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13. ABSTRACT (Maximum 100 words)

Slightly soluble, high molecular weight chemicals and Polycyclic Aromatic Hydrocarbons (PAH), are common pollutants of concern in the remediation of oil spill sites. Low volatility, coupled with hydrophobic characteristics, make them more persistent in nature. In-place biological transformation is believed to be the most effective process for their removal. The hydrophobic nature of the contaminants results in a partition onto the soil matrix. In most cases this can account for 95-99% of the total contaminant mass. This limits the biological transformation by reducing the soluble concentration, thereby, making them unavailable on the microbial population. Thus a well-designed bioremediation process should consider a way of mobilizing the contaminants from the soil surface to make them available to the microbial population. Surfactants have been found to be effective in mobilizing hydrophobic contaminants from soil surface [Ellis W.D. et al. (1985)]. Mobilization of contaminants by surfactants depends on the surfactant-soil-contaminant interactions [Vigon and Rubin (1989)]. Edwards et al., (1991) developed a model for the prediction of the mobilization of low solubility organic contaminants by surfactants in soils. Surfactants are known for their capability in enhancing biodegradation of oil spills in open waters, by reducing

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the surface tension and therefore the droplet size, which increases the rate of dissolution [National Research Council, (1989)]. A few field experiments have indicated a potential for enhanced biodegradation of sub-surface PAH contaminants in presence of surfactants [Rittmann and Johnson (1989)]. Aronstein et al. (1991) reported similar results from laboratory experiments using low surfactant concentrations. On the contrary Laha and Luthy (1991) observed a strong inhibition of the biodegradation of phenanthrene in presence of some non-ionic surfactants. The available literature lacks a systemic study towards gaining an understanding of the effects that surfactants have on the bioavailability of low solubility organic pollutants in soils. Our objective is to try to understand the mechanism of biodegradation of a contaminant which has been mobilized using surfactants. This information can be used to design surfactant-enhanced bioremediation processes, selecting dose and type of surfactants, and determining the overall amount of electron acceptors needed.

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**SURFACTANT-ENHANCED INSITU BIODEGRADATION OF STRONGLY SORBING
ORGANIC SUBSTANCES IN SOIL ENVIRONMENTS**

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Introduction

Slightly soluble, high molecular weight chemicals and Polycyclic Aromatic Hydrocarbons (PAH), are common pollutants of concern in the remediation of oil spill sites. Low volatility, coupled with hydrophobic characteristics, make them more persistent in nature. In-place biological transformation is believed to be the most effective process for their removal. The hydrophobic nature of the contaminants results in a partition onto the soil matrix. In most cases this can account for 95-99% of the total contaminant mass. This limits the biological transformation by reducing the soluble concentration, thereby, making them unavailable to the microbial population. Thus a well-designed bioremediation process should consider a way of mobilizing the contaminants from the soil surface to make them available to the microbial population. Surfactants have been found to be effective in mobilizing hydrophobic contaminants from soil surface [Ellis W.D. et al. (1985)]. Mobilization of contaminants by surfactants depends on the surfactant-soil-contaminant interactions [Vigon and Rubin (1989)]. Edwards et al., (1991) developed a model for the prediction of the mobilization of low solubility organic contaminants by surfactants in soils.

Surfactants are known for their capability in enhancing biodegradation of oil spills in open waters, by reducing the surface tension and therefore the droplet size, which increases the rate of dissolution [National Research Council, (1989)]. A few field experiments have indicated a potential for enhanced biodegradation of sub-surface PAH contaminants in presence of surfactants [Rittmann and Johnson (1989)]. Aronstein et al. (1991) reported similar results from laboratory experiments using low surfactant concentrations. On the contrary Laha and Luthy (1991) observed a strong inhibition of the biodegradation of phenanthrene in presence of some non-ionic surfactants.

The available literature lacks a systemic study towards gaining an understanding of the effects that surfactants have on the bioavailability of low solubility organic pollutants in soils. Our objective is to try to understand the mechanism of biodegradation of a contaminant which has been mobilized using surfactants. This information can be used to design surfactant-enhanced bioremediation processes, selecting dose and type of surfactants, and determining the overall amount of electron acceptors needed.

Scope

The goal of our research for the first year was to:

- Screen a selected number of surfactant based on the available information about their chemical structure and solubilizing properties.

- Characterize the interactions of the soil, surfactant, non-ionic pollutant system.
- Evaluate the effects of surfactants on the microbial degradation of non-ionic pollutants, and standardize the procedure for bacterial degradation experiments in the presence of surfactants.

Surfactants Tested to Date

A series of surfactants were selected. Some of them have a well defined chemical formula while others are mixtures of different surfactants. Table 1 lists these surfactants along with their chemical formula, wherever known. Cationic surfactants sorb onto clay by cation exchange and are therefore less useful for the mobilization of contaminants. For this reason, all of our work was conducted with non-ionic and anionic surfactants.

A typical surfactant molecule (monomer) has a two component molecular structure (Figure 1a). One component is hydrophilic while the other is hydrophobic. When we add surfactant monomers to water, the surface tension decreases as the concentration of monomers increases. At a specific concentration, the surface tension stops decreasing any further. This is called the Critical Micelle Concentration (CMC), which is characteristic of the type of surfactant being used. Above CMC, monomers combine to form micelles, where each monomer is oriented with its hydrophilic end projected towards the outside and its hydrophobic end towards the center of the micelle. This forms a hydrophobic core at the center of the micelle where hydrophobic pollutants can partition into (Figure 1b).

Soils Tested

Two types of soils representing a very low organic content aquifer material (Jordan Sandstone) and a medium organic content surface soil were tested (Tables 2a & b).

The Jordan sandstone used in this study was obtained from a subsidiary of J. L. Shiely Company, Minnesota Frac Sand Company, which mines Jordan sandstone near Jordan, Minnesota. This sand is almost pure quartz characterized by a low foc (<0.01%). The sand was washed, oven-dried and sieved. The sand fraction used for all experiments was that passing through sieve #30 and that retained on sieve #100, in order to collect the fraction representing the materials in sand aquifer. The sand was sterilized by autoclaving prior to all experiments. Autoclaving was carried out at a temperature of 121°C and 15 psi pressure.

The surface soil/sediment (Table 2b) has been characterized by Witkowski (1990). Figure 2 shows a sorption isotherm of phenanthrene on this soil, and illustrates that most of the phenanthrene mass will be sorbed by this soil from solution.

Organic Contaminants Tested

Phenanthrene was used as a representative PAH compound. It is a strongly hydrophobic compound with very low aqueous solubility of 1.29 (+/- 0.07) mg/l at 25°C [Stephen and Stephen (1963)]. Although volatile in the aqueous solution, very little volatilization loss (< 0.01%) was reported [Park et al. (1990)] from soil sample after 48 hours of incubation. It is easily biodegraded in presence of appropriate microbial population.

In addition, studies were carried out to characterize aqueous solubility enhancement of octadecane as a model compound. Octadecane is representative of a large class of slightly soluble straight chain hydrocarbons found in soils contaminated by petroleum products and it has a relatively low solubility of 0.007 mg/L in water at 25°C.

Section I - Solubility Enhancement

I-A. Abstract-Solubility Enhancement of Phenanthrene

The aqueous solubility enhancement of phenanthrene by commercial non-ionic surfactants was investigated both below and above their critical micelle concentrations (CMCs). The solubility of phenanthrene was greatly enhanced above the CMC of all the surfactants. (See for example Figure 3.) The data below the CMC did not indicate a significant increase in apparent solubility as illustrated in Figures 4 through 6.

The effectiveness of a surfactant in solubilizing a hydrocarbon is indicated by the molar solubilization ratio (MSR). This number represents the ratio of the number of moles of organic compound solubilized per mole of surfactant added to the solution [Edwards et al. (1991)]. Thus the MSR in the presence of excess hydrocarbon may be obtained from the slope of the curve when solubilize concentration is plotted against surfactant concentration. PAH partitioning between micellar and monomeric surfactant solutions can be represented by a micelle/aqueous phase partition coefficient K_m . This partition coefficient is the ratio of the mole fraction of the compound in the micellar pseudophase to the mole fraction of the same compound in the aqueous pseudophase. Values of MSR and K_m determined for 8 surfactants are listed in Table 3.

The observed order of phenanthrene solubility enhancement above the CMC for the 8 surfactants listed in Table 3 is as follows: Tween40>Triton114> Corexit 8685>Corexit 0600> Brij35>Corexit 7665>Triton 405. The observed differences in $\log K_m$ values is attributed to the nonpolar group content of the surfactants. The nonpolar content in increasing order for the surfactants is as follows: Tween 40> Triton 114> Brij 35> Triton 405. Kile and Chiou (1989) have also attributed the differences in K_m values to the nonpolar content of the surfactants rather than to the size of the micelles. Generally, the inner nonpolar core of the micelle is responsible for solute solubilization.

This study indicates that at concentrations above the CMC, surfactants will have a significant impact on the mobility, transport and fate of a wide range of organic pollutants. Lower concentrations of surfactants will affect very insoluble solutes as pointed out by Kile and Chiou (1989).

I-B. Abstract-Solubility Enhancement of Octadecane

Studies were carried out to characterize aqueous solubility enhancement of octadecane by addition of non-ionic surfactants (above and below their CMC) as part of a broader study aimed at facilitating biodegradation of slightly soluble hydrophobic chemicals. Octadecane was used as a model compound because it is representative of a large class of slightly soluble straight chain hydrocarbons found in soils contaminated by petroleum products and it has a relatively low solubility of 0.007 mg/L in water at 25°C. Four commercially available surfactants, representing some of the major chemical classes of non-ionic surfactants, were tested.

Total solubilities were measured after centrifugation to remove excess solid phase octadecane. In addition, solute size distributions were determined from concentration measurements after sequential filtration through a 0.2 μm filter and molecular filters with nominal molecular cutoffs of 100,000, 30,000, 10,000, and 3,000 (molecular diameters of 62Å, 41.2Å, 28.5Å, and 19.2Å, respectively).

The effect of surfactant concentration on total octadecane solubility is illustrated in Figure 6 for Corexit 0600. Similar results were seen with the other surfactants, namely, progressively higher total octadecane concentrations with increasing surfactant concentrations. At low surfactant concentrations (below CMC), Brij 35 showed the greatest enhancement (up to approximately 1 mg/L octadecane at CMC), whereas Tween 40 only increased solubility to 0/2 mg/L at CMC. At surfactant concentrations above CMC, octadecane solubilities increased to approximately 2 mg/L, except for Tween 40 which showed essentially no enhancement above its CMC. Thus surfactant addition can be used to increase total solubility of octadecane from its saturation concentration of 0.007 mg/L in pure water to 2.+ mg/L.

Filtration resulted in significant removal of octadecane in all cases with progressively more octadecane being removed with smaller sized filters. It was shown that removal was by size exclusion and not by adsorption on the filter. The 0.2 micron filter removed up to 50% of the octadecane from high surfactant concentration solutions, suggesting that the solubility enhancement obtained by using high surfactant concentrations is partly due to dispersion of relatively large aggregates of surfactant stabilized octadecane.

As shown in Table 4, filtration through smaller size opening filters removed most of the surfactant solubilized octadecane as shown by the fact that solubility was reduced to levels that are only marginally larger than the reported water solubility of 0.007 mg/L. These results show

that the molecular solubility of octadecane was not enhanced significantly by addition of surfactants with the possible exception of Tween 40 which showed slightly higher octadecane concentrations in the 3000 MW filtrate (up to 0.037 mg/L). The effect with Tween 40 needs to be checked by other means to eliminate the modifications in the properties of the filter.

The finding that surfactant induced enhancement of solubility is primarily due to formation of quasi-stable dispersions raises important practical questions, namely:

- How does the formation of stable aggregates affect transport of octadecane through soils via groundwater flow?
- How does the presence of surfactant stabilized aggregates affect rates of biodegradation of octadecane? (This question is discussed further in the latter part of this progress report.)
- A related question is whether the dispersion of octadecane into small aggregates will increase rates of solubilization by increasing the exposed surface area of octadecane thus making it more readily available for biodegradation. This aspect is being addressed in ongoing work. As of this writing, the physical-chemical composition of the aggregates has not been established. However, some data regarding the associations between octadecane and surfactant molecules are being generated in ongoing experiments.

Section II - Surfactant Effects on Rates of Biodegradation

Three types of batch reactor tests were carried out to characterize the effects of surfactants on rates of biodegradation of two model chemical pollutants at concentrations above, and below the chemical's solubility.

- A) Screening studies where phenanthrene was used to examine a series of commercially available non-ionic and anionic surfactants in order to identify the most promising ones.
- B) Kinetic studies were carried out to measure rates of biodegradation of phenanthrene and to obtain insight on the mechanisms by which biodegradation is enhanced.
- C) Parallel kinetic studies were carried out with octadecane.

II-A Effect of Surfactants on the Degradation of Phenanthrene at Concentrations Below Solubility in the Presence of a Sorbing Soil

II-A-1. Screening Studies-Experimental Methods

Experimental setup for screening experiments is shown in Figure 7. The C-14 labeled phenanthrene solution, seed, surfactant and soil were placed in the main reaction chamber. Total volume of reactants was kept at 15ml. 250ml of a 1N KOH solution was placed in the KOH cup. Reaction bottles were sealed and placed on magnetic stirrer to mix the reactant continuously.

This mixing was required to ensure that the degradation was not limited by transport. At the end of the incubation time, 200ml of concentrated sulfuric acid were added to the main reaction chamber, to stop the reaction and to release any dissolved carbon dioxide. Two hours later, samples were taken from the KOH cup and from the reaction chamber and analyzed for radioactivity, to quantify the carbon dioxide produced from the phenanthrene degraded and the overall decrease in the phenanthrene concentration (loss, oxidation, and incorporation into biomass).

II-A-2. Screening Studies-Results

The goal of this experiment was to screen surfactants to determine their effect on the bacterial degradation of phenanthrene at concentrations below solubility. This type of experiment can best be understood by comparison with a Bernoulli trial. We are interested in assigning a (+) ve or (-) ve (1 or 0) to each surfactant. This has been achieved as explained in the following paragraph and illustrated in Figures 8a through 8h.

A series of reaction vessels had been set up for different doses of surfactant. Parallel sets were run for both in absence and presence of soil (5%, w/w). Blanks were also set up with no surfactant. Production of carbon dioxide had been measured after 15 days. These type of experiments were conducted for each of the surfactants.

Some of the surfactants do not show a significant effect on the degradation of phenanthrene (i.e. results shown in Figures 8a to 8d) while others reduce the degradation dramatically (i.e. results shown in Figures 8e to 8h). We label the 1st set as (+)ve and the later as (-)ve.

II-A-3. Screening Studies-Conclusions

A screening test procedure has been developed and standardized for testing surfactants on specific chemical pollutants. Screening tests indicate a great potential for a few of these surfactants to be used in practical applications in in-situ bio-reactor/slurry reactor. Further work in this direction using the testing methods developed and the correlated values of molar solubilization ratio (MSR) and partition coefficients (K_m) as described in Table 3, will lead to specific design criteria for the selection of surfactants in relation to the physical chemical properties of the polluting chemicals and the properties of the soil.

II-B Effect of Surfactants on the Degradation of Phenanthrene at Concentrations in Excess of the Solubility

A modified Hach BOD apparatus (Figure 9) was used to measure kinetics of biodegradation in the presence and absence of selected surfactants in the presence of an excess of

phenanthrene. This apparatus allows measuring concurrent production of cell mass and oxygen utilization as a function of time. Phenanthrene biodegradation was tested under conditions in which it was added as a suspended separate phase and as a coating on soil surfaces.

II-B-1. Effects of Surfactants on Phenanthrene Biodegradation-Experimental Methods

Acclimated enrichment cultures capable of degrading phenanthrene were developed from soil and sewage. Samples of petroleum contaminated soil were collected from the Bemidji (Minnesota) Oil Spill Site and the return sludge from the activated sludge treatment plant at the Metropolitan Waste Control Commission Wastewater Treatment Plant in St. Paul, Minnesota.

Biodegradation of phenanthrene was calculated from the oxygen uptake measurements throughout the course of each experiment. A major advantage of oxygen uptake as a measure of biodegradation is that it is easily measured on a continuous basis. Glass bottles were constructed in such a way that soil contained in stainless steel baskets could be suspended at the center of the bottles without touching the stirbar at the bottom. This prevented grinding of the sand due to the continuous movement of the magnetic stirbar. These bottles were then placed on the Model 2173 A, HACH Manometric System (Hach Chemical Co., Loveland, Colorado). Carbon dioxide was scrubbed out by lithium hydroxide suspended in a rubber cup. The bottles were stirred constantly by a magnetic stirrer.

Cell mass present in the solution phase and on the soil was calculated from protein concentration measurements by the method of Lowry et al. (1951) on a spectrophotometer (Bausch and Lomb, Spectronic 1001). Bovine serum albumin (Sigma Chemicals, St. Louis, MO.) was used as the protein standard.

Analysis for phenanthrene metabolic intermediates was carried out because there was visual evidence of color at certain stages of the batch tests. Biochemical studies indicate that many of the byproducts are hydroxylated aromatic compounds (10-12). Thus culture supernatants were analyzed for the presence of phenolic compounds by a modification of the Folin-Ciocalteu reaction by Box et al. (1983). Absorbance at 750^oA was measured in a spectrophotometer (Bausch and Lomb, Spectronic 1001); resorcinol was used as a standard and all results are reported in milligrams of resorcinol equivalents per liter.

Effects of surfactant addition were tested by adding particulate phenanthrene and by coating phenanthrene on soil surfaces. (Coating procedures are described elsewhere). Oxygen uptake data with particulate phenanthrene (no soil) and soil coated phenanthrene are illustrated in Figures 10a, 10b, 10c, and 10d. The corresponding increase in cell mass concentration (measured as protein) for selected experiments are shown in Figures 11a and 11b. The time dependent concentrations of metabolic intermediates is shown in Figures 12a and 12b as resorcinol equivalents.

II-B-2. Effects of Surfactants on Phenanthrene Biodegradation-Results and Discussion

All six surfactants enhanced the mineralization rates both in the presence and absence of soil. This is due to the increased rate of dissolution of phenanthrene due to surfactant influence on wetting and surface tension. Earlier experiments had indicated that the presence of soil changes the aqueous surfactant concentration due to adsorption of the surfactant to the soil. Batch sorption experiments were conducted to determine the partition coefficients of all six surfactants to the sand. These results and experimental details have been reported in another paper in details [Jahan, Ph.D. thesis]. The isotherms for all the surfactants could be represented by the Freundlich isotherm model. Surfactants were thus added such that the aqueous phase concentration was at 25 mg/L after sorption in the soil-water system.

None of the systems showed any inhibitory effects due to the presence of the surfactants. Inhibition of phenanthrene mineralization by Triton X-100, Tergitol NP-10 and Brij 30 at doses in excess of 1.0% has been reported by Laha and Luthy (1991). Other reports [Kile and Chiou (1989)] have indicated that dilute surfactant solutions can increase the rate of mineralization of slightly soluble, hydrophobic compounds in aqueous systems by rendering them more bioavailable. None of the surfactants indicated any mineralization during the time course of the experiments.

A more complete discussion of the results is in preparation [Jahan, Ph.D. Thesis] and will be published.

II-B-3. Effects of Surfactants on Phenanthrene Biodegradation-Conclusions

The batch reactor test data clearly show that addition of 25 mg/L of surfactant in presence and absence of soil increased biodegradation rates. Phenanthrene solubility in water alone at room temperature was found to be 0.825 mg/L. Solubility enhancement studies with the same surfactants at 25 mg/L [Jahan, Ph.D. Thesis] indicated slight increases in the apparent solubility of phenanthrene. The presence of surfactants tends to stabilize suspensions of particulates thereby exposing more surfaces of solid phase phenanthrene for solubilization as reported by other investigators. Larger surface area facilitates mass transfer at the interface. Thus the microorganisms had more soluble substrate available as compared to the control. This also helps explain the higher concentration of intermediate metabolite production in the presence of surfactants. It is believed that intermediates are formed when available soluble substrate is higher, resulting in higher transport rates of substrate into cells which in turn results in production of more intermediates. Linear growth is observed in all cases indicating growth is limited by availability of transportable soluble substrate.

II-C. Effect of Surfactants on the Degradation of Octadecane at Concentrations in Excess of the Solubility

Biodegradation of octadecane was studied in the presence of four non-ionic surfactants: Brij 35, Corexit 0600, Triton X-114, and Tween 40. Octadecane was chosen as the model compound because of its low solubility, 0.007 mg/L in water at 25°C.

The experiments were carried out in batch reactors (Hach BOD apparatus). Oxygen utilization and protein production were measured as indication of growth. Octadecane was added in the particulate form. The experiments included test bottles without surfactant, and with each surfactant at two concentrations, one below the respective surfactant's critical micelle concentration, and one above.

II-C-1. Effects of Surfactants on Octadecane Biodegradation-Materials and Methods.

Octadecane (purity = 99%) was obtained from Aldrich Chemical Company, Milwaukee, WI. Carbon-14 octadecane, labeled in the C-1 position (specific activity: 3.6 mCi per mmol; purity > 98%) was obtained from Sigma Chemical Company, St. Louis, MO.

Microbial oxidation was measured in Hach manometric BOD test apparatus, Model 2173A (Hach Chemical Company, Loveland, Colorado) which measures the utilization of oxygen manometrically. Carbon dioxide is scrubbed out by lithium hydroxide in a suspended cup. The bottles are stirred continuously by a magnetic stirrer.

270 milliliters of buffered growth medium (0.5 g/L NaNO₃, 0.65 g/L K₂HPO₄, 0.17 g/L KH₂PO₄, 0.1 g/L MgSO₄·7H₂O, 0.03 g/L CaCl₂, 0.00375 g/L FeSO₄·7H₂O in Megapure water) plus surfactant was added to each BOD bottle. Each surfactant was tested at two concentrations: 25 mg/L and 200 mg/L.

The initial octadecane concentration of 200 mg/L was added in the particulate form. The bottles were shaken at 125 rpm for 48 hours to equilibrate before adding 30 mL of octadecane-acclimated inoculum.

The inoculating culture was originally obtained by enrichment from soil from an oil spill site in Bemidji, Minnesota. The inoculum had been acclimated to octadecane through repeated transfers into fresh nutrient / buffered medium with octadecane as sole carbon source. The last transfer was from the endogenous phase of a previous experiment.

Tests included duplicate bottles of each surfactant at each of the two tested concentrations, as well as control bottles of: (1) inoculum with substrate (i.e. octadecane) and no surfactant, (2) inoculum with surfactant and no octadecane, and (3) inoculum with no surfactant and no octadecane.

The bottles were maintained at room temperature (22±1°C). As bacteria utilize substrate, oxygen is absorbed from the headspace, resulting in a decrease in pressure which is recorded as a

rise in mercury in the manometer tube. The bottles were periodically opened and the oxygen supply replenished by purging with pure oxygen.

Cell mass production was determined by measuring protein production throughout the experiment. Protein content was determined by the Lowry method. Detailed procedures are described in Le Thai's Thesis.

II-C-2. Effects of Surfactants on Octadecane Biodegradation-Results and Discussion

Test results with solutions of Corexit and Brij are shown in Figures 13 (a & b) and 14 (a & b) as plots of oxygen uptake versus time. All of the oxygen uptake vs. time curves are plotted for the whole course of the experiment and also for the first 150 hours of the experiment where uptake rates are highest. Protein versus time plots are also shown (Figures 13c & 14c). All data are averages of duplicate samples, except for the plots of the Corexit experiment, where the duplicate data points are plotted and the average curves are shown.

Data from the control set of bottles with inoculum with no octadecane and no surfactant is not shown, as the oxygen uptake and protein production in these bottles did not increase during the course of the experiments. However, there was biodegradation of surfactants in the control bottles with 25 mg/L and 200 mg/L surfactant for two of the surfactants, namely, Corexit 0500 and Tween 40. Oxygen uptake and protein production versus time curves are shown for the control bottles with 200 mg/L surfactant (Figures 15a & b).

For the Corexit experiment (Figure 13), addition of surfactant seems to enhance octadecane biodegradation very slightly. This can be seen in the plot of oxygen uptake versus time for the first 150 hours of the experiment, as the oxygen uptake rates are slightly higher for the bottles with surfactant.

Increasing surfactant from 25 mg/L to 200 mg/L does not seem to affect oxygen utilization significantly until after approximately 70 hours, at which time the 200 mg/L Corexit curve increases at a faster rate than the 25 mg/L curve. This point coincides with the point of abrupt increase in uptake of Corexit, as can be seen on the oxygen versus time plot for 200 mg/L surfactant and no octadecane (Figure 15). Thus the increase in uptake rate after 70 hours for the 200 mg/L Corexit curve is most likely due to uptake of the surfactant.

The protein versus time curve shows a similar trend. Protein production rates are slightly higher for the BOD bottles with surfactant (for both 25 and 200 mg/L Corexit) as opposed to the bottles without surfactant up to about 70 hours; after 70 hours, protein in the 200 mg/L surfactant bottles increases more rapidly than the lower surfactant concentration. In addition, for the bottles with 200 mg/L Corexit, both total cumulative oxygen uptake and protein production at the end of the experiment are much higher than those for the control system with octadecane only, and the difference can be attributed to biodegradation of Corexit.

Octadecane biodegradation in the presence of Brij 35 is only slightly enhanced for the first 150 hours of the experiment (Figure 14). The total cumulative oxygen uptake and protein production at the end of the experiment for the three cases (1) without surfactant, (2) with 25 mg/L Brij, and (3) with 200 mg/L Brij are approximately the same. Brij 35 is not biodegraded by our octadecane-acclimated culture during the course of the experiment, as there was no significant oxygen uptake or protein production in the bottles with 25 or 200 mg/L Brij and no octadecane.

II-C-3. Effects of Surfactants on Octadecane Biodegradation-Conclusions

In batch reactor tests in which octadecane (200 mg/l) was added as particulates, the presence of the four non-ionic surfactants that were tested in this phase of the study all enhanced biodegradation rates slightly.

A secondary effect of surfactant addition was noted with Corexit, namely that this surfactant was biodegraded concurrently with octadecane after 70 hours of incubation and contributed to oxygen utilization as well as the accumulation of active biomass as measured by protein accumulation. The practical implications of surfactant biodegradation are potentially detrimental and beneficial. Increased oxygen demand is seen as detrimental in subsurface environments where oxygen is likely to be limiting. However, because kinetics of biodegradation are first order in biomass it follows that higher biomass accumulations are potentially beneficial in environments in which the available biomass is very low and removal is limited by kinetics. This situation would apply in pristine subsurface environments that have not been previously enriched in microbial cell mass by exposure to similar organic chemicals.

Section III - Conclusions

III-A. Solubility Enhancement of Phenanthrene

It has been shown that commercial non-ionic surfactants can be used to enhance the dispersion of sparingly soluble hydrocarbons. The apparent aqueous solubility of phenanthrene increased linearly above the CMC of all the surfactants. This slope could be used to determine the molar solubilization ratio and the partitioning of the compound between the micelle and the aqueous phases.

III-B. Solubility Enhancement of Octadecane

Surfactant addition can be used to increase apparent solubility of octadecane from its saturation concentration 0.007 mg/L to 2.4 mg/L. Most of the increase is in the form of surfactant stabilized molecular aggregates that are retained on 3000 molecular weight filters.

The dispersed octadecane affords high interfacial contact with the water phase which facilitates transport and solubilization but aggregates are not directly available for biodegradation.

III-C. Biodegradation Screening Study

A screening test procedure has been developed and standardized for testing surfactants on specific chemical pollutants. Screening tests indicate a great potential for a few of these surfactants to be used in practical applications in in-situ bio-reactor/slurry reactor. Further work in this direction using the testing methods developed and the correlated values of molar solubilization ratio (MSR) and partition coefficients (K_m) as described in Table 3, will lead to specific design criteria for the selection of surfactants in relation to the physical chemical properties of the polluting chemicals and the properties of the soil.

III-D. Effects of Surfactants on Phenanthrene Biodegradation

The batch reactor test data clearly show that addition of 25 mg/L of surfactant in presence and absence of soil increased biodegradation rates. Phenanthrene solubility in water alone at room temperature was found to be 0.825 mg/L. Solubility enhancement studies with the same surfactants at 25 mg/L [Jahan, Ph.D. Thesis] indicated slight increases in the apparent solubility of phenanthrene. The presence of surfactants tends to stabilize suspensions of particulates thereby exposing more surfaces of solid phase phenanthrene for solubilization as reported by other investigators. Larger surface area facilitates mass transfer at the interface. Thus the microorganisms had more soluble substrate available as compared to the control. This also helps explain the higher concentration of intermediate metabolite production in the presence of surfactants. It is believed that intermediates are formed when available soluble substrate is higher, resulting in higher transport rates of substrate into cells which in turn results in production of more intermediates. Linear growth is observed in all cases indicating growth is limited by availability of transportable soluble substrate.

III-E. Effects of Surfactants on Octadecane Biodegradation

Some enhancement of rates of biodegradation was observed with all four of the surfactants tested at both 25 and 200 mg/L. Increased rates were most noticeable during the first 150 hours when particles of octadecane were largest. There was no evidence of toxicity due to surfactant addition. It appears that addition of surfactants enhanced dispersion thereby creating additional surface contact with the water phase resulting in higher rates of dissolution and accelerated biodegradation rates. The implication is that the rate of dissolution is the rate limiting factor.

Two of the surfactants (Corexit 0600 and Tween 40) were biodegraded concurrently with octadecane. The resultant increase in oxygen demand is potential undesirable in subsurface environments. However, the associated increase in active microbial cells may be beneficial in soils that have low microbial population densities. The net impact of surfactant biodegradation therefore depends on the characteristics of the site and should be assessed by modeling the related effects of oxygen utilization and biomass accumulation.

Section IV - Ongoing/Future Work

IV-1. Kinetics of Bacterial Degradation of Phenanthrene.

Parameters for bacterial degradation kinetics are currently being determined using batch experiments with the experimental setup shown in Fig. 1. Methods have been standardized. The data will be correlated in terms of the chemical properties of surfactants and pollutant to be used as a design tool in choosing surfactants for specific application.

IV-B. Model for Enhanced Mobility and Degradation.

As discussed elsewhere, presence of surfactant changes the distribution of contaminant in the system. This requires changes to be made in the more traditional biodegradation model of contaminant transport in Soil-Water-Biomass system. Appropriate model has been framed to cater to the contaminant transport in Soil-Water-Surfactant-Biomass system.

IV-C. Optimum Dose-Response Relationship.

It appears that adding more surfactant can mobilize more contaminant from the soil surface, which in turn can make the process of clean up much faster. But there is a possibility that higher dose of surfactant will adversely affect the bacterial kinetic parameters giving rise to slower degradation rate. There is a need to find out the optimum dose of surfactant for most efficient clean up.

IV-D. Basis for Selection of Surfactant.

From the screening experiment, it is seen that some of the surfactants do work while others fail. These experiments are designed to explore the reason for this phenomena, possibly depending on the structure of surfactant molecule and the contaminant involved.

IV-E. Specificity of Surfactant Towards Pollutants.

Here we will explore the possibility of our result being specific to the particular contaminant used. A few representative experiments with other contaminants can provide us

with the information about the type of specificity, if at all present. This, in conjunction with the 'basis for selection of surfactant', will lead us to design for most general cases.

IV-F. Extension of Model into Porous Media Situation.

Model for enhanced mobility and degradation will be extended for porous media case. This can be used to design/predict the field/prototype experiments.

IV-G. Applicability in In-Situ Bio-Reactor/Slurry Reactor.

This will be done in laboratory column/tank reactor simulating field condition. Above model will be used to simulate the results.

IV-H. Octadecane Solubilization

Preliminary data from ongoing experiments in which octadecane was introduced as a coating on the soil grains thereby creating large surface area exposure to the water phase gave significantly higher rates of biodegradation with and without surfactants. These results support the conclusion that solubilization is a rate limiting factor. Ongoing research is therefore aimed at developing environments for acceleration of solubilization. Two approaches will be pursued namely, to examine alternative methods for modifying the physics of solubilization (the physics of solubilization are strongly dependent on energy inputs). A second approach is based on recently published information showing that microbiologically produced biosurfactants (rhamnolipids) are significantly more effective in dispersing octadecane than the commercially available surfactants that we are using.

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Table 1: Structures and Properties of Selected Commercial Surfactants

Surfactant	Structure	MW	CMC mg/L	HLB #
Triton X-114	$C_8H_{17}-C_6H_4-O-(CH_2CH_2O)_nH$ $n=7.5$	538	110	12.9
Triton X-405	$n=40$	1986	620	17.9
Triton X-100	$n=9.5$	628	130	13.5
Corexit 0600	Blend of Surfactant Esters		40	15.0
Corexit 7665	.		60	15.0
Corexit 8600	.		100	15.0
Tween 40	Monopalmitate Polyoxyethylene Sorbitan	256	30	15.6
Triton X-102	$C_9H_{19}-\text{C}_6\text{H}_4-O-(CH_2CH_2O)_nH$ $n=9.5$		150	
Triton N-101	$C_9H_{19}-\text{C}_6\text{H}_4-O-(CH_2CH_2O)_nH$ $n=9$		63	
Tergitol NP-10	$C_9H_{19}-\text{C}_6\text{H}_4-O-(CH_2CH_2O)_nH$ $n=10.5$		52	
Polyoxyethylene 10 Lauryl Ether			48	
Triton CF-21			210	
Tergitol 15-S-9			70	
Tergitol 15-S-20			105	
Tergitol TMN-10			1200	
Brij 35	23 Lauryl Ether $C_{12}H_{25}(OCH_2CH_2)_{23}OH$	1200	74	16.9

Table 2a: Jordan Sand Characteristics

SiO₂	99.62 %	Al₂O₃	0.04 %
Fe₂O₃	0.053 %	CaO	0.014 %
MgO	0.003 %	Na₂O	0.01 %
K₂O	0.01 %	TiO₂	0.01 %
MnO	0.001 %	SrO	<0.01 %
BaO	< 0.01 %	Loss on Ignition	0.17 %
Organic Carbon	0.01 %	Cation Exchange Capacity	0.2 meq/100g
Total	99.93 %	pH, H₂O Extract	7.1

(Data provided by J.L. Shiely Company)

Table 2b. Physical Characteristics of the Soil

Sample Fraction	Size Range	Percent by Weight	Percent Organic Carbon Content
Bulk	< 2mm		2.60
Sand	2mm - 62μm	17	0.58
Silt	62μm - 2μm	25	1.95
Clay	< 2μm	58	3.51

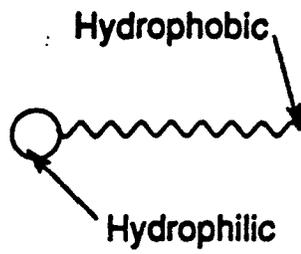
Table 3: Calculated Values of MSR and Log Km for Selected Surfactants

Surfactant	MSR	Log Km
Triton X-114	0.0313	5.221
Triton X-405		
Triton X-100	0.0119	4.986
Corexit 0600	0.0098	5.041
Corexit 7665	0.0086	4.723
Corexit 8600	0.0118	5.231
Tween 40	0.0241	5.371
Brij 35	0.0062	4.782

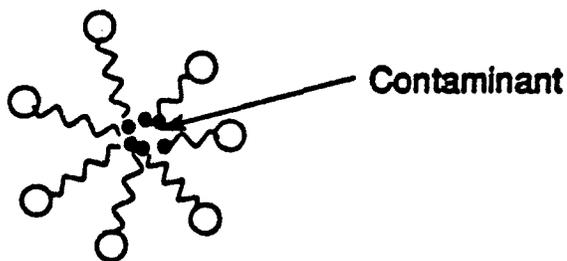
Table 4: Concentration of Octadecane (mg/L) in Filtrates of Corexit 0600 Solutions.

Corexit 0600 Concentration	0.2 μm^* Filtrate	100,000 MW* Filtrate	30,000 MW* Filtrate	10,000 MW* Filtrate	3,000 MW* Filtrate
No Surfactant	0.020	0.006	0.005	0.004	0.003
5 mg/L	0.050	0.008	0.006	0.006	0.004
25 mg/L	0.177	0.018	0.018	0.014	0.011
50 mg/L	0.756	0.019		0.015	0.012
80 mg/L	1.266	0.018	0.013	0.011	0.009
120 mg/L	1.599	0.016		0.013	0.009
200 mg/L	1.625	0.022	0.015	0.012	0.009
400 mg/L	2.091	0.029	0.018	0.015	0.012

*0.2 μm = 2000 \AA ; 100,000MW = 62 \AA ; 30,000MW = 41.2 \AA ; 10,000MW = 28.5 \AA ; 3,000MW = 19.2 \AA .



a. Non-ionic Surfactant Monomer



b. Contaminant Partitioned in Micelle

Figure 1. Non-ionic Surfactant Monomer and Micelle Formation

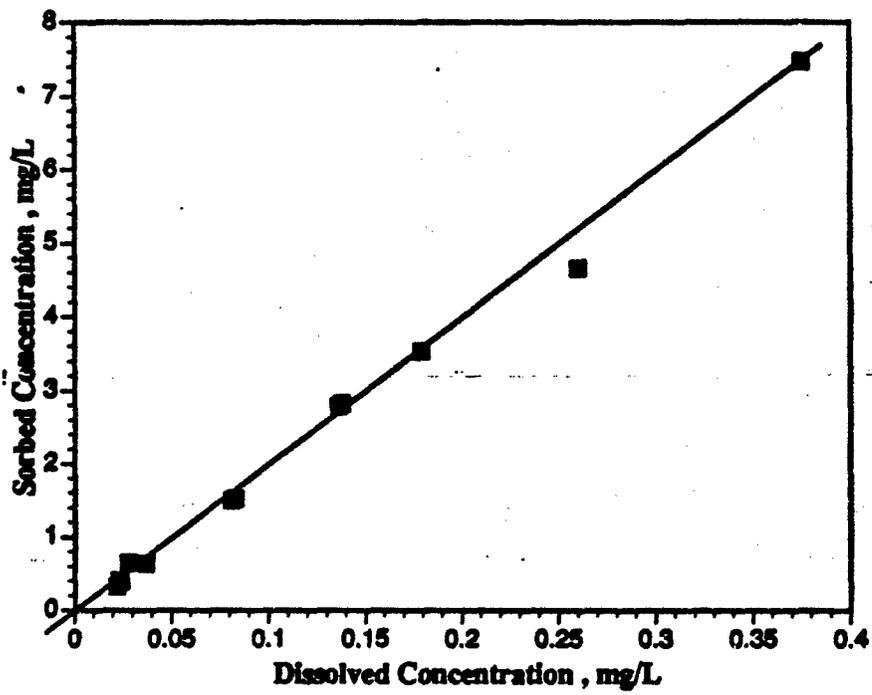


Figure 2. Sorption Isotherm for the Soil
(5% Soil, w/w)

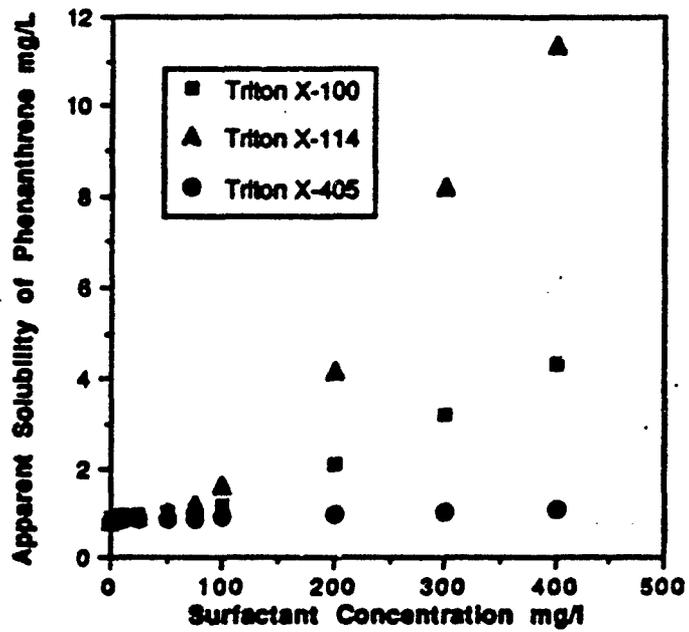


Figure 3. Plot of the apparent solubility of phenanthrene versus the Triton surfactant concentrations.

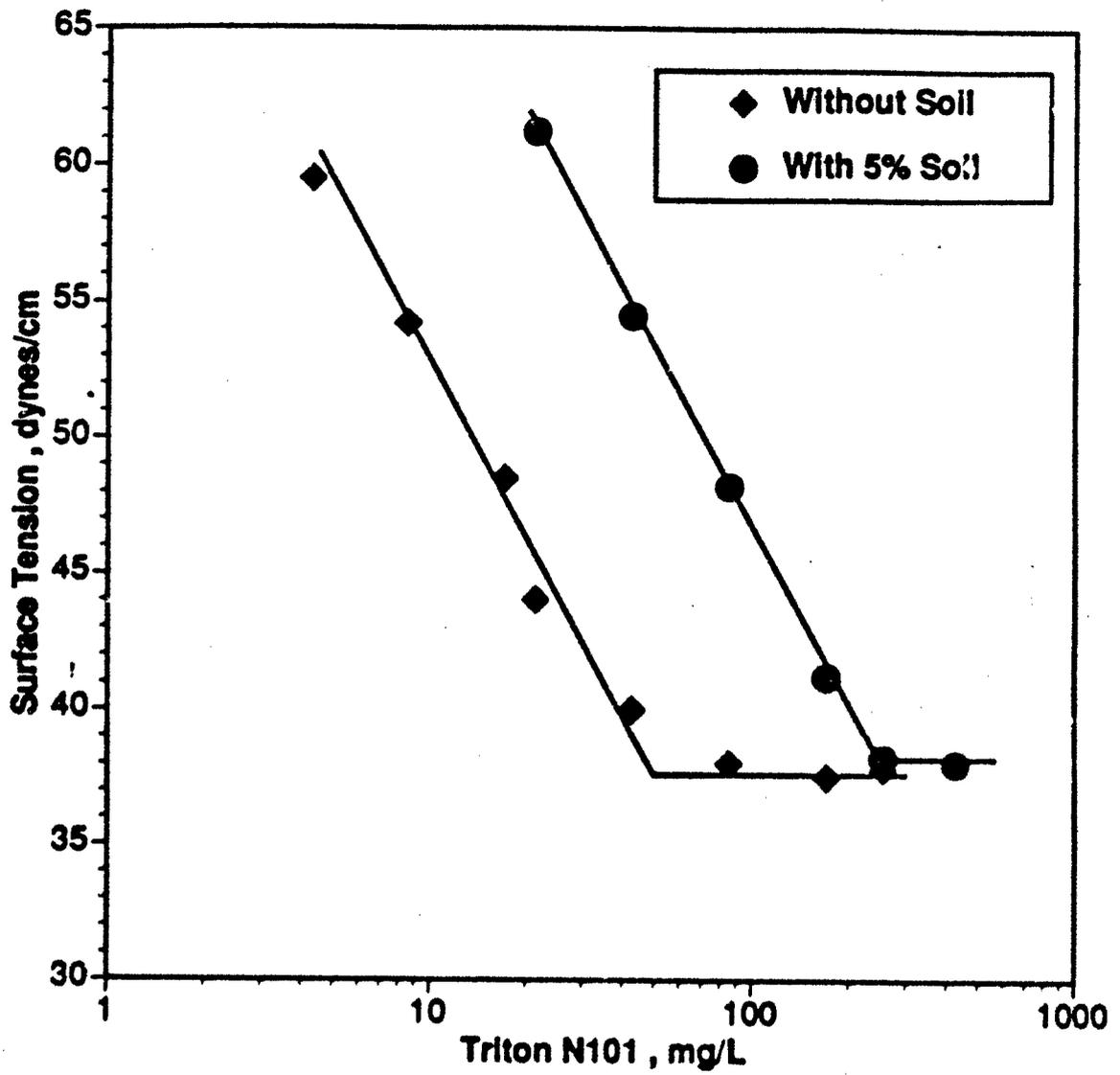


Figure 4. Determination of CMC for Triton N101

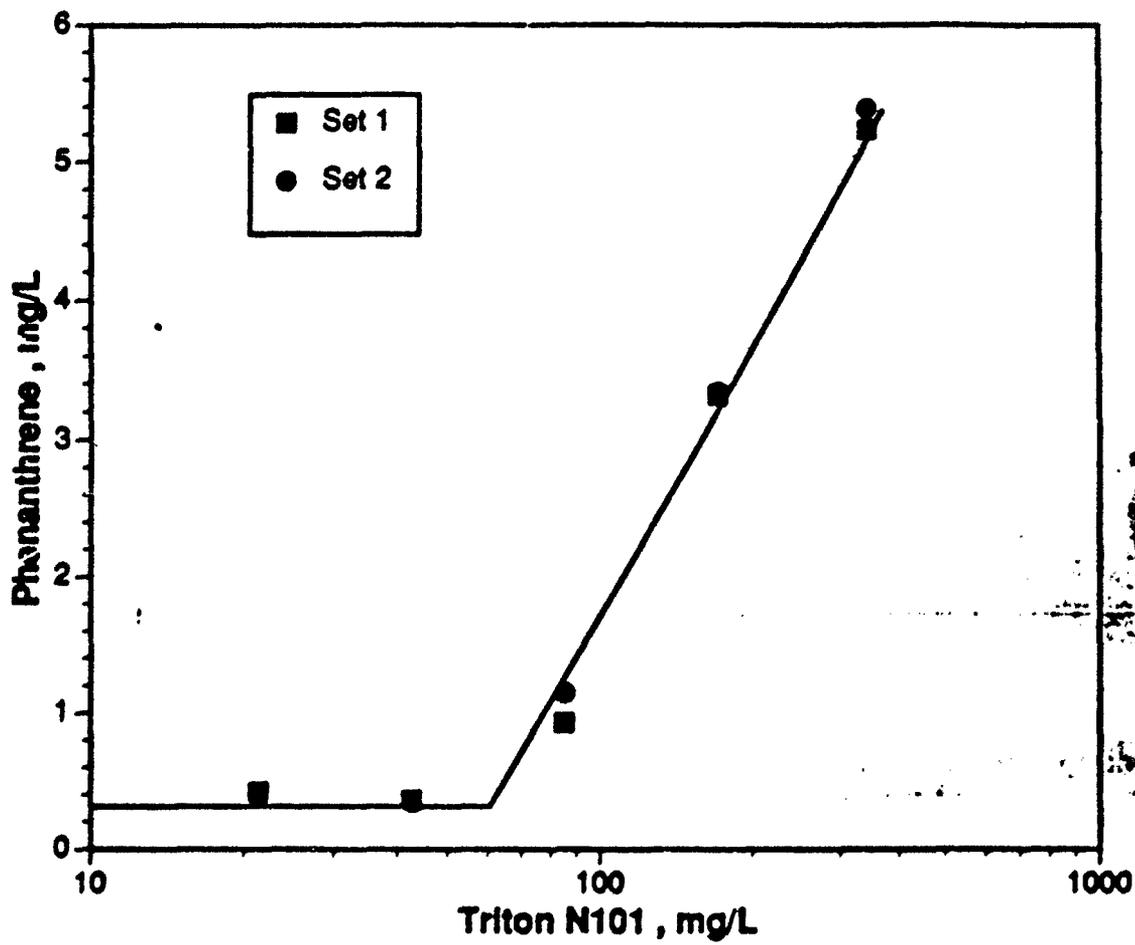


Figure 5. Solubility Enhancement By Triton N101

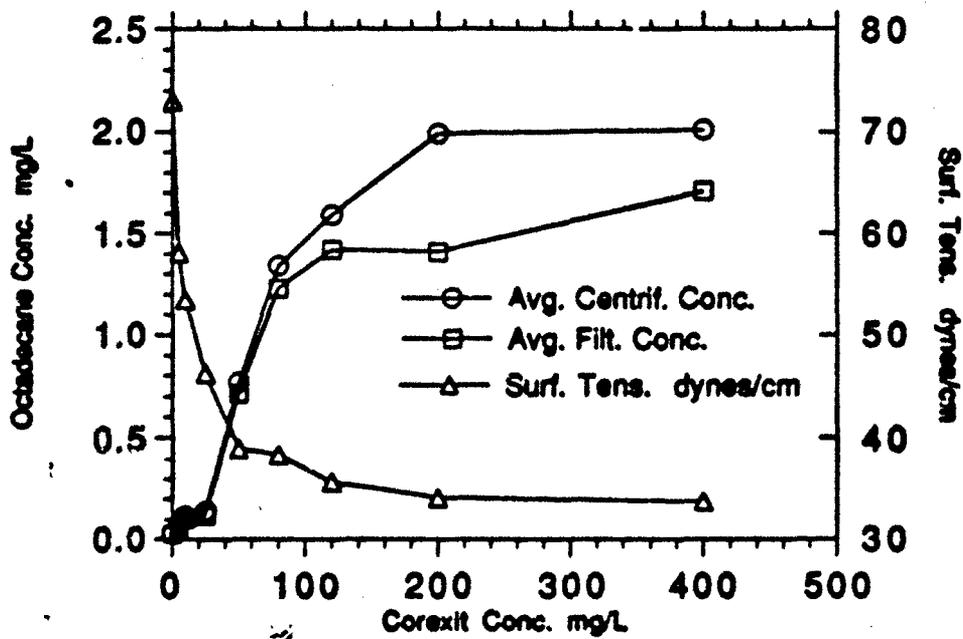


Figure 8. Apparent Octadecane Solubility & Surface Tension vs. Corexit 0500 Concentration.

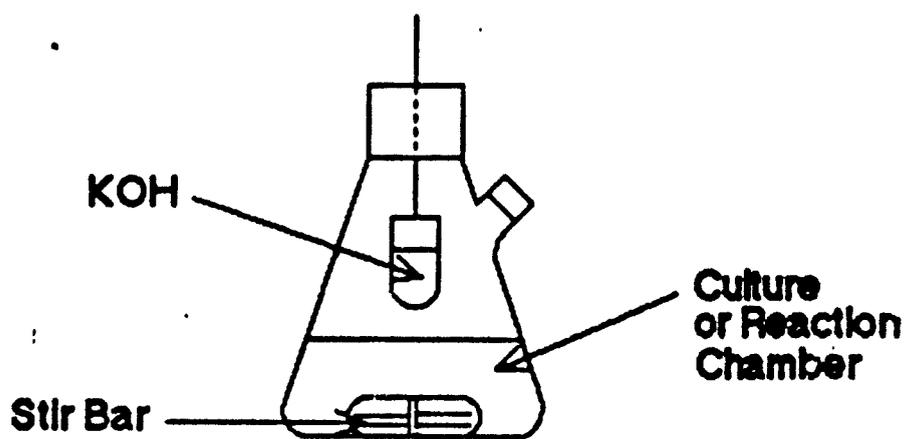


Figure 7. Experimental Setup

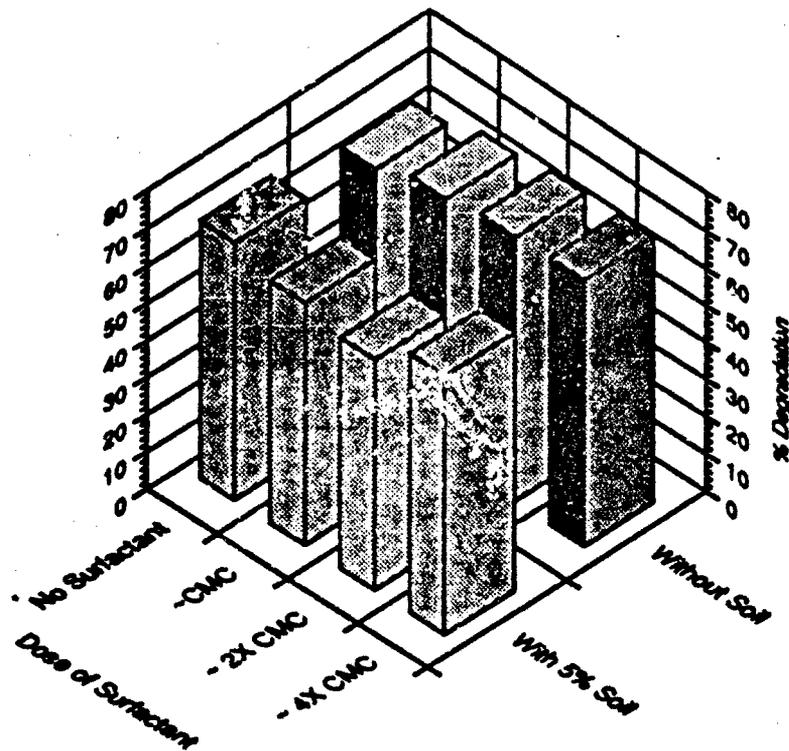


Figure 8a. Triton N-101

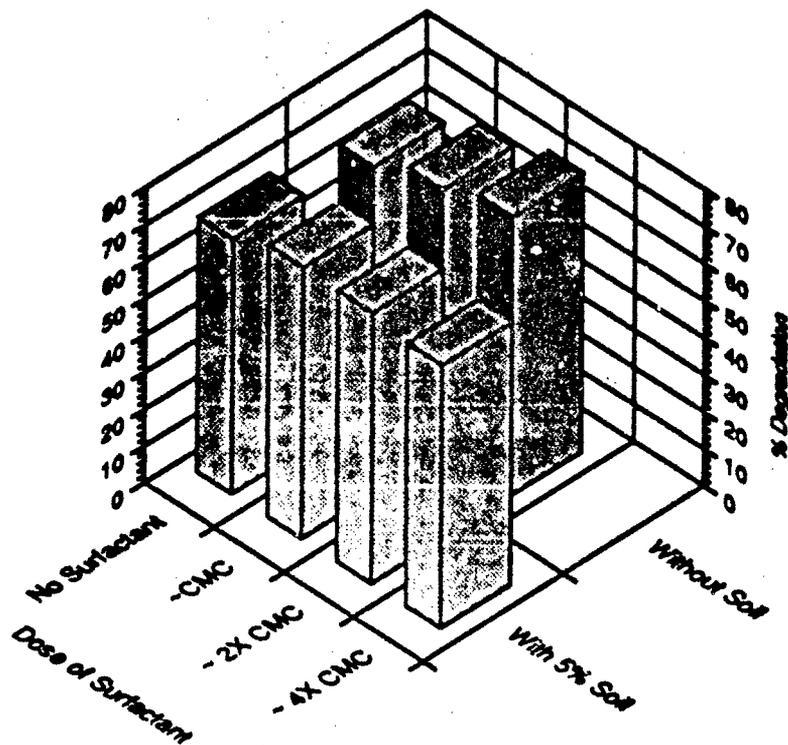


Figure 8b. Triton CF-21

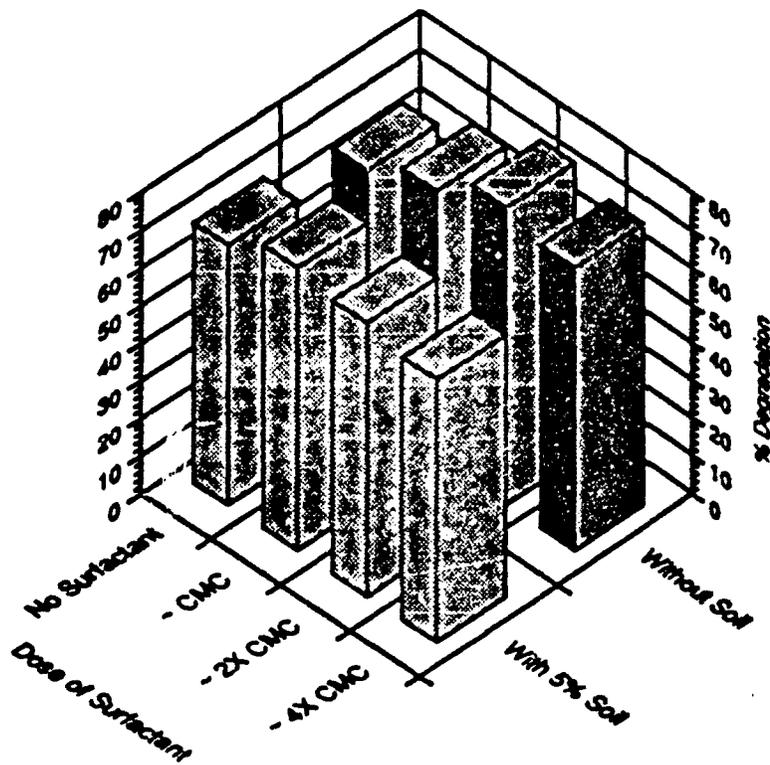


Figure 8c. Tergitol 15-S-9

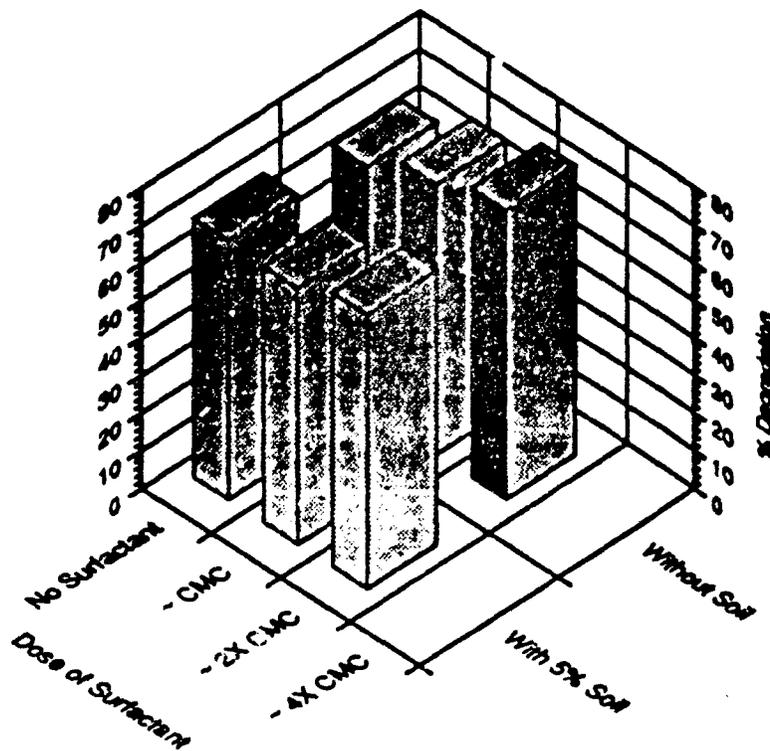


Figure 8d. Polyoxyethylene 10 Lauryl Ether

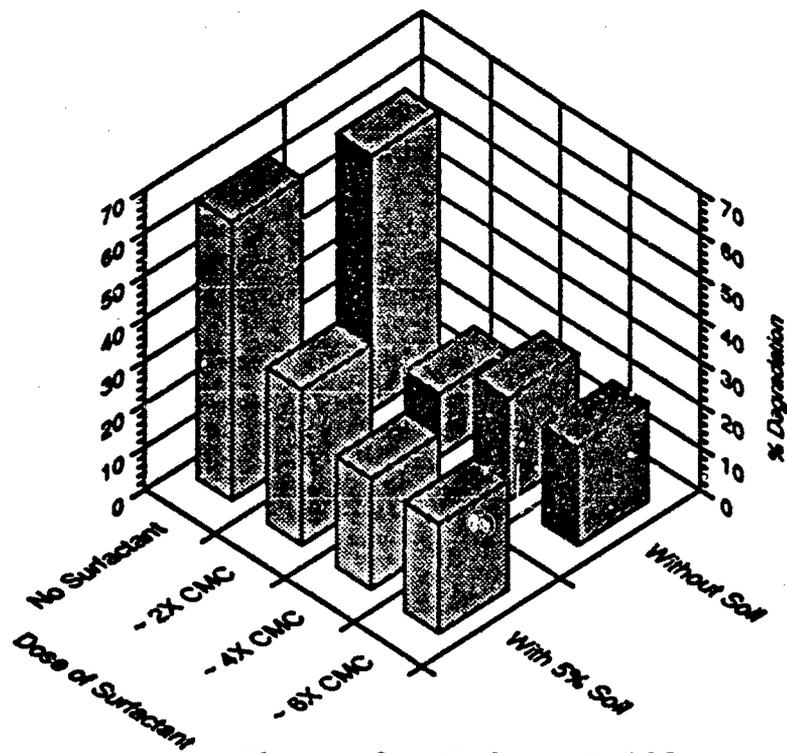


Figure 8e. Triton X-102

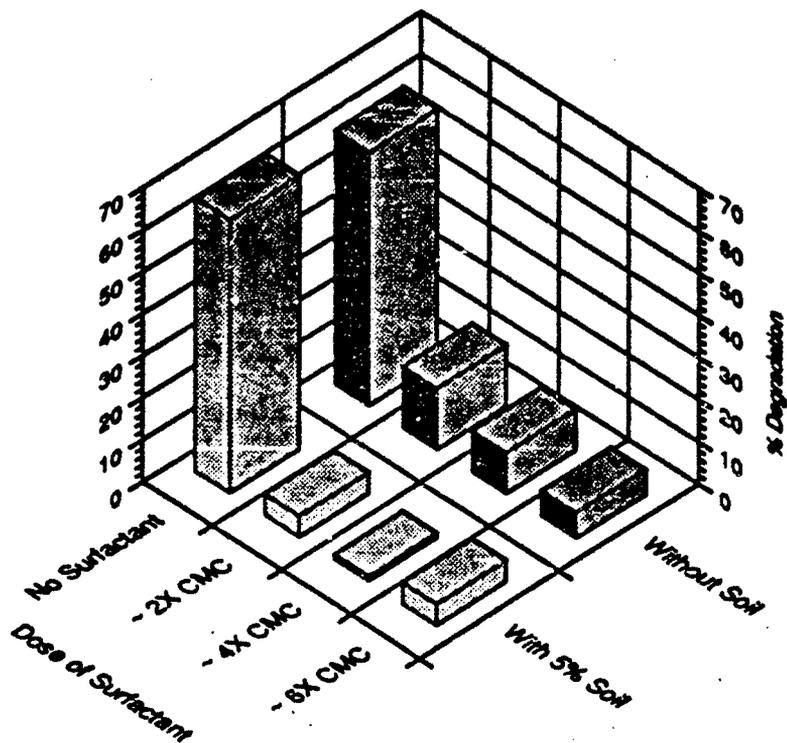


Figure 8f. Tergitol 15-S-20

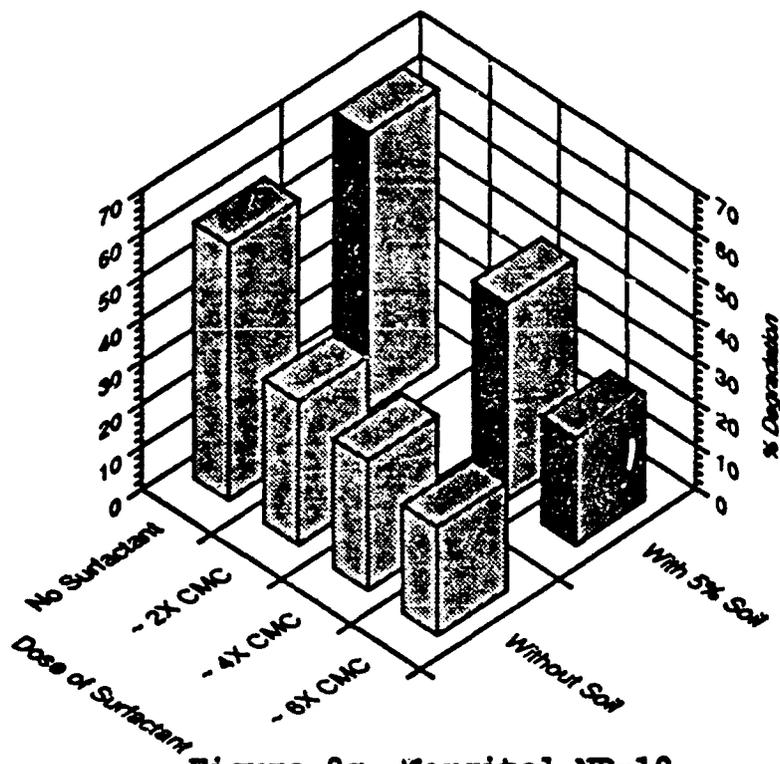


Figure 8g. Tergitol NP-10

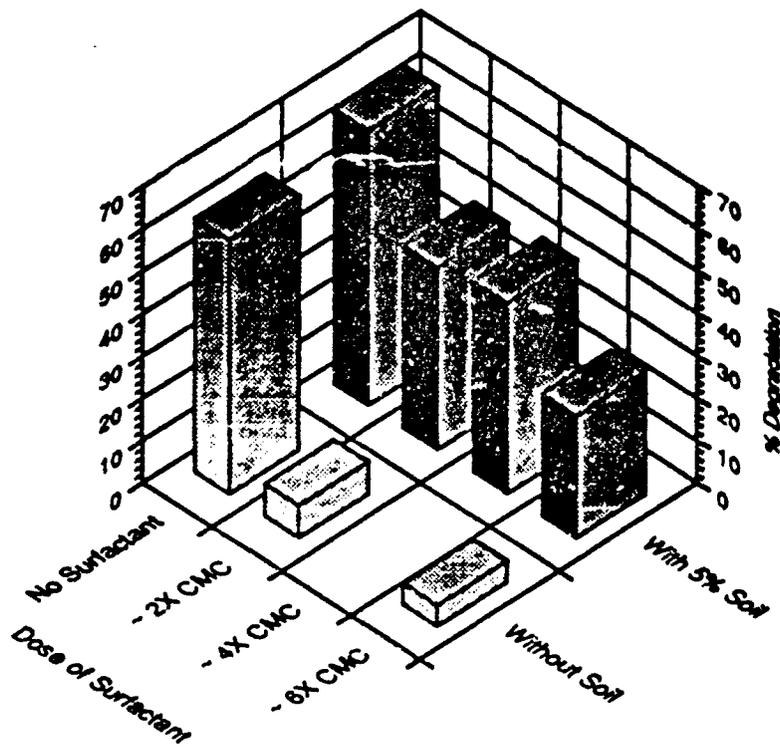
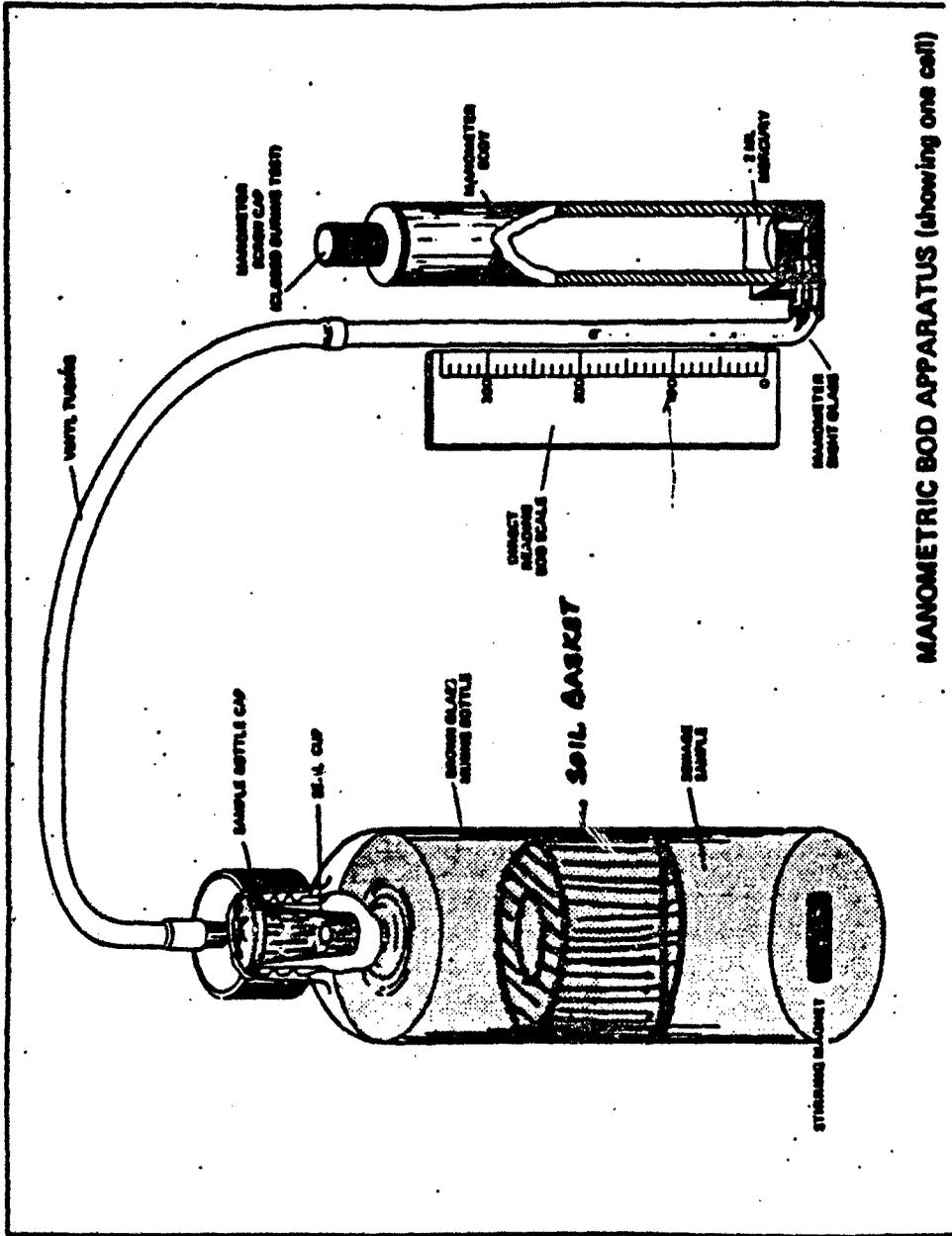


Figure 8h. Tergitol TMN 10



MANOMETRIC BOD APPARATUS (showing one cell)

Figure 8.

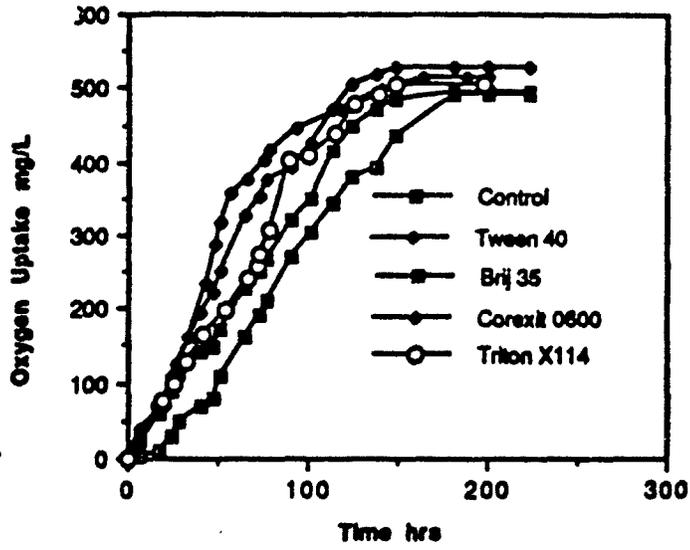


Figure 10a. Oxygen Uptake vs. Time. Phenanthrene Biodegradation in the Presence of Surfactants

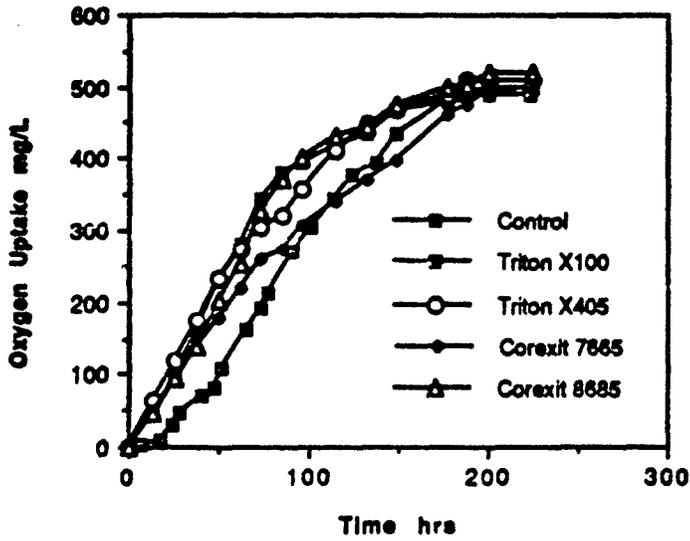


Figure 10b. Oxygen Uptake vs. Time. Phenanthrene Biodegradation in the Presence of Surfactants.

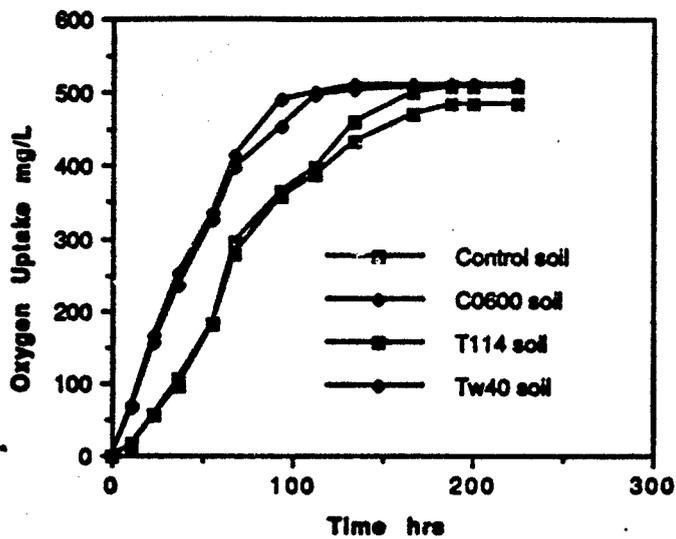


Figure 10c. Oxygen Uptake vs. Time. Phenanthrene Biodegradation (w/ Surfactants) in Presence of Soil.

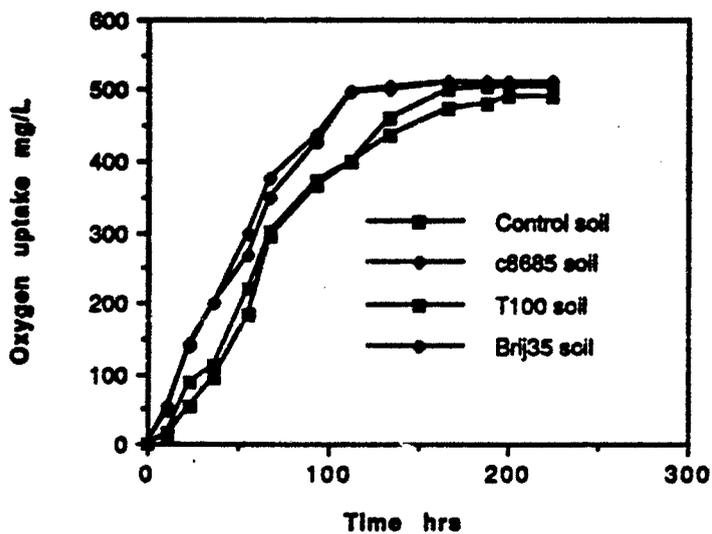


Figure 10d. Oxygen Uptake vs. Time. Phenanthrene Biodegradation (w/ Surfactants) in the Presence of Soil.

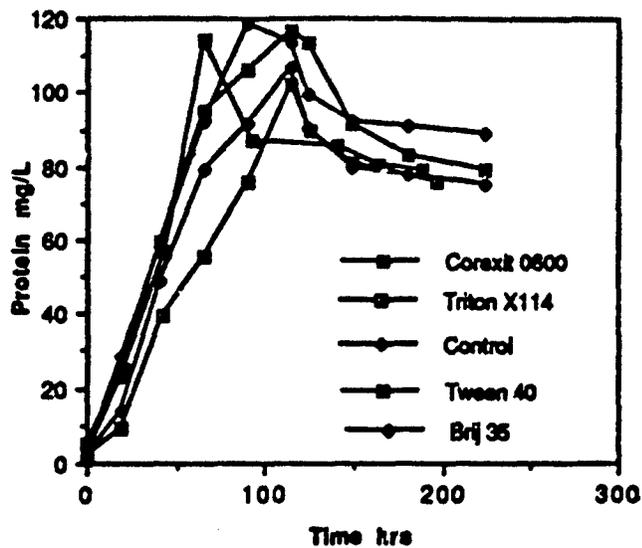


Figure 11a. Protein Production vs. Time. Phenanthrene Biodegradation in the Presence of Surfactants.

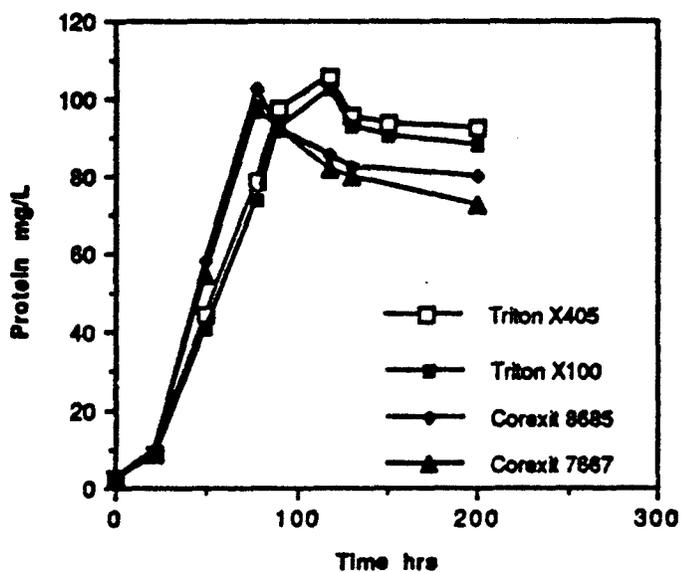


Figure 11b. Protein Production vs. Time. Phenanthrene Biodegradation in the Presence of Surfactants.

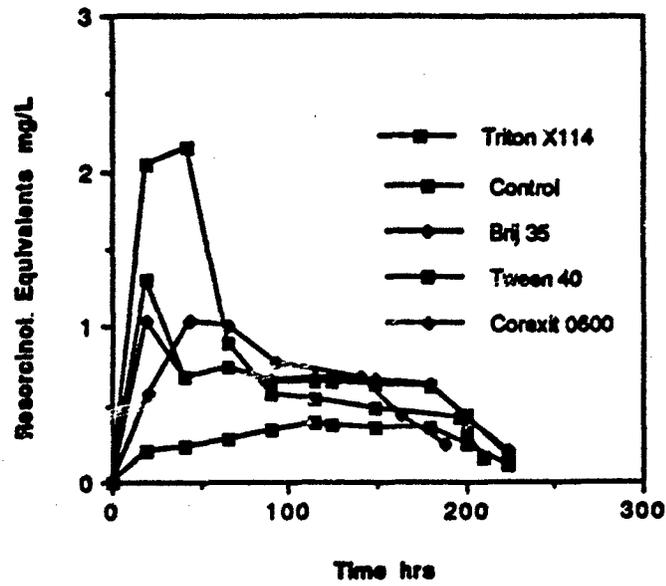


Figure 12a. Resorcinol Production vs. Time. Phenanthrene Biodegradation in the Presence of Surfactants.

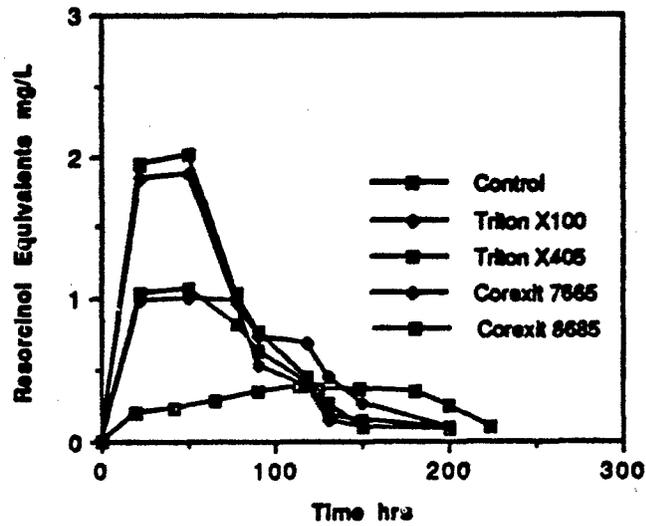


Figure 12b. Resorcinol Production vs. Time. Phenanthrene Biodegradation in the Presence of Surfactants.

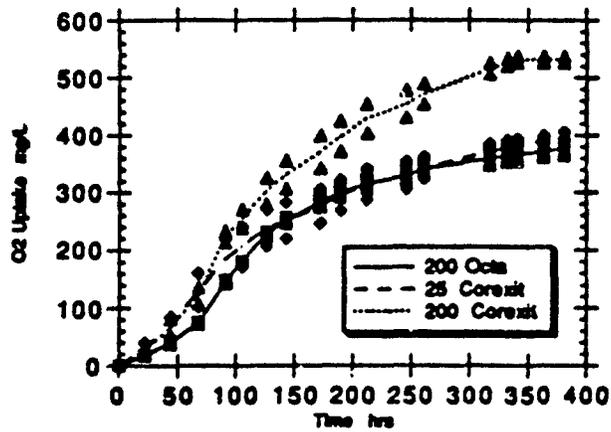


Figure 13a. Oxygen Uptake vs. Time. Octadecane Biodegradation in the Presence of Corexit 0800.

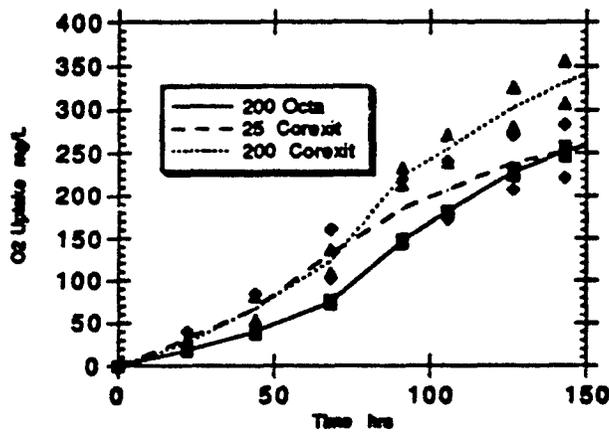


Figure 13b. Oxygen Uptake vs. Time-1st 180 hours. Octadecane Biodegradation in the Presence of Corexit 0800.

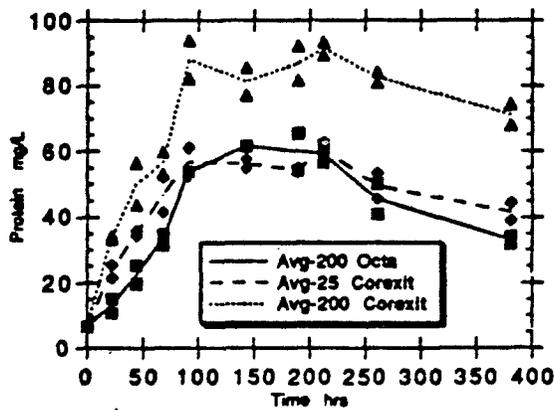


Figure 13c. Protein Production vs. Time. Octadecane Biodegradation in the Presence of Corexit 0800

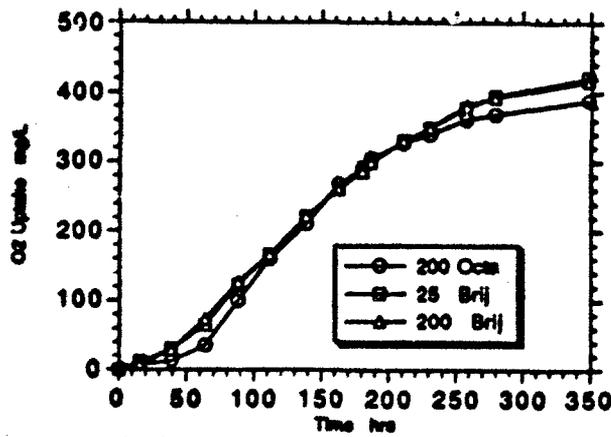


Figure 14a. Oxygen Uptake vs. Time. Octadecane Biodegradation in the Presence of Brij 35.

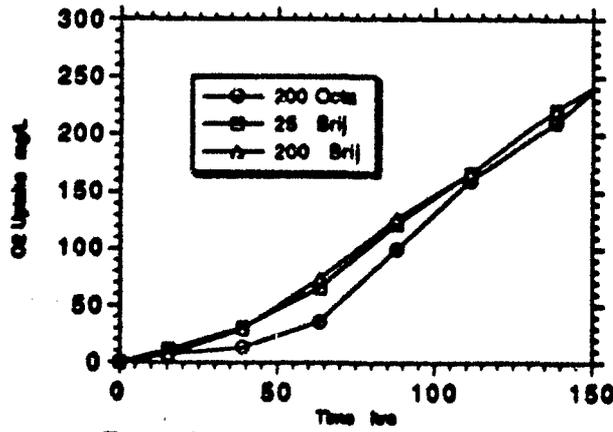


Figure 14b. Oxygen Uptake vs. Time-Start 150 hours. Octadecane Biodegradation in the Presence of Brij 35.

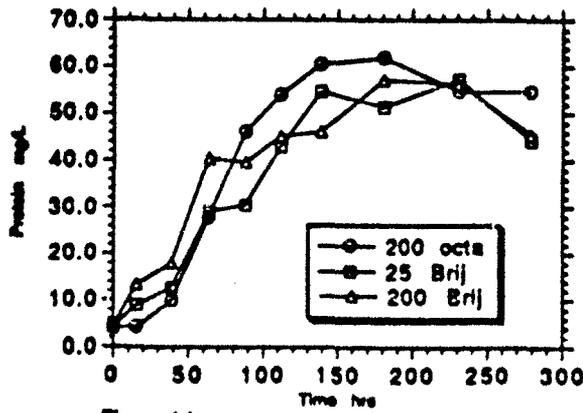


Figure 14c. Protein Production vs. Time. Octadecane Biodegradation in the Presence of Brij 35.

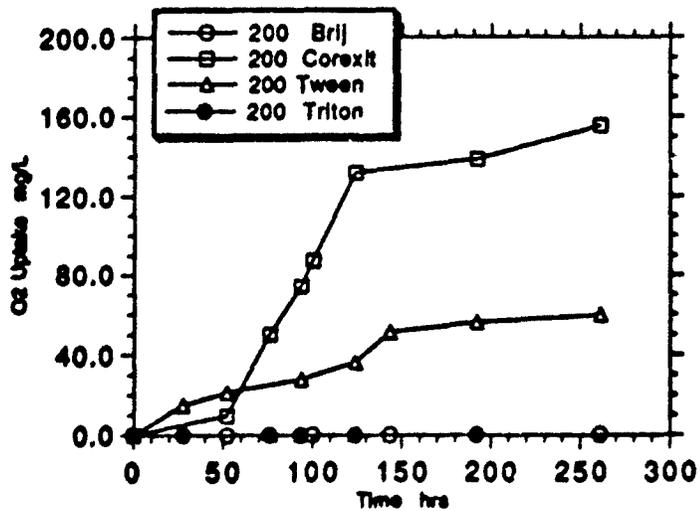


Figure 15a. Oxygen Uptake vs. Time. Biodegradation of 200 mg/L Surfactant (No Octadecane)

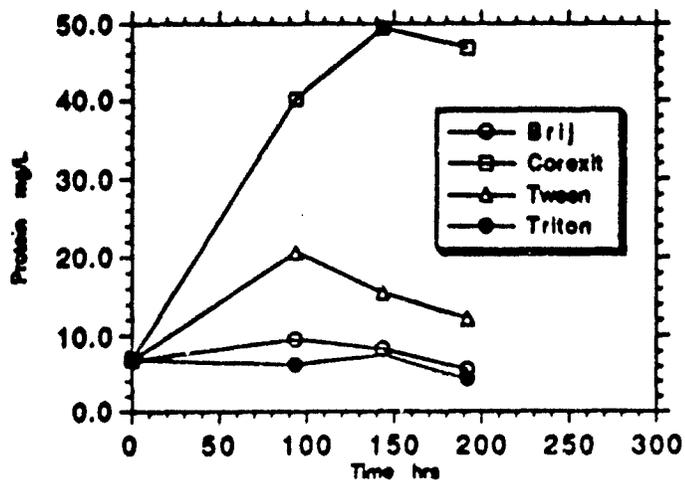


Figure 15b. Protein Production vs. Time. Biodegradation of 200 mg/L Surfactant (No Octadecane).