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A Model for diffusion controlled bioavailability of crude oil components

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Abstract

Crude oil is a complex mixture of several different structural classes of compounds including alkanes, aromatics, heterocyclic polar compounds, and asphaltenes. The rate and extent of microbial degradation of crude oil depends on the interaction between the physical and biochemical properties of the biodegradable compounds and their interactions with the non-biodegradable fraction. In this study we have systematically altered the concentration of non-biodegradable material in the crude oil and analyzed its impact on transport of the biodegradable components of crude oil to the microorganisms. We have also developed a mathematical model that explains and accounts for the dependence of biodegradation of crude oil through a putative bioavailability parameter. Experimental results indicate that as the asphaltene concentration in oil increases, the maximum oxygen uptake in respirometers decreases. The mathematically fitted bioavailability parameter of degradable components of oil also decreases as the asphaltene concentration increases.

Introduction

Crude oil is a complex mixture of several different structural classes of compounds such as alkanes, aromatics, heterocyclic polar compounds and asphaltenes. The rate of microbial degradation of crude oil depends on the interaction between the physical and biochemical properties of these compounds. Also, the transport and solubility of these substances dictate their bioavailability and biodegradation. We have systematically studied the impact of transport limitation on the utilization of the biodegradable fractions of crude oil and have quantified and interpreted the results through a mathematical model.

The distribution of the various structural classes and compounds present in petroleum influences the biodegradability of individual hydrocarbon components. Walker et al. (1976) compared the biodegradation of No. 6 fuel oil (Bunker C oil), which has significant amounts of sulfur, nitrogen, nickel, vanadium, aromatics, resins, and asphaltenes, to the biodegradation of No. 2 fuel oil, which has a high aromatic content. Gas-liquid chromatography revealed that 55% of the No. 2 oil was biodegraded compared to only 11% of No. 6 oil. They also compared the biodegradation of South Louisiana crude with Kuwait crude oil. Biodegradation of light South Louisiana crude oil, which is a low sulfur crude oil rich in saturates, was found to be 82%, whereas Kuwait crude oil, which is a high sulfur oil rich in aromatics and resins, was degraded by only 51%. They also reported major differences in biodegradability of identical compounds within the context of different hydrocarbon mixtures. Mulkins-Phillips et al. (1974a) found that 94% of the n-alkanes in Arabian light crude oil were biodegraded compared to only 77% of the n-alkanes present in Venezuelan crude oil. From both these studies it can be inferred that in addition to the different concentrations of the various compounds in an oil, the distribution of the various oil fractions may play a key role in influencing the availability of the biodegradable components.

Westlake et al. (1974) reported that the ability of mixed microbial cultures to utilize hydrocarbons present in four crude oils depends not only on the concentration of the n-saturated fraction but also on the asphaltene and nitrogen, sulfur, and oxygen (NSO) fraction of the oil. For example, even though the composition of saturates in crude from Prudhoe Bay and a crude oil from Lost Horse Hill is comparable, 16-23% of saturates from Lost Horse crude were degraded compared to only 6 to 10% in Prudhoe Bay. They attributed this to the presence of higher polar NSO content in Prudhoe Bay crude.

To further understand the interactions noted above, we studied the effect of the concentration of the hexane insoluble, non-biodegradable fraction (asphaltene) on the biodegradation of the asphaltene-free fraction of crude oil in an oil-water system. The asphaltene free fraction consists of thousands of hydrocarbons, some of which are biodegradable. We used cumulative oxygen uptake to model the biodegradation of oil and measured the residual hydrocarbons to confirm the extent of biodegradation. The mathematical model we developed explains and accounts for the dependence of cumulative oxygen uptake on a bioavailability parameter (which consists of a diffusivity term and a surface area term) of biodegradable oil from the oil droplet. Values of the bioavailability parameter have been obtained by fitting the experimental data to the model.

Material and methods

Preparation of the substrate

A 550 ml volume of Prudhoe Bay crude oil was artificially weathered by unforced evaporation under a fume hood for two weeks. As a result of evaporation of the lighter constituents, the volume of the oil decreased by 100 ml. The final weight was 385.3 g. This weathered oil was diluted with 2000 ml of optima grade hexane (Fisher Scientific, USA) and allowed to stand in a freezer for three hours. It was subsequently centrifuged at 2000 $\times$ g for 15 minutes at 4 °C in a Sorvall RT6000B centrifuge (DuPont Company, Wilmington, Delaware, USA). The asphaltene fraction formed a pellet at the bottom of the centrifuge tubes, and the supernatant was decanted and stored overnight in a freezer. This supernatant was centrifuged again under the same conditions as before to recover any additional precipitate formed. The supernatant was then diluted with hexane (1:5 by volume) and vacuum filtered through 60-200 mesh silica gel. Asphaltenes that escaped centrifugation were adsorbed onto the silica gel. The hexane extract was placed in a rotary evaporator to drive off the hexane leaving behind the deasphaltened oil. The mass of deasphaltened oil was 224.2 g (yield = 58.18%). The pellet of asphaltene from the centrifuge tube was dissolved in optima grade methylene chloride (Fisher Scientific, USA), and the solution was transferred to a beaker. The solvent was evaporated to recover the asphaltene powder. The yield of asphaltenes was 6.5%. About 35% of the weathered crude oil was lost on the silica gel.

Reconstituted crude oil was prepared by adding varying amounts of asphaltenes to 500 mg of deasphaltened oil. The amount of asphaltene added was 0 mg, 62.5 mg, 125 mg and 250 mg corresponding to concentrations of 0, 11.1, 20.0 and 33.3% by weight, respectively.

Respirometry

The effect of asphaltenes on the degradation of deasphaltened oil was evaluated using analytical respirometers (model WB512, N-CON Systems, Larchmont, NY). Each respirometer flask was furnished with an inlet port for dispensing oxygen and a syringe and valve assembly to permit changing the KOH solution in the carbon dioxide traps. Pure oxygen was supplied to the headspace of each flask in response to the pressure drop resulting from microbial oxygen consumption. Calibrated solenoid valves metered oxygen into the flasks to balance pressure against temperature-controlled reference cells. A computer monitored the solenoid valve pulses, recorded the data, and calculated the oxygen uptake on a cumulative basis. The KOH solution (20 ml) was changed when the pH indicator, Alizarin Red S (10 mg/L), changed color from purple to pink. The amount of carbon dioxide trapped was determined by measuring the pH change in the KOH solution.

Culture preparation

An undefined oil-degrading consortium of microorganisms (BS48), isolated from the National Seashore south of Corpus Christi, Texas, was grown and maintained (subcultured every 3 weeks) in Bushnell-Haas medium (2% NaCl) supplemented with Prudhoe Bay crude oil ($\sim$ 5 g/L). On the day of an experiment, the liquid culture was centrifuged, washed, resuspended

in fresh Bushnell-Haas medium without oil, and used as the inoculum for the respirometry experiments.

Most probable number analysis of microbial population

A Most Probable Number (MPN) procedure was used to estimate the population density of oil degrading microorganisms in aqueous solutions (Haines et al., 1995). Duplicate plates were prepared for each respirometer flask. Microtiter 96-well plates were processed with a Beckman Biomek 1000 laboratory workstation (Beckman Instruments, Fullerton, CA). The workstation automatically filled each well with 180 μL Bushnell Hass medium (BH) (Difco Products, Detroit, MI) supplemented with 2% NaCl, performed 10-fold serial dilutions of the sample, and added 2 μL No. 2 fuel oil as the carbon source to the inoculated wells. Plates were incubated for 14 days at 20degC. Positive wells were scored by observing the pink or red color formed after adding 50 μL iodonitrotetrazolium violet (INT) to each well. INT competes with oxygen for electrons in the electron transport chain of aerobic organisms (Packard, 1971), and it is reduced to an insoluble formazan that deposits as a red precipitate in the presence of actively respiring microorganisms. The MPN values were calculated using a personal computer-based program (Klee, 1993) that automatically corrects for the bias associated with the maximum likelihood estimator (Salama et al. 1978).

Chemical analysis

To determine actual biodegradation of hydrocarbons, reactor contents were analyzed for specific saturated hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), and sulfur heterocyclic constituents by extracting in 100 ml of dichloromethane (DCM). A portion of the DCM extract was solvent exchanged to hexane. A 1.0 μL aliquot of the hexane extract was then injected into a Hewlett-Packard 5890 Series II gas chromatograph equipped with an HP 5989A Mass Selective Detector (Mass Spectrometer) operating in the selective ion monitoring mode (SIM) to analyze for the target compounds. Details of chemical analysis can be found elsewhere (Venosa et al. 1996).

Experimental setup

Duplicate respirometer flasks were filled with 250 ml of Bushnell-Haas medium and 500 mg of deasphaltened oil supplemented with varying amounts of the asphaltene fraction (0, 11.1, 20.0 and 33.3%). The flasks were inoculated with 1.0 mL of the oil-degrading culture and then sealed with caps furnished with oxygen and KOH exchange ports. Oxygen uptake was recorded hourly by a computer, and the carbon dioxide produced due to mineralization of oil was measured periodically, as needed. The experiment was concluded after 20 days. At the end of the experiment, a 5.0 mL sample of reactor contents was analyzed for oil-degrader MPN.

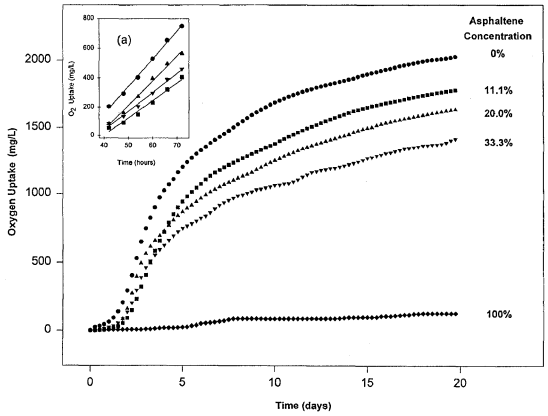

Figure 1. Oxygen uptake curves from biodegradation of crude oil that has been deasphaltened and then reconstituted with various concentrations of asphaltenes. Inset (Figure 1a) shows initial data on an expanded scale along with linear regression fits.

Results

Figure 1 summarizes the average cumulative oxygen uptake with time for deasphaltened oil, crude oil reconstituted with 11.1, 20, and 33.3% asphaltenes, and for the asphaltene fraction alone. The insert (Figure 1a) shows oxygen uptake for short time periods following the lag period on an expanded scale for deasphaltened and reconstituted oils. No substantial oxygen consumption took place in reactors containing the asphaltene fraction alone, indicating that asphaltenes are not degraded by the microorganisms in the 20 day time period of the experiment (previous results from unpublished experiments revealed that no oxygen uptake occurred even after three months of incubation in the presence of the asphaltene fraction). Biodegradation commences with the breakdown of crude oil from the surface of the oil droplet. As the surface concentration decreases, oil from bulk of the oil droplet diffuses to the surface, where it is degraded by microorganisms.

Table 1. Growth of iol degraders in reconstituted oil

Asphaltene content,%	Oil Degrader MPN/mL	Lower 95% Confidence limit MPN/ml	Upper 95% Confidence limit MPN/ml
0	9.5×10^7	2.5×10^7	4.1×10^8
11.1	1.2×10^8	1.9×10^7	7.1×10^8
20.0	5.2×10^7	4.7×10^7	4.6×10^8
33.3	6.2×10^7	2.6×10^7	4.9×10^8

Table 2. Concentration of target analytes after 20 days as a function of asphaltene content

Asphaltene content,%	Total Alkanes[1] ng/mg Oil	Total PAHs[2] ng/mg oil	Total Target Hydrocarbons, ng/mg oil
0	0	235.1	235.1
11.1	0	418.7	418.7
20.0	247.3	2991.1	3238.4
33.3	4111.2	2397.7	6509.9

[1] = 128260.1 ng/mg oil
[2] = 11271.5 ng/mg oil

The oxygen uptake patterns for the other flasks containing reconstituted oil showed a lag period of about 2 to 3 days followed by a rapid increase in oxygen uptake for the next 4 to 5 days and then a slowly decreasing uptake response until termination. As the asphaltene content of the oil increased from 0 to 33.3%, the cumulative oxygen uptake in the reactors decreased.

Even though the amount of deasphaltened oil (and hence the biodegradable fraction) was the same in all the flasks, the presence of asphaltenes at increasing concentrations resulted in an overall decrease in the observed oxygen uptake (Figure 1). This suggests that the asphaltenes were either toxic to the oil degraders or they limited the availability of the degradable components to the oil-degrading populations. The slopes of the curves in Figure 1a represent the initial rates of oxygen uptake, which reflect the degradation of oil on the surface of the droplets. The initial rates computed from Figure 1a are 18.7, 12.1, 16.8 and 13.0 mg/L-hr for deasphaltened oil and reconstituted oil with 11.1%, 20.0% and 33.3% asphaltenes, respectively. Since these rates are comparable, the microbial activity for various experimental conditions is believed to be similar. Table 1 summarizes the MPNs and the upper and lower 95% confidence limits of the microbial populations after 20 days. The wide confidence limits are typical of the imprecise nature of the MPN method. Thus, a large difference in oxygen uptake between the deasphaltened oil and the oil reconstituted with 33.3% asphaltenes (Figure 1) is unlikely to have been detectable by MPN estimates. Although we cannot say with certainty that the asphaltenes were not toxic, the fact that we did not observe a huge decrease in MPNs with higher asphaltene content suggests that the asphaltenes did not significantly affect microbial growth. The lack of any evidence of change in the population of the degraders, coupled with the fact that initial oxygen uptake rates were unaffected by the presence of the asphaltic components, supports the hypothesis that asphaltenes act by inhibiting the diffusion of biodegradable components through the oil droplet.

Table 2 summarizes the concentration of target analytes remaining in the reactor at the end of each experimental run (20 days). The amount of total target hydrocarbons (sum of alkanes and PAHs measured by GC/MS) remaining in the reactors increased from 235 to 6,509 ng/mg oil as the asphaltene concentration increased from 0% to 33.3%. This supports the premise that as the concentration of asphaltenes increases, the bioavailability of substrate decreases, resulting in less degradation, lower oxygen uptake, and higher residual hydrocarbons remaining in the reactors. During the latter stages of the experiment, the high microbial population and presence of undegraded target hydrocarbons indicate that the observed oxygen uptake can be attributed mainly to substrate utilization. It was observed in previous experiments (Haines et al. 1997) that microbial growth during crude oil biodegradation is characterized by a rapid and significant increase in the number of alkane degraders. This growth is followed by a rise in population density of PAH degraders during a concomitant decline in the alkane degraders. The PAH degrading microorganisms remain fairly constant until at least 28 days after startup. Although it could be argued that the slow increase in oxygen uptake during the latter stages of crude oil biodegradation could be due to endogenous respiration of the decaying biomass, our experience supports the assumption that most of the oxygen uptake observed in this period is due to continued growth on the less easily degradable PAHs.

The rate and extent of biodegradation of crude oil depends not only on the microbial population density but also on (i) the diffusivity of various components of crude oil within the oil droplet, and (ii) the size of oil droplets. Addition of asphaltenes introduces

inhomogeneity in the oil droplet, giving rise to surface interactions between asphaltenes and oil components. Also the effective path for the diffusion is now 'cramped and tortuous' and is longer than it would be for homogenous material (Cussler, 1984). The term diffusivity here includes the effects arising from multicomponent and multi-phased systems. These effects tend to decrease bioavailability of the oil to microorganisms.

We present below a mathematical model to evaluate the effect of diffusivity and droplet size with a single parameter. The bioavailability parameter is defined as the ratio of diffusivity of oil to the surface area available for microbial attack (D/a^2). Microorganisms produce surfactants for the uptake of insoluble hydrocarbons. Once the concentration of surfactant reaches its critical micelle concentration (CMC, usually between 10 to 100 mg/L), droplet size will be independent of surfactant concentration (Coulaloglou and Tavlarides, 1976). It has been found that the amount of oil dispersed in the aqueous phase increases with time during the lag phase, resulting from the production of cell bound and extracellular surfactants by the microorganisms (Horowitz *et al.* 1975, Rosenberg et al. 1992). Beyond the lag period, the amount of oil dispersed in the aqueous phase does not change appreciably, indicating a stable emulsion of oil in water (Reisfeld et al. 1972). Further, the likelihood of coalescence of emulsion drops would be low due to stabilizing effects arising from the presence of biosurfactants at the oil-water interface (Hunter, 1987). Hence, any effect of asphaltene on the degradation of oil will be purely due to the change in diffusivity of the biodegradable components through the oil droplet.

Model for evaluation of bioavailability

Development of the model

During the course of an experiment, the mixing energy imparted by the magnetic stirrers causes small droplets of oil to be suspended in the medium. Each oil droplet can be viewed as comprising a porous, non-biodegradable asphaltene matrix filled with biodegradable and non-biodegradable fractions of deasphaltened oil. Since the aqueous solubility of oil is low (Wodzinsky and LaRocca, 1974), microorganisms mineralize the bulk oil at the oil-water interface (Atlas, 1981). The rate of degradation of the biodegradable fraction of oil depends on its reaction rate constants and concentration of oil. Since the concentration of the deasphaltened oil was the same in each of the experimental treatments, the reaction rate constants for the biodegradation of oil should be the same. Changes in the asphaltene concentrations, however, could lead to changes in the diffusivity (and hence bioavailability) of the biodegradable constituents of oil through the droplet. We propose a diffusion controlled model for the biodegradation of oil with the following simplifying assumptions: (i) the droplet size distribution in the respirometer flask is narrow and is uniform during the course of the experiment or between treatments, (ii) the density of degrading populations is ample for biodegradation to proceed unhindered except for the initial lag period, (iii) following the incubation period, the concentration of the substrate at the oil-water interface is very low, (iv) the diffusivity of the degradable fraction of oil through an oil droplet is independent of substrate concentration, spatial location in the droplet, and time, (v) diffusion of the biodegradable components within the oil droplet is only in the radial direction, and (vi) no back diffusion of oil occurs within the droplet.

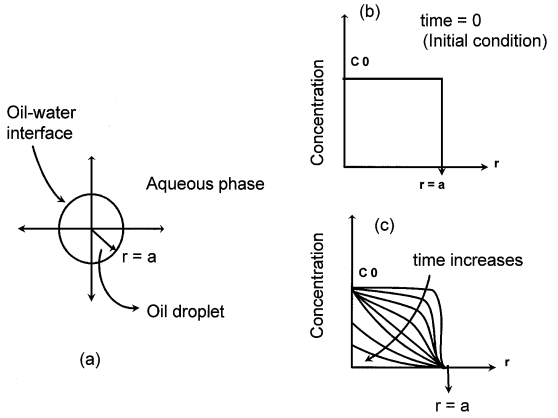

Figure 2. Schematic of an oil droplet (a). Initial concentration profile for oil in the droplet is shown in (b), and the oil concentration profile at different times is shown in (c).

Figure 2a shows a schematic of an oil droplet of radius 'a' in the respirometer flask. The initial concentration C_0 of the biodegradable fraction of the oil within the oil droplet is uniform (Figure 2b). Due to biodegradation of oil at the oil-water interface (r = a), the concentration of the degradable fraction of oil at the oil-water interface decreases to 0. This leads to radial diffusion of oil resulting in time dependent concentration profiles within the oil droplet as shown in Figure 2c. The unsteady state diffusion equation for the biodegradable fraction of oil can be written as:

$$\frac{\delta C}{\delta t} = D\left\{\frac{\delta^2 C}{\delta r^2} + \frac{2}{r}\frac{\delta C}{\delta r}\right\} \qquad (1)$$

Substituting u for rC, the above expression reduces to

$$\frac{\delta u}{\delta t} = D\frac{\delta^2 u}{\delta r^2} \qquad (2)$$

The initial condition required to solve equation (2) can be written as

$u = C_0\, r \quad t = 0 \quad 0 \leq r \leq a$

and the boundary condition as:

$u = 0 \quad r = a \quad t > 0$

$u = 0 \quad r = 0 \quad t \to \infty$

The cumulative mass of substrate diffusing out of the droplet for short time periods and for long time periods can be expressed as a series solution (Crank, 1975). The cumulative oxygen uptake resulting from the breakdown of this substrate at short times can be written as:

$$\frac{M_t}{M_\infty} = 6\left(\frac{Dt}{a^2}\right)\left(n^{-1/2} + 2\sum_{n=1}^{n=\infty} ierfc\frac{na}{\sqrt{Dt}}\right) - 3\frac{Dt}{a^2} \qquad (3\text{ a})$$

where

$$ierfc\, x = \int_x^\infty erfc\zeta d\zeta = \frac{1}{\sqrt{\pi}}e^{-x^2} - x\, erfc\, x$$

and

$$x = \frac{n\, a}{\sqrt{Dt}}$$

For longer time periods, the solution to eq(2) is:

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{n=\infty}\frac{1}{n^2}e^{-n^2\pi^2\frac{Dt}{a^2}} \qquad (3\text{ b})$$

where M_t corresponds to the total oxygen uptake at time t, M_∞ is the oxygen uptake corresponding to complete biodegradation of the deasphaltened oil, n is the number of terms in series expansion, a is the radius of the oil droplet, and t is time.

Estimation of parameters for the model

The model above was used to fit the experimental data obtained for the cumulative oxygen uptake resulting from the degradation of deasphaltened oil supplemented with differing amounts of asphaltenes. This experiment was conducted to help explain the effect of asphaltene content on the diffusivity and hence the bioavailability of the biodegradable constituents of oil. Since the droplet size is unknown, one cannot explicitly estimate diffusivity within the oil droplet; instead, the ratio of diffusivity to the square of droplet radius (D/a^2), the bioavailability parameter, is evaluated. The second parameter in this model is the maximum oxygen uptake M_∞ , which is the oxygen uptake required for the breakdown of all biodegradable constituents of the oil.

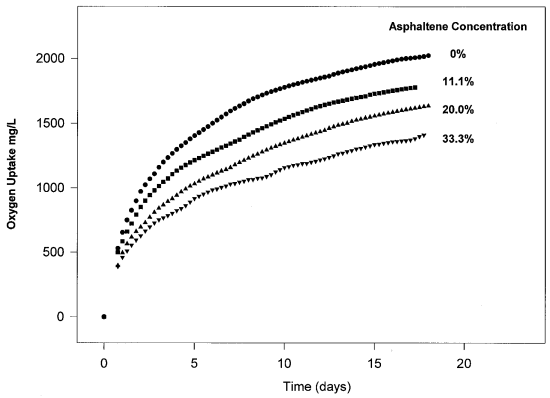

Figure 3. Oxygen uptake curves from biodegradation of deasphaltened oil and reconstituted oil after lag periods were eliminated.

The initial growth of microorganisms during the lag period is associated with increase in cell mass (Westlake, et al. 1974) and the production of the cell-bound (Neufeld, 1984) and extracellular surfactants (Kawashima et al. 1983; Li et al. 1984) used by the cell to facilitate the uptake of insoluble hydrocarbons (Rosenberg et al. 1992). Production of surfactants by the microorganisms is associated with changes in the droplet size of the oil and subsequent emulsification of the substrate (Reisfeld et al. 1972; Margaritis et al. 1979; Schulz et al. 1991) followed by the uptake of the substrate. Formation and coalescence of droplets in a pure system have been studied extensively in the chemical engineering literature (Becher, 1975; Coulaloglou and Tavlarides, 1976; Clift et al. 1978). The growth of microorganisms, production of surfactants, and the variation of droplet sizes during the lag period are extremely difficult to incorporate into a biodegradation model. Since our model cannot predict behavior during the initial lag period of the experiment, the data in this period are ignored, and the time axis is adjusted accordingly. The modified oxygen uptake curves are shown in Figure 3.

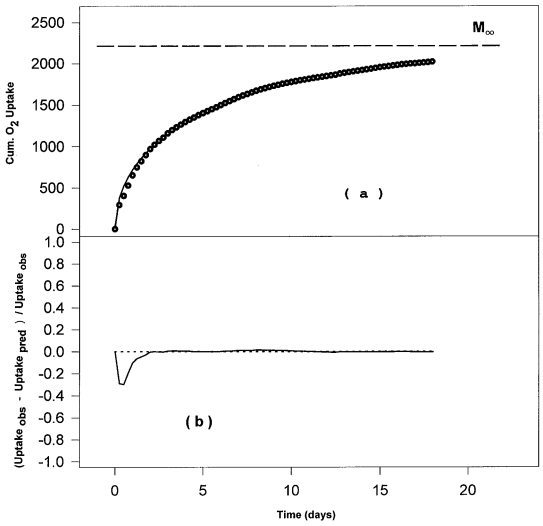

Figure 4. Comparison of the experimental data for oxygen uptake for deasphaltened oil with the fit obtained from the model is shown in the top panel (a). The bottom panel (b) shows the apparent difference between the experimental and model oxygen uptake values expressed as fraction of the observed oxygen uptake. Model parameters are $D/a^2 = 0.0113$ day $^{-1}$ and $M_\infty = 2214$ mg/L.

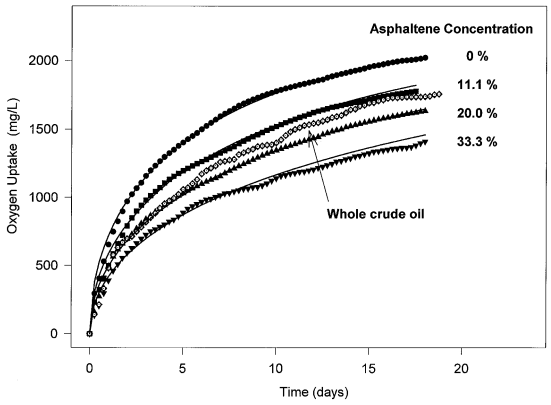

Figure 5. Comparison of the experimental oxygen uptake data with model predictions. The parameter value for M_∞ is 2214 mg/L for all cases, whereas the values of D/a^2 are given in Figure 6.

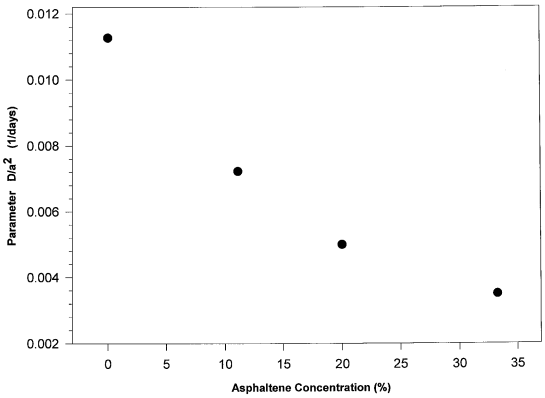

Figure 6. Effect of asphaltene concentration on the bioavailability parameter, D/a^2.

The parameters M_∞ and D/a^2 are obtained by fitting the model solution to the modified oxygen uptake data for *deasphaltened oil*, using Gauss' method in procedure NLIN of SAS (1987). The NLIN (Non-LINear) procedure produces least squares estimates of the parameters of a non-linear model. In addition to the model, the first partial derivatives of the model with respect to the parameters are provided along with the bounds and grid for the parameters. Procedure NLIN first examines the starting value specifications of the parameters and evaluates the residual sum of squares at each combination of values to determine the best set of values to start the algorithm. The iterative method then regresses the residual to the partial derivatives of the model with respect to the parameters until estimates converge. Since the deasphaltened oil contains both biodegradable and non-biodegradable constituents, the parameter D/a^2 represents mass transfer resistance offered by the non-biodegradable fraction to the biodegradable fraction. The M_∞ value obtained here corresponds to the oxygen required for breakdown of the biodegradable constituents of deasphaltened oil, and since the amount of deasphaltened oil was the same for all experimental conditions, the value of M_∞ determined for the deasphaltened oil was adopted for all other experiments. Consequently, the *only* parameter to be determined for the reconstituted oil is D/a^2.

Five terms were included in the series expansion of equation 3a and equation 3b. The applicability of model solutions for short and long time periods was optimized by least square estimates. Results of the fit for deasphaltened oil are shown in Figure 4a. Figure 4b shows the deviation of the predicted values from the observed data ((observed - predicted) / observed x 100). The values determined for the maximum biodegradation potential (M_∞) and diffusivity term are 2214 mg/l and 0.0113 days^{-1}, respectively. Careful examination of the experimentally observed rates of oxygen uptake revealed that even after 20 days, the degradation of oil was continuing albeit at a very slow rate. This is accounted for in the model. The fitted value of M_∞ (2214 mg/L) is higher than that obtained for the deasphaltened oil after 20 days (2020 mg/L).

The model fit to the data for reconstituted oils is shown in Figure 5. For the sake of comparison, we

have included the experimental oxygen uptake data for the whole oil (after eliminating the lag period). Although the asphaltene fraction of the whole oil was approximately 20%, the oxygen uptake data are closer to oil containing 11.1% asphaltenes rather than 20% asphaltenes. Since the recovery of deasphaltened oil was only about 65% during the fractionation process, the reconstituted oil containing 20% asphaltenes had a different composition from the whole oil, which could explain the difference in the oxygen uptake pattern observed.

The values of the D/a^2 parameter for the various reconstituted oils are shown as a function of asphaltene concentration in Figure 6. Clearly, the D/a^2 parameter decreases as the asphaltene content in the oil increases. The asphaltenes can be considered as a matrix that retains oil. Increasing the asphaltene content results in the matrix becoming more dense, leading to slower diffusivity of the degradable compounds through it.

One may argue at this stage that the decrease in the bioavailability of biodegradable constituents in oil as a result of increasing concentrations of asphaltenes is not due to unfavorable interactions between the asphaltenes and oil but is a consequence of the increase in the size of the oil droplet. Assuming that all the asphaltene added to the oil dissolved uniformly in the deasphaltened oil, we calculated the increase in droplet size due to added mass of asphlaltene [$a_2/a_1 = (m_2/m_1)^{1/3}$, where m refers to mass and the subscripts 1 and 2 refer to deasphaltened oil and oil reconstituted with asphaltenes, respectively]. We then estimated the effect of this increase in droplet size on the parameter D/a^2 ($D/a_2{}^2 = D/a_1{}^2 \times (m_1/m_2)^{2/3}$) and plotted it in Figure 7 along with the values obtained from fitting the experimental data to the model. The effect of droplet size on the change in D/a^2 as a function of asphaltene concentration is small compared to the actual observed change. We conclude that the observed decrease in D/a^2 parameter is primarily due to unfavorable interactions between the substrates and the asphaltenes leading to diminished substrate availability rather than any change in the droplet size and hence the surface area of the droplet.

Figure 8 shows the effect of the bioavailability parameter on model predictions of oxygen uptake for a constant M_∞ of 2500 mg/L. As can be seen from the figure, increasing bioavailability of oil in the matrix (increasing D/a^2) results in higher oxygen uptake m_t. It should be noted here that only at sufficiently high D/a^2 values (i.e., 0.03 days $^{-1}$) does complete degradation of substrate take place within about 20 days. m_t for other values of D/a^2 will eventually approach M_∞ but only after very long time periods.

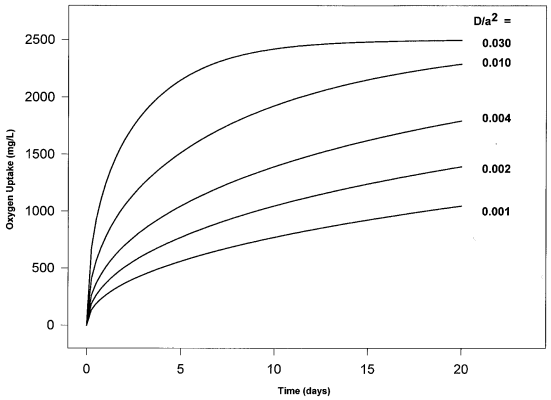

Figure 7. Model predictions of oxygen uptake for different bioavailability parameters, D/a^2, at a constant M_∞ of 2500 mg/L.

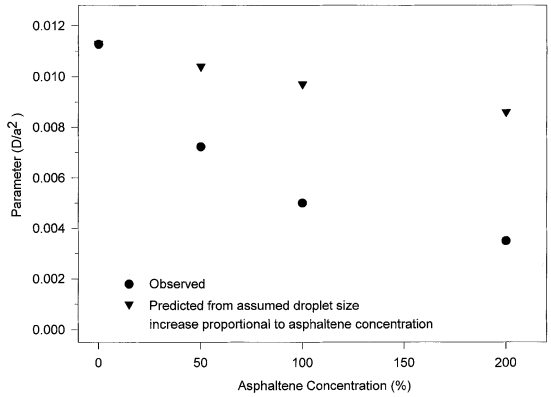

Figure 8. Effect of oil droplet size on the bioavailability parameter at different asphaltene concentrations. The bioavailability calculated from the effect of size is denoted by ▼ whereas the experimentally obtained values are depicted by ●.

Conclusions

Experimental data indicated that addition of asphaltenes to deasphaltened oil reduces the cumulative oxygen uptake by the microorganisms and results in higher residual hydrocarbons remaining in the flasks. It was also found that the asphaltenes were not inhibitory to the microorganisms. Hence, we postulated that the asphaltenes give rise to transport limitations within the oil droplet. We developed a two-parameter mathematical model for diffusion-controlled transport of the biodegradable fraction of the oil to the oil-water interface where it becomes available for the microorganisms

to degrade it. From the model, we found that diffusivity of the oil decreased with increasing asphaltene concentration in the reconstituted oil. We also mathematically showed that this decrease was not due to size effects of the oil droplets but rather due to the inhibitory effect of the asphaltene on transport (Figure 7). Model simulation results indicated that the higher the parameter D/a^2, the higher is the rate of biodegradation of the substrate.

Surfactants can theoretically be used to enhance the biodegradability of crude oil hydrocarbons. Atlas and Bartha (1973) and Robichaux and Myrick (1974) observed in laboratory experiments that addition of surfactants increased the rate and/or extent of oil biodegradation. They postulated that the surfactants acted by decreasing the size of the oil droplets, which increased the surface area available to the hydrocarbon degraders. Mulkins-Phillips and Stewart (1974b), however, observed quite the opposite. For four dispersants tested, they found that emulsified oil was less biodegraded than the oil that was least emulsified. These conflicting results demonstrate that addition of surfactants to emulsify hydrocarbons does not necessarily correspond to increased biodegradation. The data presented herein suggest that the reason why investigators have found surfactant addition in some cases enhances biodegradation while in other cases does not may be due to the interaction of the surfactant with the asphaltic components of crude oil. Based on our modeling results (Figure 7), it is likely that increasing the surface area by decreasing the oil droplet size will not have much effect on biodegradability. Research to increase the bioavailability of the biodegradable components by studying the factors that enhance the diffusivity in crude oil may be fruitful. One factor is temperature. Low temperatures will negatively affect transport within oil droplets and biodegradation kinetics. Another factor is the type of oil; oils with higher levels of asphaltenes should be less biodegradable than those with lower levels, given the same concentration of biodegradable constituents. A third factor is the type of surfactant. Biosurfactants produced by microorganisms actively metabolizing crude oil are more likely to increase the bioavailability of oil than synthetic surfactants that simply increase the surface area by affecting droplet size. In addition to increasing the surface area for microbial attack, surfactants can enhance bioavailability through solubilizing the compounds in aqueous phase, by stabilizing the substrate in micelles (Edwards et al. 1991), or by facilitating the transport of pollutant from the solid phase to the aqueous phase by a number of phenomena such as lowering of surface/interfacial tensions of pore water in soil particles and interactions with solid surfaces and pollutants (Volkering et al. 1995). Studies to understand how such mass transport enhancement takes place as a result of biosurfactant/asphaltene interactions might provide significantly better ways to effect bioremediation of oil spills contaminating shorelines.

List of Symbols

C	= concentration of degradable fraction of oil in the droplet, Kg/m^3
r	= radial distance, m
t	= time, days
t_0	= initial time
C_0	= initial concentration of oil in the droplet, Kg/m^3
a	= radius of the droplet, m
M_t	= Cumulative oxygen uptake at time t, mg/L
M_∞	= Cumulative oxygen uptake for complete degradation of deasphaltened oil, mg/L
m	= mass of substrate, Kg
D	= diffusivity of deasphaltened oil in the oil droplet, m^2/day

References

Atlas RM (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. 45: 180–209

Atlas RM & Bartha R (1973) Effect of some commercial oil herders, dispersants and bacterial inocula on biodegradation of oil in sea waters. In: Ahearns D.G. & Myers S.P. (Eds) Microbial degradation of oil pollutants, Louisiana State University publication-number LSU-SG-73-01, Baton Rouge, Louisiana

Becher P (1965) Emulsion: Theory and Practice, 2^{nd} edition. Reinhold, New York

Clift R, Grace JR, Weber ME (1978) Bubbles, drops and particles, Academic Press, New York

Coulalglou CA & Travlarides LL (1973) Drop size distribution and coalescence frequencies of liquid-liquid dispersions in flow vessels. AIChE J. 22: 289–297

Crank J (1975) Mathematics of Diffusion, 2^{nd}edition. Clarendon Press, Oxford (England)

Cussler EL (1984) Diffusion: Mass Transfer in Fluid Systems, Cambridge University Press, New York (USA)

Edwards DA, Luthy RG & Lui Z (1991) Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. Environ. Sci. Technol. 25: 127–133

Haines JR, Wrenn BA, Holder EL, Strohmeier KL, Harrington RT & Venosa AD (1995) Measurement of hydrocarbon degrading

microbial populations by a 96-well plate most-probable-number procedure. J. Ind. Microbiol. 16: 36–41

Haines JR, Holder EL & Venosa AD (1997) Assessment of mixed microbial cultures for bioremediation product testing. Fourth In situ and on-site bioremediation symposium 4: 419–424

Horowitz A, Gutnick D & Rosenberg E (1975) Sequential growth of bacteria on crude oil. Applied Microbiology 30:10–19

Hunter RJ (1987) Foundations of Colloid Science, Volume 1, Clarendon Press, Oxford

Kawashima H, Nakahara T, Oogaki M & Tabuchi T (1983) Extracellular production of a mannosylerthritol lipid by a mutant of Candida sp. from n-alkanes and triacyl glycerols. Journal of Fermentation Technology 61: 143–149

Klee AJ (1993) A computer program for the determination of most probable number and its confidence limits. J. Microbiol. Methods 18: 36–41

Li ZY, Lang S, Wagner F, Witte L & Wray V (1984) Formation and identification of interfacial active glycolipids from resting microbial cells. App. and Environ. Microbiol. 48: 610–617

Margaritis A, Zajic JE, Gerso DF (1979) Production and surface active properties of microbial surfactants. Biotechnol. and Bioeng. 21: 1151–1162

Mulkins-Phillips GJ & Stewart JE (1974a) Effect of environmental parameters on bacterial degradation of Bunker C oil, crude oil, and hydrocarbons. App. Microbiol. 28: 915–922

Mulkins-Phillips GJ & Stewart JE (1974b) Effect of four dispersants on biodegradation and growth of bacteria on crude oil. App. Microbiol. 28: 547–552

Neufeld RJ & Zajic JE (1984) The surface activity of Acinetobacter calcoaceticus sp. 2CA2". Biotechnol. and Bioeng. 26: 1108–1113

Packard TT (1971) The measurement of respiratory electron-transport activity in marine phytoplankton. J. Mar. Res. 29: 235–244

Procedure NLIN (1987). SAS Release version 6 Edition, SAS Institute, Cary, NC, USA

Reisfeld A, Rosenberg E, Gutnick D (1972) Microbial degradation of crude oil: Factors affecting the dispersion in sea water by mixed pure cultures. App. Microbiol. 24: 363–368

Robichaux TJ & Myrick HN (1972) Chemical enhancement of the biodegradation of crude oil pollutants J. Petrol. Technol. 24: 16–20

Rosenberg E, Legmann R, Kushamaro A, Taube R, Adler E & Ron EZ (1992) Petroleum bioremediation- A multiphase problem. Biodegradation 3: 337–350

Salama IA, Koch GG & Tolley HD (1978) On the estimation of most probable number in a serial dilution technique. Commun. Stat. Theor. Methodol. A7: 1267–1282

Schulz D, Passeri A, Schmidt M, Land S, Wagner F, Wray V & Gunkel G (1991) Marine biosurfactants I: Screening for biosurfactants among crude oil degrading marine microorganisms from the North sea. Z. Naturforsch 46 C: 197–203

Venosa AD, Suidan MT, Wrenn BA, Strohmeier KL, Haines JR, Eberhart BL, King D, & Holder E (1996) Bioremediation of an experimental oil spill on the shoreline of Delaware bay. Environ. Sci. and Technol. 30: 1764–1775

Volkering F, Breure AM, van Andel JG & Rulkens WH Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol. 61: 1699–1705

Walker JD, Petrakis L & Colwell RR (1976) Comparison of biodegradability of crude and fuel oils. Can. J. of Microbiol. 22: 598–602

Westlake DWS, Jobson A, Phillippe R & Cook FD (1974) Biodegradability and crude oil composition. Can. J. of Microbiol. 20: 915–928

Wodzinsky RS & LaRocca D (1977) Bacterial growth kinetics on diphenylmethane and napthalene-heptamethlynonane. App. Environ. Microbiol. 33: 660–665