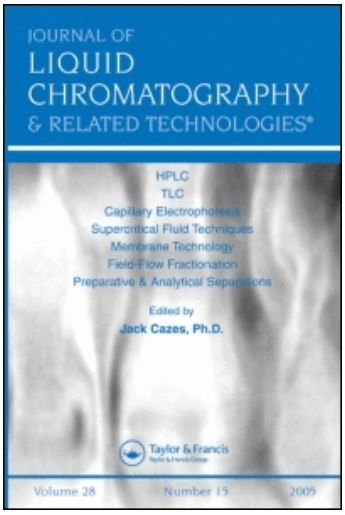

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Applicability of an HPLC System in the Analysis of Biodegraded Crude Oil Components

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APPLICABILITY OF AN HPLC SYSTEM IN THE ANALYSIS OF BIODEGRADED CRUDE OIL COMPONENTS

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ABSTRACT

High performance liquid chromatography has been used for the separation of biodegraded crude blend into fractions using back flushing procedure. The stationary phase was Energy analysis (3.9mm x 30cm) NH_2 column and the detection mode was UV absorptiometry. The method was compared with the liquid chromatography method.

INTRODUCTION

The importance of hydrocarbon degrading microbial communities of oil polluted marine environment is well established (Atlas, 1975; Vanderlinden, 1978; Fedorak and Westlake, 1981; Floodgate 1984). Class separation of biodegraded crude oil was studied through liquid chromatographic technique (Jobson et.al., 1972; Atlas, 1975; Teschner and Wehner, 1985; and Rotani et.al., 1986). Subsequently the fractions were subjected to mass spectrometry, gas chromatography, and HPLC analysis by many researchers (Fedorak and Westlake, 1981; Teschner and Wehner, 1985; Rotani et.al., 1986; Ajisebutu, 1988).

Enough background information of alumina silica column chromatography is available in literature especially in procedures developed by Snyder (1979). Current techniques of Liquid chromatography (LC) Provide the basis for the development of class analyses of the fractions of crude oil to determine the biodegradability and to establish preferential degradation fractions of crude blend.

EXPERIMENTAL

A crude blend from Mathura refinery (a mixture of Arab mix; Iranian mix and Kuwait mix) was used as a substrate. Two species of bacteria , Curtobacterium sp. and Enterobacter sakazaki isolated from the effluents of Mathura refinery were used as type specimens. Preliminary studies established their versatility in degrading oil. The studies were carried out in synthetic sea water (Fedorak and Westlake, 1981) with inputs of nitrogen and phosphorus. Each experimental protocol contained 100 ml sea water 0.6% crude oil blend, 200 bacterial seed alongwith controls in Erlenmeyer flaks in a protracted time domain of 30 days. Inoculated and control flasks were removed at different time intervals i.e. 0, 2, 4, 6, 8, 10, 20 and 30th days for detailed analyses.

EXTRACTION PROCEDURE

The contents of flasks with bacterial inoculum after a defined period were carefully transferred into a sterilized separating funnel. Crude oil was extracted thrice with, 10 ml of petroleum ether Benzene mixture (1:3 v/V). The oil solvent mixture was transferred to a sterilized weighed glass beaker. The solvent was evaporated for 24 hrs at 25^{o}C under a stream of nitrogen (Atlas, 1975).

Separation procedures as defined by Jobson and Westlake, 1972; Atlas, 1975; and Bhosle, 1981; were followed for liquid chromatography.

HIGH PRESSURE LIQUID CHROMATOGRAPHY

The HPLC system utilised was :

Detector : UV absorbance detector (Water Associates Milford, MA) model 481 at 254 nm, 0.5 AUFS.

Pump : Solvent delivery system model 501 with a 10 µl fixed loop injector.

Stationary phase : Porous 3.9mm x 30cm especially packed with an amino propylsilane chemically bonded to 10 µ fully porous silica particles.

Backflush : Six port valve with 1/16" filling for back flushing (Water Associates Milford, MA).

Valve controller : Valve controller module and pneumatic switch which translate a switch closure signal from the model 740 data module into a pneumatic signal to move the six port valve.

Solvents : Lichosorb hexane.

Flow rate = 1.6 ml/min gradually increased to 2 ml/min after 8 minutes of injection.

Air and nitrogen : Air at 35 psi and nitrogen (GC quality).

The asphaltene component from the residual oil (RO) was removed by dissolving RO in hexane and filtering it through a millipore Syringe filter (0.5 µ). The filter was dried and asphaltene determined gravimetrically. The filtrate was resolved through LC & HPLC.

RESULTS AND DISCUSSIONS

Separation of alkanes, aromatics, and polar compounds usually depends on the stationary phase. LC on silica alumina gel with a polar solvent such as n-hexane gives good separation. Separation of components was achieved on HPLC and saturated compounds were eluted in less than 4.2 min., whereas aromatics took 6.2. The polars took 14.35 min. to elution which included 10 min back flushing (Fig. II). The comparative results, signifying separation through LC and HPLC of crude blend are presented in Table I.

Table 1 - Crude Oil Composition

	LC	HPLC
Saturate	40.03%	43.36%
Aromatic	14.20%	12.84%
Polars	25.55%	26.37%
Asphaltenes	16.6%	16.6%
Total Recovery	96.38%	99.17%

Degradation of each of these fractions by microbes is reflected in gradual reduction of various components of crude oil. Fig. II presents comparison of results of LC and HPLC. The compounds that seems to be more resistant to microbial degradation are polar and asphaltene. The bar diagrams reveal that even on the 30th day of incubation there is appreciable decrease of total crude oil though the reduction was of the order of 57.13% by Curtabacterium spp. and 52.84%, by E. sakazaki. The saturate and aromatic profiles Fig. 1 show significant decrease whereas. Polars they show a different behaviour are more resistant to E. sakazaki than curtobacterium. spp. In E. sakazaki there is an increase in the polars which is evidenced by the results both LC and HPLC of the order of 18.65 and 12.65%. This could only be due to an unexplained biotransformation of residual components. This is in agreement with the observations of Rotani et al. 1984.

The resolution of biodegraded crude blend carried in HPLC was possible in 20 minutes, where as LC needs one day. Further the recovery is higher in HPLC and the solvent used is much smaller. These observations each the findings of Suatoni et al. 1975; Bollet and Escalier 1981; and Grizzle and Sablotny, 1986.

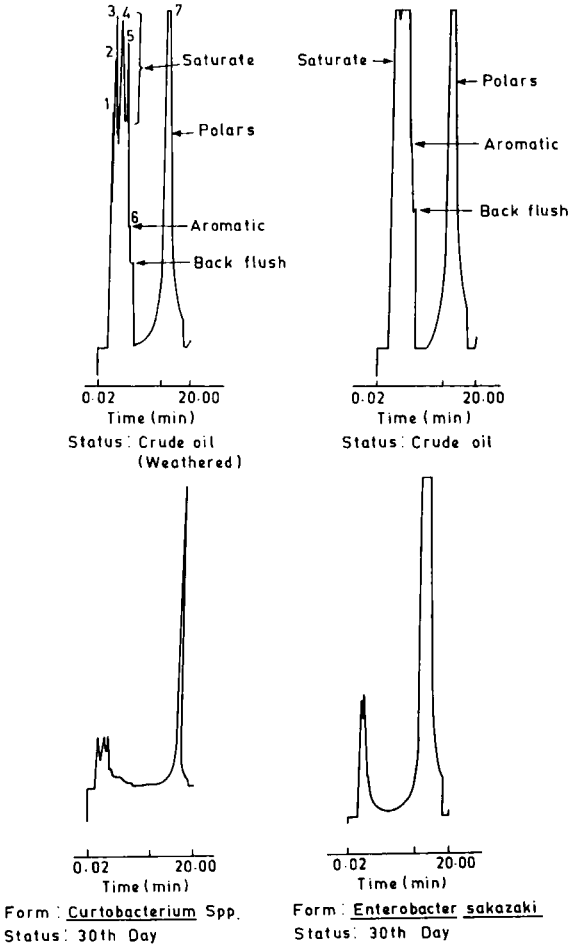

FIG. I HPLC CHROMATOGRAMS

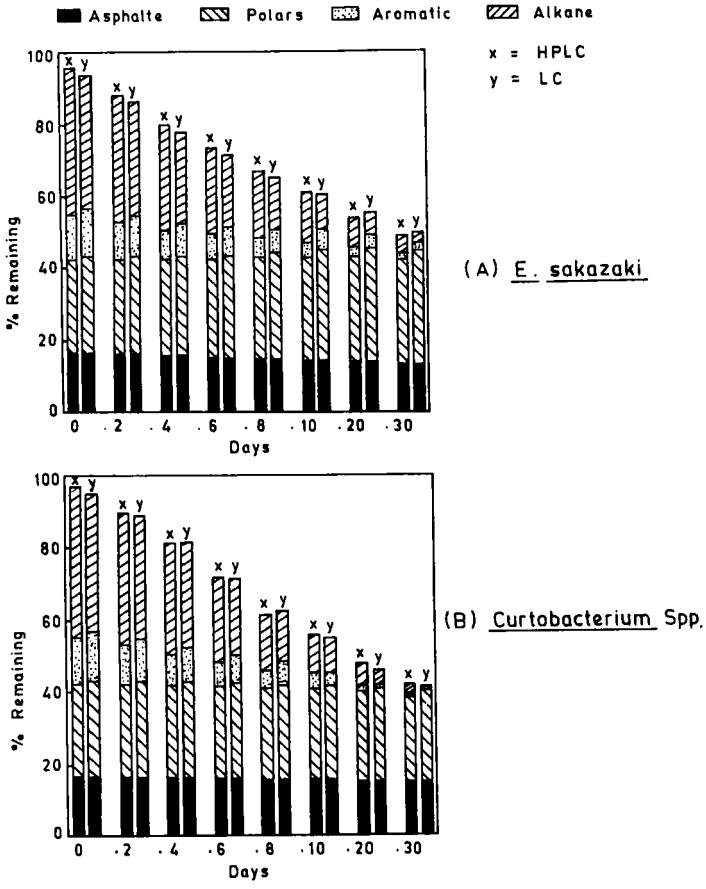

FIG. II HPLC AND LC ANALYSIS OF DEGRADED CRUDE OIL

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