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USE OF ADSORPTION AND GAS CHROMATOGRAPHIC TECHNIQUES IN ESTIMATING BIODEGRADATION OF INDIGENOUS CRUDE OILS

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ABSTRACT

Indigenous crude oils could be degraded and emulsified upto varying degree (visual observation) by locally isolated bacteria. Degradation and emulsification was found to be dependent upon the chemical composition of the crude oils. Tando Alum and Khashkeli crude oils were emulsified in 27 and 33 days of incubation respectively. while Joyamair crude oil did not emulsify even mainly due to high viscosity of this oil. Using adsorption chromatographic technique, oil from control (uninoculated) and biodegraded flasks was fractioned into the deasphaltened oil containing saturate, aromatic, NSO (nitrogen, sulphur, oxygen containing hydrocarbons) and soluble asphaltenes. Saturate fractions from control and degraded oil were further analysed by gas liquid chromatography. From these analyses, it was observed that saturate fraction was preferentially utilized and the crude oils having greater contents of saturate fraction were better emulsified than those low in this fraction. Utilization of various fractions of crude oils was in the order saturate > aromatic > NSO.

Crude oil or petroleum is an extremely complicated mixture of chemical compounds which is composed of hydrocarbons alongwith smaller amounts of organic compounds of nitrogen, sulfur and oxygen. The hydrocarbon content may range from 95-98% and as low as 50% for heavy crude oils. The hydrocarbons present in crude oil belong to three main classes namely paraffins (alkanes and isoalkanes); cycloparaffins (cycloalkanes and nepthenes). and aromatics (mono, di-and polycyclic). Crude oil is generally composed of gasoline, kerosene and lubricant fractions. The high viscosity of oil is due to the lubrication fraction which is characterized by high content of nepthenic and aromatic compounds (Gruse and Stevens)(1). Crude oil which is a complex mixture of chemical compounds may provide a variety of substrates for microbial population. These micro organisms chew up some of the fractions and change the composition of oil, for which there must be some analytical methods to study the residual oil. A number of chromatographic methods have been used in the analysis of petroleum and well reviewed by Camin and Raymond(2). In a previously published study, techniques proposed for evaluating the microbial degradation of petroleum were discussed by Walker and Colwell(3). Attempts have been made to fractionate the oil by applying chromatographic methods to determine the fate of hydrocarbons present in different fractions of oil (4) Fedorack and Westlake(5), Kator et al(6) used the column of activated silica gel G to separate saturates, aromatic and asphaltic fraction. Jewell et al(7) have used the alumina column to fractionate the saturates and aromatics.

The present research work has been undertaken to study the contents of different fractions present in the indigenous crude oils and the extent of utilization by mixed bacterial population. The method described by Jobson et al(8) with slight modifications has been applied for the fractionation of oil and gas liquid chromatography (GLC) for the assessment of microbial utilization of the saturate fraction.

MATERIALS AND METHODS

The crude oil previously used for biodegradation studies by Kokub et al (9) were fractionated by the chromatographic techniques to establish the utilization of different fractions by mixed bacterial culture.

Extraction of the residual oil

The residual crude oil was extracted from the culture broth after 7,18 and 26 days of incubation by washing three times with n- hexane in 1:2 ratio. The organic layer was evaporated to dryness by a rotary evaporator under vacuum at 25-30 °C and then flushed with a stream of nitrogen until constant weight. The residual oil obtained after different incubation periods was compared with the uninoculated oil obtained through the same procedure.

The work was carried out at the Biotechnology Labs.of the Nuclear Institute of Agri. and Biology, Faisalabad

Analytical Methods

At each incubation period, residual crude oil from inoculated as well as from their uninoculated control flasks was fractionated by adsorption chromatographic techniques (8) into different fractions of oil and compared with similar fractions of crude oil i.e. at 0 day incubation.

Liquid chromatographic fractionation of crude oils

Control as well as the residual crude oil extracted with n-hexane was placed in a fume hood at 32-35°C for 24 hours. This treatment removed the volatile materials leaving behind a weight referred to as the "topped weight of the oil". One gram of this oil suspended in n-hexane was applied to the celite (Johns-Manville) column and sequentially eluted with 125 ml of n-hexane and 100 ml of benzene to elute the deasphaltened oil and benzene soluble asphaltenes (Waxy asphalt). The fractions were concentrated by a rotary evaporator and further dried to constant weight by flushing with a stream of nitrogen. The benzene insoluble asphaltenes remaining on the column were calculated by difference. The deasphatened oil was fractionated by adsorption chromatography by using a dual phase column containing, 180.5 g activated silica gel (70-230 mesh) in the lower half and 201.0 g activated aluminium oxide-90 (70-230 mesh) in the upper half. Both phases were packed after suspending in n-hexane. The deasphaltened oil in n-hexane was layered on the top of column and eluted sequentially with 165 ml n-hexane; 250 ml benzene and 250 ml benzene and methanol (1:1) mixture to elute the saturate, aromatic and NSO fractions respectively (Figure 1) These factions were dried to constant weight.

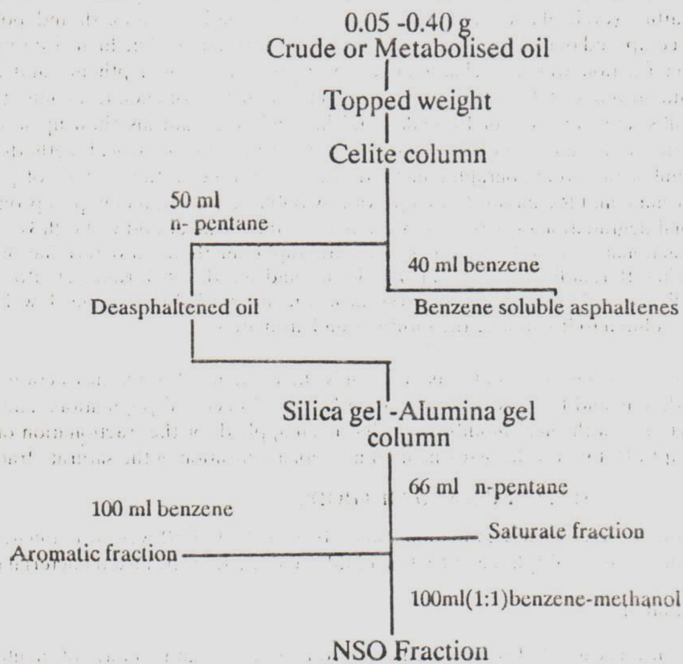

Figure 1: Flow sheet for liquid chromatographic separation of crude oil fractions.

Gas liquid chromatography of saturate fraction

Saturate fraction of control as well as of residual oil at each incubation period was analysed using Hitachi chromatograph (Model-163) equipped with flame ionization detector (FID) and SE-30 (methyl silicone) glass capillary column (25 m x 0.5 mm I.D) precoated with Silanox 101.

Other conditions were as follows: Linear temperature progamme 50-250°C with 5°C/minute increase; Injector and Detector temperature 300°C; Nitrogen flow rate 4 ml/min. Reduction in peak height of the components of this fraction in comparison to the control was taken as measure of bacterial utilization of this fraction.

RESULTS AND DISCUSSION

Fraction of crude oils

Chromatographic fractionation of different oils revealed that the saturate fraction was the major component which ranged from 56-79%. The aromatic fraction ranged from 0.14-0.25% while the NSO fraction was only 0.04-17%. The residual oils were also fractionated by the same technique as to know the utilization of differ ent fractions of oil.

Utilization of different fractions of oil by mixed bacterial culture

Changes in the chemical composition of various crude oils after growth of mixed bacterial culture after 7, 18 and 26 days of incubation are shown in Table I. It has been found that saturate fraction from all crude oils was found to be the preferred carbon source by mixed bacterial culture. Utilization of the components of saturate fraction from different crude oils by mixed culture was followed by gas liquid chromatography. The results are presented in Figure 2. The reduction in peak height of various components of saturate fraction in comparison to similar components of saturate fraction in uninoculated control flasks indicated bacterial utilization of these components. These results also confirm the results obtained gravimetrically (Table I). Decrease in the weight percent of aromatic fraction has also been observed after 18 or 36 days of incubation indicating bacterial utilization of this fraction by mixed culture which has also been confirmed by gravimetric method (Table I). Decrease in weight percentage of saturate and aromatic fractions indicate bacterial utilization (5) and (10), while increase in weight percent of NSO fraction may be due to extracellular accumulation of polar compounds derived from the metabolism of aliphatic hydrocarbons and to lesser extent due to aromatic hydrocarbons (11).

Table I. Changes in various oil fractions as a result of bacterial growth.

	Incu-bation period	Satu-rates	% Change*	Aro-mates g/g oil	% Change* g/g oil	NSO** fraction g/g oil	% Change*
Tando Alum	0	0.79		0.14		0.04	
	7	0.69	-12.7	0.14	0.0	0.05	25.0
	18	0.59	-25.3	0.18	28.6	0.09	125.0
Khashkeli	0	0.69		0.21		0.08	
	7	0.60	-13.0	0.13	-38.1	0.23	187.5
Sakessar formation	0	0.60		0.25		0.12	
	7	0.54	-10.0	0.31	24.0	0.14	16.7
	18	0.51	-15.0	0.33	32.0	0.14	16.7
Balkassar	0	0.59		0.20		0.17	
	7	0.53	-10.2	0.23	15.0	0.19	11.8
	26	0.26	-55.9	0.18	-10.0	0.36	111.8
Fimkassar	0	0.56		0.24		0.17	
	7	0.52	-7.1	0.23	-4.2	0.19	11.8
	26	0.46	-17.9	0.23	-4.2	0.24	41.2

* = Values with (-) sign indicate "Utilization" and without it are for the "Production" over 0 day observation.
**NSO = Nitrogen, sulphur and Oxygen containing hydrocarbons.

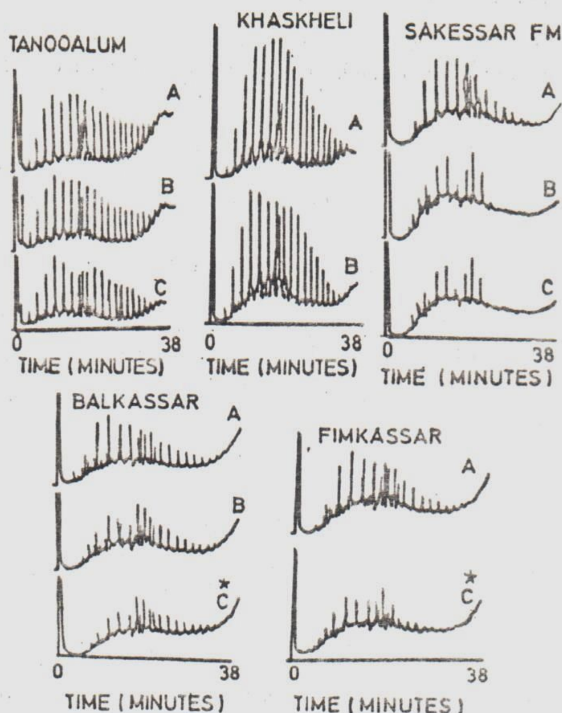

Figure 2: Liquid chromatographic analysis of residual saturate fractions after sequential utilization of different crude oils, by mixed bacterial culture at room temperature: A. control ; B. 7 days; C.18 day; C*. 26 days of incubation.

CONCLUSIONS

The crude oil utilization by bacteria is mainly dependent upon the proportion of the saturate fraction in oil and persistance was found to be mainly due to high viscosity. Degradation by mixed culture was in the order of saturate aromatic NSO fractions.

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