction alone (2.56 mg).

From a comparison of Fig. 1a and 1b, it appears clear that the microorganism is moderately capable of removing the organic sulfur compounds of the substituted benzothiophene and dibenzothiophene groups present in deasphaltenated heavy oil. We also observed a small yet significant selectivity of the microorganism in degrading the less substituted benzothiophenes, i.e. di- and tri-benzothiophenes with respect to the more substituted ones and benzothiophenes.

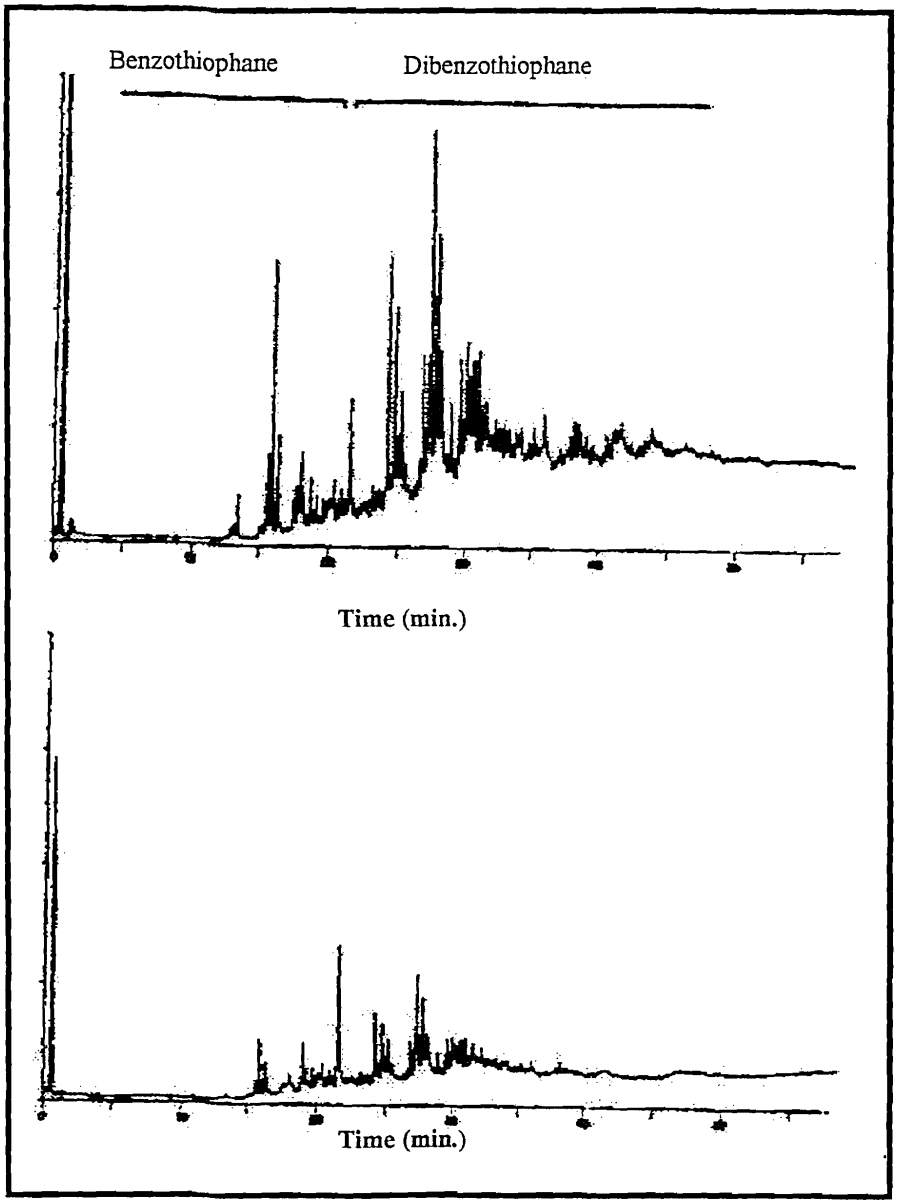

Fig. 1: a,b Gas-chromatographic analysis of the sulfur aromatic compounds in the deasphaltenated heavy oil (a) and in the relative biodegradated residue by strain CP80 (b)

DISCUSSION

The largest quantities of removed DBT dispersed in *n*-dodecane can be probably attributed to co-metabolism between the assimilation of the hydrocarbon and that of the sulfur compound (Table I). The microorganisms of the *Pseudomonas* sp. exhibit a marked capability to degrade *n*-alkanes, as has been amply demonstrated by experiments conducted on the aerobic degradation of crude oils (Nyholm 1990; Fava et.al 1993). It follows that the use of the aliphatic hydrocarbon for dispersing DBT in the soil may enhance the bioavailability of the sulfur compound itself. Two main factors are probably behind this increased bioavailability. Firstly, it is worth noting that, even as an amorphous precipitate, the DBT dispersed with DMFA is nevertheless present in the soil in the solid form, so that the microorganism must attack the compound in its most stable thermodynamic phase when it exhibits its lowest surface area, (a particularly difficult condition even for a hexoenzyme), and/or in its smallest quantity, which was dissolved in the culture medium (1 to 10 ppm). Secondly, it should be noted that DBT in the solid phase does not guarantee its constant saturation in the aqueous phase over time. A second reason that may account for the increased bioavailability of the sulfur compound is the capability of the microorganism to adhere to the organic phase of the hydrocarbon (Tiehm and Fritzsche,1995) thus facilitating transfer of DBT in the direction of the concentration gradient which arises between the organic phase and the wall of the bacterium itself.

Findings also showed a significant increase in the removal of DBT dispersed in *n*-hexadecane (Table I). The different results obtained using the two hydrocarbons, C12 and C16, can be attributed to two concomitant phenomena. The first is related to the chemical and physical differences between the two *n*-alkanes. In fact, compared to *n*-dodecane, *n*-hexadecane exhibits a greater relative viscosity and a lower solubility and diffusivity in the culture medium (Ong, et.al 1991 Sparling and West 1990). Moreover, thanks to its greater surface tension, *n*-hexadecane also exhibits a lower dispersability. The second phenomenon is of a biochemical nature and concerns the differing capabilities of the microorganism to assimilate the two hydrocarbons, a fact which obviously affects cell growth in the culture (Mac Kinnon and Boerger1991). This phenomenon, however, was kept under control by the presence of benzoate, which, as a source of carbon, ensured the same cell concentration in all trials.

The amount of organic sulfur removed from the aromatic fraction is lower than that removed from the deasphaltenated heavy oil. This can be accounted for by the presence of *n*-alkanes in the saturated fraction of heavy oil, which play an important role in removing sulfur compounds.

Fedorak and Peakman (1992) have assumed that several strains of *Pseudomonas* are capable of degrading the fractions which make up crude oil according to the following priority scale: saturated fractions, aromatic fractions, resins, asphalthenes. Boerger et al. (1992) have suggested that the degradation of heterocyclic sulfur compounds may be concomitant with that of the aromatic hydrocarbons rather than with the *n*-alkanes that make up the saturated fraction and that are generally found in great amounts in crude oil.

These results have shown that the amount of sulfur compounds that is degraded depends on the presence of *n*-alkanes. These findings are extremely interesting as they suggest the existence of co-metabolism between the aliphatic and the aromatic hydrocarbons by the microorganism. This hypothesis is also supported by the results of the biodegradation trials of asphaltenes conducted by Tiehm & Fritzche, (1995) and Verbeek et al. (1993). These authors observed that degradation of asphaltenes is favoured by the presence of a co-substrate of saturated hydrocarbons (*n*-paraffin C12-C18), which, in addition to ensuring the growth of the culture, also permits the solubilization and the emulsibility of the substrate.

REFERENCES

Boerger, H , Mackinnon, M., van Meer, T., and Verbeek, A. 1992. Wet landscape option for reclamation of fine tails. In Environmental Issues and Waste Management in Energy and Minerals Production: Second International Conference on Environmental Issues and Management of Waste in Energy and Minerals Production. Calgary, Alta., September 1-4, 1992. Edited by R.K. Singhal. Bakkema, Rotterdam. pp. 1249-1261.

Fava, F.; Di-Gioia, D.; Marchetti, L.; Quattroni, and Marraffa, V. (1993) Aerobic mineralization of chlorobenzoates by natural polyclorinated biphenyl degrading mixed bacterial culture. Appl. Microl. Biotechnol., 40 (4): 541-548.

Fedorak, P.M., and Peakman, T.M. 1992. Aerobic microbial metabolism of some alkylthiophenes found in petroleum. Biodegrad. Nat. Synth. Mater. 2: 223-236.

MacKinnon, M.D., and Boerger, H. 1991. Assessment of a wet landscape option for disposal of fine tails sludge from oil sands processing. In proceedings of the Petroleum Society of CIM and AOSTRA 1991 Technical Conference, Banff, Alta., April 21-24, 1991. Alberta Oil Sands Technology and Research Authority (AOSTRA), Edmonton, Alta. AOSTRA-CIM Pap. No. 91-124.

MacKinnon, M., and Sethi, A. 1993. A comparison of the physical and chemical properties of the tailings ponds at the Syncrude and Suncor oil sands plants. In Oil Sands: Our petroleum Future Conference, Edmonton, Alta., April 4-7, 1993. Alberta Oil Sands Technology and Research Authority, Edmonton, Alta. Pap. No. F-2.

Nyholm, N. 1990. Biodegradability testing of poorly soluble compounds by means of manometric respirometry. Chemosphere, 21: 1477-1488.

Ong, S.K., Hinchee, R., Hoeppel, R., and Scholze, R. 1991. In situ respirometry for determining aerobic degradation rates. In In situ bioreclamation: applications and investigations for hydrocarbon and contaminated site remediation. Edited by R.E. Hinchee and R.E.

Sparling, G.P., and West, A.W. 1990. A comparison of gas chromatography and differential respirometer methods to measure soil respiration and to estimate the soil microbial biomass. Pedobiologia, 34: 103-112.

Stolp, H. and Godkari, D. (1991). In the prokaryotes,Stan, M.P., Stolp, H., Truper, H.G., Ballows, A. and Schiegel, H. G., Eds. Springer - Verlag, Berlin, pp. 719-743.

Tiehm, A. and Fritzsche, C. (1995). Utilization of solubilized and crystalline mixtures of polycycli aromatic hydrocarbons by Microbacterium sp. Appl. Microbial. Biotechnol., 42 (6): 964-968.

Verbeek, A., MacKay, W., and MacKinnon, M. 1993. Isolation and characterization of the acutely toxic compounds in oil sands process water from Syncrude and Suncor. In Oil Sands: Our Petroleum Future Conference, Authority,Edmonton, Alta., April 4-7.