

**Study of Hydrocarbon Waste Biodegradation and the Role of Biosurfactants  
in the Process**

by

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## **(ABSTRACT)**

Two types of oily waste sludges generated by a railroad maintenance facility were studied to reduce the volume of hydrocarbon waste. The specific goals of this laboratory study were to evaluate rate and extent of microbial degradation, benefits of organism addition, role of biosurfactant, and dewatering properties.

The oily waste sludges differed in characteristics and contained a mixture of water, motor oil, lubricating oil, and other petroleum products. Degradation was measured using COD, suspended solids, GC measurements of extractable material, and nonextractable material concentration. Biosurfactant production was characterized using surface tension and polysaccharide measurements.

Degradation of ten percent waste oil showed that the removal in a 91 day experiment was 75 percent for COD and suspended solids, 98 percent for extractable oil, and negligible for non-extractable material. It was concluded that methylene chloride extraction could be used to estimate degradation potential of a hydrocarbon waste. Addition of organisms increased the rate and extent of degradation over 22 days, but did not provide any benefits over 91 days.

Data suggested that microorganisms degraded simple compounds first, then produced biosurfactants. It was thought that the biosurfactants remained attached to the organism membrane and increased solubility, stimulating the degradation of difficult to degrade waste oil. After oil was degraded the biosurfactants became ineffective.

The dewatering properties of 10 percent oily sludge deteriorated with the production of biosurfactant and improved after the surfactant was degraded due to changes in oil solubility.

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## **EXECUTIVE SUMMARY**

The objective of this study was to test the feasibility of using aerobic microbial degradation to treat and reduce volume of hydrocarbon wastes from a railroad maintenance facility. The specific objectives of this study were: to study the rate and extent of active microbial degradation of the hydrocarbon waste, to compare the performance of native and introduced bacterial species, to investigate the role of bio-surfactant in the biodegradation process, and to evaluate the dewatering properties of the waste.

Two types of oily waste sludges were studied in this series of experiments. The sludges differed in characteristics and contained a mixture of water, motor oil, lubricating oil, and other petroleum products. Waste oil #1 had a lower COD, lower suspended solids, and lower methylene chloride extractable fraction than waste oil #2.

Several experiments were performed to study degradation and dewatering properties. In all experiments the waste oil was diluted to 10 percent and studied separately or as a mixture of the two waste oil sources. Nutrients and oxygen were added to create favorable conditions for organism growth. Degradation was measured using COD, suspended solids, GC measurements of extractable material, and nonextractable material concentration. Biosurfactant production was characterized using surface tension and polysaccharide measurements.

After the initial experiments were performed, a problem with the COD measurements was discovered. The potassium hydrogen phthalate standard recovery was only about 10 percent and the measured COD increased with dilution of the sample and reaction time at 150°C. In this series of experiments the COD values discussed below are the experimental values and they are used for comparative purposes only. To minimize the variability in the measurements the test conditions were constant for each of the experiments. The suspended solids, polysaccharides, surface tension, and extractable and non-extractable oil were not effected by oil interferences.

In the 10 percent aerated batch reactor experiments, the waste oil partitioned into a liquid phase oil fraction and attached oil fraction. The attached oil was positioned on the walls of the reactor and was not sampled. The attached oil entered the liquid phase and

caused difficulties in the interpretation of results. The liquid phase oil was dissolved or suspended in the liquid and this fraction was sampled for 91 days. The liquid phase of the reactor showed an average of 75 percent reduction for COD and suspended solids, 98 percent for extractable oil, and negligible for non-extractable material. The extent of degradation varied based on the initial concentration of oil and organisms used.

Four different organism types were tested in this experiment. The indigenous organisms growing in the waste oil, a mixture of pure cultures of hydrocarbon degraders grown on diesel fuel, anthracene, and naphthalene, a consortium of soil organisms grown on diesel fuel and motor oil, and a pure culture of *Rhodococcus* species grown on diesel fuel. Addition of organisms increased the rate and extent of degradation over 22 days, but did not provide any benefits over 91 days. Since the extractable oil showed substantial degradation and the non-extractable material did not degrade, it was concluded that methylene chloride extraction can be used as the first estimate degradation potential of a hydrocarbon waste.

A filtered oil experiment was performed with a low concentration of oil to avoid problems associated with attached oil. In this experiment the oil degraded in four stages. First the easily degradable oil measured as filtrate COD and filtrate polysaccharides was degraded. Next biosurfactants measured by surface tension and polysaccharides were produced. The data suggested that the biosurfactants remained attached to the organism membrane and increased solubility, stimulating the degradation of difficult to degrade waste oil. After the oil was degraded the biosurfactants became ineffective.

The dewatering properties were studied using a 10 percent oily sludge reactor. The properties measured were capillary suction time and specific resistance. In the dewatering experiment it was found that the dewatering properties deteriorated with the production of biosurfactant. These properties improved after the oil was removed and the surfactant was no longer effective.

The dewatering properties were directly related to the amount of biodegradable material in the reactor. The waste oil #2 reactor, which contained more biodegradable material, showed a larger deterioration in the dewatering properties and the final dewatering properties were well above the initial values.

It is recommended that the waste oil be diluted prior to biodegradation to reduce potential toxic effects to the biomass and reduce the oxygen demand. In order to facilitate biodegradation of waste oil, addition of nutrients along with oxygen is recommended. Addition of commercial microorganisms produced minimal benefit over the indigenous microbial culture. The indigenous microorganisms are acclimated to the waste oil and will result in removal of hydrocarbons when provided favorable conditions. For fresh oil that has not had the opportunity for natural seeding, organism addition may be beneficial. For this system in which oil was stored prior to treatment microbial amendments were not beneficial.

The recommended reactor configuration includes a plug flow reactor or a series of continuously stirred reactors. The continuously stirred reactor series could consist of four reactors. In the first reactor of the series, easily degradable material would be removed. In the second reactor, biosurfactants would be produced. In third, the emulsified compounds would be removed, and in the fourth the biosurfactant would be inactivated to produce a more easily dewatering sludge.

## **I. INTRODUCTION**

The accumulation of hydrocarbons, especially polycyclic aromatic hydrocarbons (PAH), in the environment is a major health concern. These compounds have been shown to exhibit toxic, mutagenic, and carcinogenic effects (Fujikawa et al. 1993 in Juhasz et al. 1996). Hydrocarbons, especially petroleum products, are used extensively in many industries including transportation, chemical, and textile industries. The frequent and extensive use of hydrocarbons presents a risk of environmental contamination from the time they are mined through product formation and use to waste disposal.

Traditional hydrocarbon waste management methods are often difficult and costly processes. These methods include landfill disposal, incineration, thermal evaporation, extraction, chemical oxidation, adsorption, and immobilization techniques (Shailubhai 1986 in Jain et al. 1992). However, in the past decade bioremediation has proven to be a relative inexpensive and effective alternative to traditional waste management techniques.

The main advantage of bioremediation is its flexibility in handling various composition waste types in both water and soil environments. Other advantages include low capital and operational costs and complete degradation of hydrocarbons to nontoxic products (Bucens et al. 1996). The main disadvantage of this technology is the inability to predict success of one operation based on observations from another operation (Bucens et al. 1996). Bioremediation's success is dependent upon the specific chemical make-up of the waste, the presence of waste degrading organisms, and the environmental conditions. This site-specific performance presents the need for laboratory and pilot testing.

This study was conducted on request of an industry to test the feasibility of using microbial degradation to treat hydrocarbon wastes from a railroad maintenance facility. The current waste management at this facility involves the collection of the waste from various sources on site and storage of the combined waste prior to landfill disposal. This costly practice can be improved by reducing the waste oil volume through the use of bioremediation to treat the entire mixed waste or the individual sources of the waste.

This laboratory study was focused on two waste sources, which account for 63 percent of the total hydrocarbon waste volume at the facility. These two waste sources

contain a mixture of water, motor oil, lubricating oil, and other miscellaneous petroleum products. The petroleum products are heavy, long chain, and polyaromatic hydrocarbons with low volatility and limited solubility.

The specific objectives of this study were:

- To study the rate and extent of active microbial degradation of the hydrocarbon waste.
- To compare the performance of native and introduced bacterial species.
- To investigate the role of biosurfactants in the biodegradation process.
- To evaluate the dewatering properties of the waste.

## II. LITERATURE REVIEW

### A. Hydrocarbon Properties and Characteristics

Hydrocarbons are organic compounds made of carbon atoms bound to each other forming a backbone with hydrogen atoms attached to the remaining sites on carbon. The carbon backbone can be straight or normal, branched, or cyclic (Olah and Molnar 1995). Compounds containing only carbon and hydrogen are often referred to as parent compounds. Compounds containing substitutions of other elements or smaller carbon and hydrogen groups onto the original carbon backbone in place of the hydrogen are called derivative compounds (McMurry 1988). Hydrocarbons are classified based on the chemical structure of parent compounds or their physical properties.

The chemical structure classification includes alkanes, cycloalkanes, alkenes, cycloalkenes, and alkynes. Alkanes, or paraffins, contain single carbon to carbon bonds in a straight backbone structure. Cycloalkanes, or naphthenes, contain single carbon to carbon bonds in a ring structure. The single carbon to carbon bond is characteristic of saturated hydrocarbons. Alkenes, or olefins, contain double carbon to carbon bonds in a normal backbone. Cycloalkenes, or aromatics, contain double bonds in a cyclic structure. Alkynes, also referred to as olefins, contain a triple carbon to carbon bonds in their backbone. The double and triple carbon to carbon bond characterizes unsaturated hydrocarbons (McMurry 1988, Ratledge 1978, Olah and Molnar 1995).

The physical properties classifications vary depending on the literature source and include: gases, gasoline, kerosene, heating oil, mineral oil, waxes, resins, and asphaltenes. Gases include compounds with one to six carbons and have a boiling point below 30°C. Gasoline includes compounds with four to twelve carbons and has a boiling point from 30°C to 200°C. Kerosene and heating oil include compounds with eleven to fifteen carbons and have a boiling point from 200°C to 300°C. Mineral oil and waxes include fifteen to twenty-five carbons and have a boiling point from 300°C to 400°C. The asphaltenes contain more than twenty-five carbons and have a boiling point above 400°C (Olah and Molnar 1995, Prince 1993).

The physical property classification above is derived from the crude petroleum oil separation. Most of the hydrocarbon compounds used by industry are derived from crude petroleum oil, coal, and natural gas. However, hydrocarbons are natural compounds produced by animals, plants, and bacteria (Ratledge 1978). Therefore, it is not surprising that hydrocarbons can be biologically degraded by a variety of organisms.

## **B. Biodegradation Mechanisms**

In most cases biodegradation reduces the toxicity and the migration potential of hydrocarbons (Field et al. 1991). The degrading organisms utilize hydrocarbons as an energy source and a carbon source.

Most hydrocarbons used as an energy source are degraded under aerobic conditions to carbon dioxide and water. This degradation process is a catabolic process and the degradation to inorganic compounds is called mineralization. However, some hydrocarbons are not mineralized but transformed into simpler compounds (Ferrari et al. 1996). The hydrocarbons used as a carbon source are degraded to smaller compounds and incorporated into the cell materials. This degradation process is a combination of catabolic and anabolic processes (Brock et al. 1994). Cometabolism is another mode of degradation, which is observed in the degradation of hydrocarbons. In cometabolism the hydrocarbon is transformed, but the organisms does not gain any energy or nutrients (Field et al. 1991, Juhasz et al. 1996).

The specific degradation mechanisms are determined by the compound structure. Linear alkanes degrade through  $\beta$ -oxidation in which the backbone is broken up two carbons at a time and the resulting acetyl-CoA is mineralized in the TCA cycle. Some cyclic alkanes degrade through cometabolism (Juhasz et al. 1996). Aromatic compounds are generally degraded via a dioxygenase enzyme, which converts the compound to a catechol followed by ring fission in the ortho or meta positions (Prince 1993).

The degradation mechanism as well as its rate and extent are determined by a number of factors. These factors are: the chemical structure of hydrocarbons, the presence and capabilities of organisms, the presence of nutrients, the presence of electron acceptor, and

the physical availability of the substrate hydrocarbon to appropriate organisms (Huesemann 1995, Brock et al. 1994).

### **C. Effect Of Chemical Structure On Biodegradation**

Hydrocarbon biodegradation is dependent on the chemical structure of the compound or compound mixture. Riis et al. (1995) studied degradation of hydrocarbons from refinery products and soil in a liquid laboratory environment. They showed that over 95 percent of hydrocarbons with boiling points of 220°C to 400°C degraded in 42 days, while only 58 to 62 percent of oils with boiling points from 310°C to 570°C degraded in the same time period.

These results were confirmed by Chaineau et al. (1995) in a soil microcosm study. They showed that the hydrocarbon concentration decreased logarithmically over the 270 days of the experiment. There was a 95 percent reduction in the concentration of straight and branched alkanes, a 60 to 70 percent reduction in saturated and aromatic compounds, and no significant reduction in the resin fraction compounds.

A soil study by Huesemann (1995) achieved a 90 percent removal of straight and branched alkanes, and monocyclic saturated compounds in aerobic soil and slurry reactors. The study also obtained 25 to 50 percent removal of dicyclic and tricyclic saturated compounds. Tetracyclic and pentacyclic compounds did not degrade significantly in the 30-week scope of the study.

Berwick (1984) performed a study in a water environment with three *Pseudomonas* species and found that aliphatic and alicyclic compounds were degraded preferentially over single ring aromatics. Monocyclic aromatics were degraded before polycyclic aromatics, which were degraded before asphaltines.

A more detail study of aromatic hydrocarbon degradation was performed by Elmendorf et al. (1994). This study showed that naphthalene and its derivatives degraded before fluorenes, which degraded before dibenzothiophenes. Dibenzothiophenes degraded before phenanthrenes, which in turn degraded before chrysenes. In addition, parent compounds were degraded faster than their alkylated derivatives up to a substitution of four

methyl groups. Compounds with larger substitution were degraded through the side chain and did not follow the above-described order (Prince 1993).

The above studies were performed with a single substrate. The degradation of hydrocarbon mixture is a more complex process. Riss et al. (1995) observed that biodegradation of hydrocarbon mixtures stopped when the hydrocarbon concentration reached 10 to 40 percent of the original hydrocarbon concentration.

In conclusion the larger and more complex the compound, the slower and more complex the degradation. The individual degradation mechanisms depend on both the organism and environmental conditions (Prince 1993).

#### **D. Effect Of Organisms On Biodegradation**

Since hydrocarbons occur naturally in low concentrations, a variety of hydrocarbon degrading organisms are present in soil, marine, and fresh water (Huesemann 1995, Rombelarisoa et al. 1984, Geerdink et al. 1996). They include bacteria, fungi, and yeast, as well as cyanobacteria and algae (Ratledge 1978, Trudgill 1978, Prince 1993, Vrdoljak et al. 1992, and Brock et al. 1994). Bacterial hydrocarbon degraders isolated from soil using enrichment techniques included *Pseudomonas*, *Arthrobacter*, *Nocardia*, and *Rhodococcus* species (Rittmann and Johnson 1989, Smithers 1997).

Following an introduction of high hydrocarbon concentrations into these environments (for example, following a spill), the degrading organisms grow and become a dominant fraction of the population (Lindstorm et al. 1991, Venkateswaran and Harayama 1995). However, the results of organism growth and hydrocarbon degradation processes are not observed immediately. The organisms need time to adjust to the new environmental conditions and to develop a significant population resistant to any toxic effects of highly concentrated hydrocarbons (Benoit and Love 1996, Love and Hoehn 1996). This time period when no changes in hydrocarbon concentration due to microbial activity can be measured is called the lag period. The lag varies depending on organism and hydrocarbon type and the initial concentration of both (Jusz et al. 1996).

In order to shorten the lag period, known hydrocarbon-degrading organisms are added to the contaminated matrix. The effects of organism addition vary depending on organism type and environmental conditions including the growth substrate.

In a laboratory study, Jahasz et al. (1996) showed that an addition of a pure culture grown on three ring PAHs to a mix of the same three ring PAHs resulted in fast and extensive degradation. However, addition of the same organisms to four, five, and six ring compounds resulted in long lag periods and less extensive degradation. Rittmann and Johnson (1989) showed that addition of mixed organisms grown in a laboratory on lubricating oil resulted in an increase of degradation rates of the same lubricating oil in contaminated soil.

Commercially produced organisms did not meet with the same success. Moller et al. (1995) observed that addition of commercial bioaugmentation products retarded diesel oil degradation in soil microcosms. The authors reasoned that the commercial product contained a carrier material, which provided an alternative easily biodegradable source of carbon for the organisms. Only after the carrier was degraded did the organisms turned to oil degradation.

Similar results were obtained by Vendosa et al. (1992a) who showed that only two out of ten commercial microbiological products showed potential for improving biodegradation in a laboratory study. In a follow up field study, Vendosa et al. (1992b) showed that addition of the previously chosen commercially-produced microorganisms did not produce statistically significant enhancement over the endogenous population in biodegradation of crude oil in the Prince William Sound.

In conclusion, the addition of organisms produces positive results only when the organisms were grown on a chemically similar substrate in similar environmental conditions, as long as no alternative growth medium is provided.

#### **E. Effect Of Nutrients On Biodegradation**

All microorganisms require a variety of nutrients to live and grow. The most significant nutrients are carbon, hydrogen, nitrogen, and oxygen. Other micronutrients, include phosphorus, calcium, magnesium, potassium, sulfur, sodium, and iron.

Micronutrients, needed in trace amounts, include zinc, manganese, copper, molybdenum, cobalt, nickel, tungsten, and selenium (Brock et al. 1994).

Hydrocarbons are able to provide only carbon and hydrogen, the remaining nutrients must be obtained from the environment for organism growth to occur. A number of studies indicated that lack of nutrients was responsible for slow degradation rates of hydrocarbons (Collwell 1978 in Venosa et al. 1992, Foght and Westlake 1982).

In the previously mentioned study by Moller et al. (1995), the addition of nutrients alone resulted in an increase in degradation rates. The same results were obtained by Venosa et al. (1992b). The addition of commercially produced nutrients to the endogenous population increased the rate and extent of oil degradation. Bragg et al. (1994) showed that addition of nutrients in a form of liquid or granular fertilizer increased oxygen uptake rates by microorganisms and decreased oil concentration on the beaches affected by the Exxon Valdez spill. Rosenberg et al. (1996) who used an insoluble nutrient source to stimulate biodegradation of hydrocarbons confirmed these results.

Foght and Westlake (1982) showed that the presence of alternative carbon sources retarded the degradation of hydrocarbons. The absence of nitrogen and phosphate nutrient addition significantly extended the retardation time and reduced the extent of Prudhoe Bay oil degradation.

In conclusion, the presence of macro and micronutrients is essential to successful biodegradation of hydrocarbons and their addition to nutrient poor environments has been shown to increase biodegradation rates in many cases.

#### **F. Effect Of Electron Acceptor On Biodegradation**

Hydrocarbons are oxidized during the degradation process. Their counterpart, the reduced compound is oxygen in aerobic metabolism, nitrogen gas (N<sub>2</sub>) in denitrifying metabolism and iron, sulfate, carbonate, and others in anaerobic metabolism (Benoit and Love 1996, Love and Hoehn 1996).

Aerobic degradation has many advantages: it is faster than the other types and results in nontoxic products of carbon dioxide and water (Prince, 1993). Huesemann et al. (1993) showed that polynuclear aromatic compounds degraded faster in oxygen sparged than in air

sparged laboratory reactors. The degradation in nitrogen sparged reactors was slow and insignificant. Hupe et al. (1995) showed that low oxygen concentration retarded degradation in petroleum contaminated soil in mixed laboratory reactors. Anaerobic degradation is very slow and not able to degrade all compounds and the possibility of toxic product creation is higher (Prince 1993, Benoit and Love 1996). Anaerobic degradation is a problem in long term storage of aircraft fuels (Hill et al. 1978).

In conclusion, there are a number of advantages to using oxygen as an electron acceptor. Aerobic degradation results in higher degradation rates and lower product toxicity.

### **G. Effect Of Hydrocarbon Availability On Biodegradation**

Bioavailable compounds are ones the microorganisms are capable of transporting through the membrane and degrading. These compounds are usually dissolved in water or attached to the organism (Guha and Jaffe 1996, Ogram 1985 in Cornelissen et al. 1998). However, hydrocarbons are nonpolar compounds, and have low solubility in water and are easily adsorbed onto soil materials, making them not bioavailable (Guha and Jaffe 1996).

However, this phenomena has the advantage of buffering potential toxic effects of hydrocarbons. The disadvantages are an insufficient concentration to sustain bacterial growth at low concentrations and extensive bioremediation times at high concentrations (Guha and Jaffe 1996, MacDonald and Kavanaugh 1994).

These conclusions were confirmed by Cornelissen et al. (1998) who studied the relationship between PAH biodegradation and desorption in contaminated soils. They also observed that the PAH desorption took place in two stages. First rapid desorption took place in the first 20 to 50 hours then slow desorption took place in the remaining 300 hours. The rapidly desorbing two to four ring PAHs were degraded preferentially over the slow desorbing two to four ring PAHs. This degradation process was limited by the bioavailability of the compounds. The rapidly desorbing five to six ring PAHs were not degraded to a significant extent. This degradation process was limited by the microbial limitations described in the previous section.

Many organisms overcome the solubility barrier by producing surfactant molecules, which will be discussed in the following section.

## H. Effect Of Biosurfactant On Biodegradation

### 1. Biosurfactants properties and characteristics

Bacteria, fungi, and yeast (Vrdoljak et al. 1992) produce biosurfactants. They are amphiphilic compounds containing distinct hydrophilic and hydrophobic domains produced by microorganisms. The hydrophilic group can be charged positively or negatively, it can be non-ionic, or it can be amphoteric. This group consists of hydrophilic components such as saccharide, a carboxylic acid, an amino acid, or a peptide. The hydrophobic group is a saturated, unsaturated, or hydroxylated fatty acid (Georgiou et al. 1992, Lin 1996).

Depending on the hydrophobic and hydrophilic components, biosurfactant are divided into four groups. These groups are: the glycolipids containing a carbohydrate and a lipid, the phospholipids containing phosphate and alcohol substituted lipid, the lipopeptides and lipoproteins containing polypeptide and lipid, and the polymeric containing saccharide and fatty acids arranged in a polymeric structure (Healy et al. 1996). In general, biosurfactants are considered to be secondary metabolites, but they play an essential role in survival of organisms growing on low solubility substrates such as hydrocarbons (Lin 1996).

Koch et al. (1991) compared the growth of a *Pseudomonas* organism able to produce biosurfactant to the growth of a *Pseudomonas* mutant not able to produce biosurfactant. They found that the mutant organism was unable to grow on hexadecane as the sole carbon source and that growth was stimulated by the addition of purified biosurfactant produced by the original *Pseudomonas* organism.

Biosurfactants are produced on both soluble and insoluble substrates, however Falatko and Novak (1992) showed that biosurfactants are substrate specific. A biosurfactant produced on glucose in a laboratory reactor did not increase solubility of gasoline and retarded its degradation. A biosurfactant produced on gasoline had increased solubility and did not retard degradation. Also, Jain et al. (1992) found that biosurfactant enhanced degradation of low solubility hydrocarbons such as hexadecane and pristane, but did not enhance the degradation of water soluble 2-methylnaphthalene.

## 2. Performance of synthetic and biological surfactants

The main purpose of a surfactant in hydrocarbon degradation is to increase the solubility and bioavailability of the hydrocarbon. When added to the solution, the surfactant molecule achieves a solubility increase by mediating the oil water interface. The hydrophobic end aligns with the hydrocarbons, while the hydrophilic end of the molecules aligns with water forming a micelle. The micelles are solubilized in water in a form of microemulsion (Georgiou et al. 1992, Lin 1996, Guha and Jaffe 1996). The increase in solubility allows for faster in-situ bioremediation as well as more efficient pump and treat systems (Fountain et al. 1991).

The advantages of using biosurfactants over synthetic surfactants include diversity, biodegradability, resistance to environmental changes, and low production costs (Rocha and Infante 1997). The main disadvantages are the high recovery and purification costs due to low product yields and a potential antibiotic effect of some powerful biosurfactants (Nakano et al. 1988).

Synthetic surfactants are often not successful in bioremediation applications (Prince 1993). Foght and Westlake (1982) studied the effect of synthetic surfactant addition on degradation of crude oil. The authors reasoned that the surfactant provided an alternate carbon source for the organisms and retarded the degradation of crude oil. Shreve et al. (1995) showed that the purified biosurfactant was nine times as effective in restoring mutant *Pseudomonas* organism growth as the synthetic anionic surfactant by producing a better microemulsion. The higher solubility results were confirmed by Kanga et al. (1997) who worked with a *Rhodococcus* species. This study also, found that biosurfactant had lower toxicity to the organisms.

## 3. Role of biosurfactant in biodegradation

Although many studies showed the benefits of biosurfactants, the exact mechanism by which biosurfactant amplifies biodegradation is not entirely understood. Research has shown that the solubility increase alone does not account for the increase in growth of organisms.

Shreve et al. (1995) worked with wild type *Pseudomonas*, which was able to produce biosurfactant and mutant *Pseudomonas*, which was not able to produce biosurfactant. In this

study, the mutant species was provided with the biosurfactant produced by the wild species. The authors discovered that the wild type was able to grow five times faster than the mutant and concluded that biosurfactant produced by the cell remains attached to the cellular membranes increasing the cell hydrophobicity and allowing the cell to attach to the hexadecane. The attachment greatly improves the organism's ability to transport hexadecane into the cell and it was not observed when the biosurfactant or the synthetic surfactant was added to the mutant organisms. The attachment of biosurfactants to the producing cell was confirmed by Phale et al. (1995) who worked with a different *Pseudomonas* species and by Goclik et al. (1990) who worked with a *Rhodococcus* species.

Despite the lack of attachment, the increase in solubility alone has positive impact on the degradation rates. Deziel et al. (1996) worked with soil *Pseudomonas* species, which produced biosurfactants and grew on naphthalene and phenanthrene. They showed that the lag period was reduced by the addition of biosurfactants produced by the same species of *Pseudomonas* and that the appearance of biosurfactant coincided with an increase in solubility of the studied PAHs.

Oberbremer and Muller-Hurtig (1990) found that the addition of biosurfactant to a culture growing on a mixture of soil and hydrocarbons increased the rate of degradation and the rate of organism growth. The biosurfactants were degraded after the hydrocarbons were consumed (Rocha and Infante 1997).

Bruheim et al. (1997) in his study with *Rhodococcus* species found that the bacteria degraded soluble compounds before engaging in biosurfactant production. Biosurfactants were produced in the stationary growth phase and remained attached to the cell membrane, as the low solubility compounds were degraded. After the low solubility compounds were consumed the organisms turned to biosurfactant degradation. Oberbremer and Muller-Hurtig (1989) obtained the same results in an earlier study.

In conclusion, biosurfactants improve biodegradation of low solubility hydrocarbons. Biosurfactants are produced only when no low solubility hydrocarbon substrates are available and the surfactants adapt to a specific substrate.

### III. MATERIALS AND METHODS

#### A. Experimental Approach

The major goal of this project was to determine if aerobic microbial growth could produce a significant waste oil volume reduction. The concentrated sludge from the two sources used in this study is referred to as waste oil #1 and waste oil #2. The two aspects of volume reduction, oil degradation and bio-solids water separation were considered separately for the purpose of this laboratory study.

Oil degradation studies evaluated the extent and rate of microbial degradation, compared the performance of native and introduced bacterial species, and investigated the role of the biosurfactants in hydrocarbon degradation. Bio-solids and water separation studies evaluated changes in the sludge dewatering characteristics of the waste oil over time.

To encourage waste oil degradation, favorable conditions for microbial growth were created. The waste was diluted to reduce the toxic effects of highly concentrated hydrocarbons. Salts, buffers, and air were added to provide nutrients, favorable pH, and oxygen. To measure the reduction in oil, the samples were analyzed using a gas chromatograph (GC) to evaluate the amount of methylene chloride extracted oil, chemical oxygen demand (COD) test to evaluate the amount of organic material present, and suspended solids to evaluate amount of immiscible oil and organisms.

To compare the performance of native and introduced organisms, two reactor types were compared. The first reactor contained waste oil, dilution water, nutrients, and indigenous (or native) organisms. The second reactor type contained waste oil, dilution water, nutrients, indigenous organisms, and introduced organisms. The introduced organisms were a *Rhodococcus* species, or a mixture of pure cultures, or a soil organism mixture. The microorganism cultures are described later in this chapter.

To investigate the role of biosurfactants in hydrocarbon degradation, the surface tension and polysaccharide concentration were measured over time. Lowered surface tension was an indication of biosurfactant presence. Polysaccharides were used as an indication of organism growth and biosurfactant production.

To study water removal from the oily sludge, the dewatering properties of oily waste were investigated over time. The parameters used were capillary suction time (CST) and specific resistance. CST values gave an indication of the rate of dewatering. Specific resistance values also gave an indication of the rate of dewatering normalized for the solids content in the sample (APHA 1995).

**B. Waste Oil**

Waste oil #1 and waste oil #2 were the only waste oil sources used in this series of experiments. The two waste oils were derived from the spill over tanks of the two DAF units in the railroad facility. These sources differed in composition and properties.

Waste oil #1 was characterized by easy oil/water separation since; the oil was present in particulate or globular form. The waste oil #2 was characterized by a lack of oil water separation since; the oil was present primarily in a soluble or emulsified form and in large solid particles. The two sources were diluted with deionized water to obtain the COD and suspended solids values shown in Table 1.

Table 1. Characteristics of a 20 percent solution of the two waste oil sources.

	Mass fraction of methylene chloride extracted oil	Suspended solids (mg/L)	Experimental COD* (mg/L)	Calculated COD* (mg/L)
Oil source #1	small	8,300	42,800	82,100
Oil source #2	large	14,000	52,200	255,000

\* for the details on experimental and calculated COD values refer to analytical methods section

In both sources, the oil was present in three phases: dissolved oil, suspended oil in globular form, and attached solid oil on the surfaces of the reactor vessel. The waste oil division into these three phases led to variability in experimental data, since the partitioning between phases was a function of degradation time, mixing, temperature, and biosurfactant presence. This phase separation added to the heterogeneous nature of the waste oil and

caused difficulty in evaluating experimental parameters. Each of the solutions produced for this study exhibited different characteristics as measured by the experimental parameters discussed in the analytical methods section.

### C. Nutrients

The amount of nutrients added into the reactors was based on the need for projected microbial growth. The projected microbial growth was calculated using a conservative assumed growth yield of 0.6 COD units of organisms per COD unit of organic matter. This projected microbial COD value, which was used for nutrient calculations, assumed complete oil degradation.

Table 2. Nutrient requirements for microbial growth.

Nutrient	Approximate requirement ( $\mu\text{m}/\text{mg}$ formed biomass COD)	Nutrient added as	Stock solution
Nitrogen	87.2	$\text{NH}_4\text{Cl}$	Ammonium
Phosphorous	17.4	$\text{KH}_2\text{PO}_4$	phosphate
Potassium	10	$\text{KH}_2\text{PO}_4$	phosphate
Calcium	10	$\text{CaCl}_2$	chloride
Magnesium	7	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	sulfate
Sulfur	6	all compounds with S	sulfate
Chloride	3	all compounds with Cl	chloride
Sodium	3	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	sulfate
Iron	2	$\text{FeCl}_3$	chloride
Zinc	0.2	$\text{ZnCl}_2$	chloride
Manganese	0.1	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	sulfate
Copper	0.02	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	chloride
Molybdenum	0.004	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	sulfate
Cobalt	<0.0004	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	chloride

The nitrogen and phosphorous requirement was calculated based on the chemical formula for bacteria. The amount of micronutrients needed for projected bacterial COD were adopted from Grady and Daigger (1998). These nutrients were added in the form of four stock solutions: ammonium, phosphate, chloride, and sulfate solutions. The chloride stock solution had a pH value below 2 to prevent precipitation. The pH change was

accomplished through the addition of HCl. The nutrient requirements are summarized in Table 2. In addition to growth nutrients, phosphate buffer was added to maintain the pH of the solution at approximately 7.2.

#### D. Organisms

The organisms utilized in this experiment included native organisms and introduced organisms. The native organisms were the indigenous organisms in the waste. Their presence was confirmed by a microscopic examination and polysaccharide measurement. Introduced organisms were grown independently of the waste oil and added to the waste oil reactor prior to the experiment. Three types of organisms were introduced to the waste oil: *Rhodococcus* species, mixture of pure cultures, and soil organisms.

Table 3. Types and growth conditions of the introduced organisms.

Organism type	Species designation	Growth substrate	Substrate concentration	Nutrient solution
Rhodococcus	<i>Rhodococcus</i>	diesel fuel	500 ppm	sterile
Pure culture mixture	A1	diesel fuel	500 ppm	sterile
	B2	diesel fuel	500 ppm	sterile
	C3	diesel fuel	500 ppm	sterile
	D4	diesel fuel	500 ppm	sterile
	N5	naphthalene	200 ppm	sterile
	H3	anthracene	300 ppm	sterile
	<i>Rhodococcus</i>	diesel fuel	500 ppm	sterile
Soil culture	N/A	diesel fuel motor oil	500 ppm	non-sterile

The *Rhodococcus* species bacterium was a known hydrocarbon degrader and biosurfactant producer. The mixture of pure culture organisms contained a mixture of known hydrocarbon degraders supplied by Sybron Chemical Co. Each component of the mixture was grown as pure culture and combined with others before addition to the reactors. The soil culture organisms were a robust consortium of soil organisms supplied by Biosystems Inc. These organisms, originally present in soil, were grown as a mixture. The organisms and their growth conditions are summarized in Table 3.

## **E. Biological Treatment Studies**

The study of waste oil biodegradation consisted of four biological experiments.

These are described as follows:

- Aerated batch experiments compared the oil degradation by different organisms at a concentration of 10 percent of oil.
- Filtered oil experiments contributed to the understanding of oil degradation and organism growth without interference of solid oil particles in the reactor.
- Sacrificial batch experiments allowed for quantification of the oil attached to the walls of the reactors at concentrations of 10 and 20 percent oil.
- Dewatering experiments gave the opportunity to correlate the oil degradation and dewatering parameters.

All of the above reactors contained active microbial cultures. There was no abiotic control in this series of experiments.

### **1. Aerated batch experiment**

The goals of this experiment were to study oil degradation, biosurfactant production, and to compare the performance of the native and introduced organisms. In this experiment, each of the nine reactors used was prepared separately. Approximately 5 percent of waste oil from each source by weight was combined with deionized water, and nutrients. The nutrient and buffer demands were calculated based on a projected biomass COD of 3780 mg/L. This value was based on an experimental COD value for a 10 percent solution.

The bioaugmented reactors received 100 ml of organisms in their appropriate growth solutions. The reactor with indigenous organisms received 100 ml of additional dilution water. The reactor compositions are summarized in Table 4.

The reactors were mixed by a 60-rpm mixer and aerated for 24 hours before the first sample was taken. The liquid portion samples of the reaction mixture were taken every seven days. The solid oil attached to the reactor walls was not sampled. The reactor setup and division of oil into the three phases are illustrated in Figure 1.

Table 4. Composition of ten percent aerated batch reactors.

Reactor name	C	W	P	M1	M2	M3	M	Rh2	Rh1
Oil #1 (gr)	36.14	35.48	35.64	36.05	35.54	36.56	36.18	35.84	35.61
Oil #2 (gr)	39.57	35.80	35.94	35.97	35.59	35.62	35.72	35.45	35.57
Water (gr)	584.22	484.21	484.12	484.19	484.34	484.05	484.29	484.26	484.33
Organism type	none	soil culture	pure culture mix	pure culture mix	Pure Culture Mix	pure culture mix	pure culture mix	<i>Rhod-coccus</i>	<i>Rhod-coccus</i>
Organisms (ml)	N/A	100	100	100	100	100	100	100	100
Nutrients (ml)	40	40	40	40	40	40	40	40	40
Buffer (ml)	10	10	10	10	10	10	10	10	10

## 2. Filtered oil experiment

The goals of this experiment were to observe the degradation of oil, growth of organisms, and biosurfactant production without the interference of suspended and solid oil. The presence of the suspended and attached oil affected the measurements taken in the 10 percent aerobic batch experiment and made it difficult to draw conclusion regarding oil degradation and biosurfactant behavior.

In this experiment, 5 percent from each of the waste oil sources by weight was combined with deionized water and mixed vigorously for 24 hours allowing oil to enter the solution. The oil water mixture was filtered through a 250  $\mu\text{m}$  filter (Whatman #4 filter) to remove globular oil and retain soluble oil, small suspended oil particles, and organisms in solution. The filtrate was combined with nutrients and buffers, based on a biomass COD of

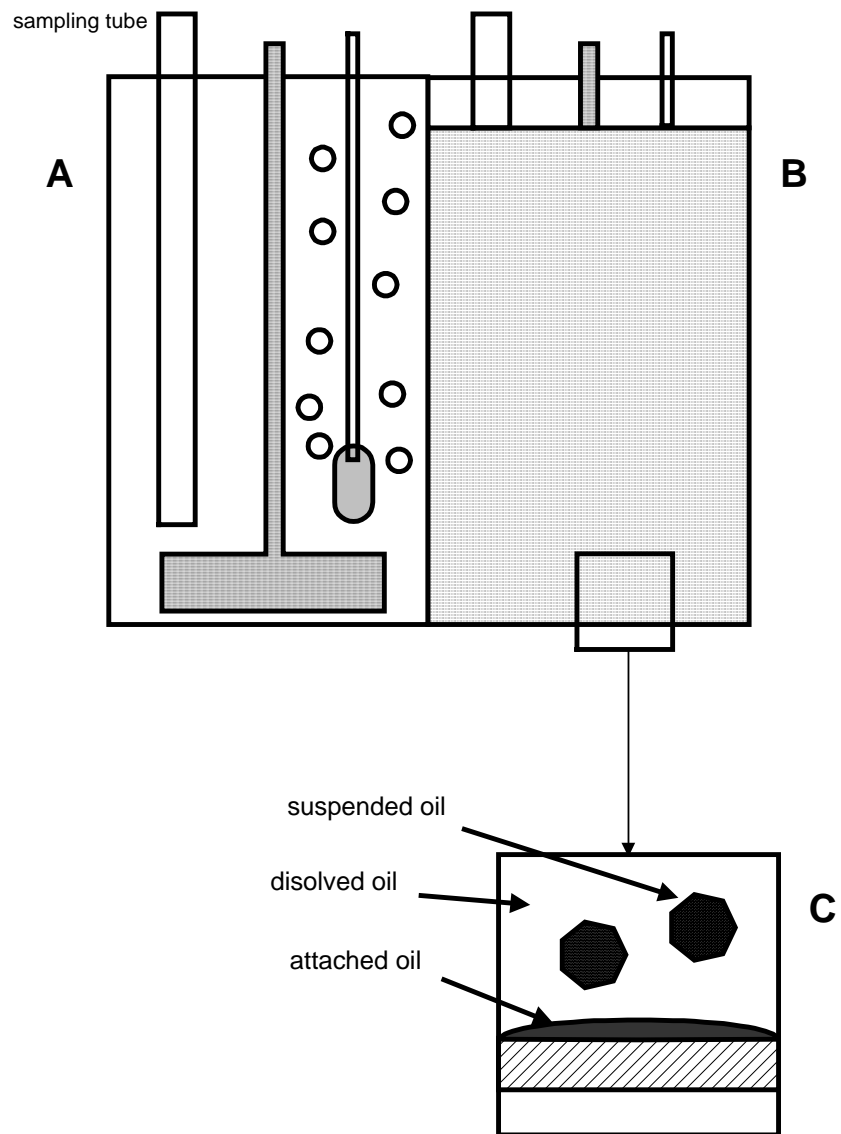


Figure 1. Experimental setup for the aerated batch reactor experiment: a) empty reactor, b) filled reactor, and c) schematic of oil phase separation.

500 mg/L, and mix of pure culture organisms, described in the previous section. The amounts of each of the components are summarized in Table 5 and the preparation is illustrated in Figure 2.

Table 5. Composition of filtered oil reactors.

	Trial one	Trial two
Filtered oil (gr)	789.02	805.10
Deionized water (gr)	263.15	61.64
Pure culture mixture (gr)	100.00	100.45
Salt stock solutions (gr)	21.60	30.97

The total experimental solution volume was approximately one liter. This solution was mixed on a stir bar mixer and aerated. Samples were taken every two or three days for the initial 2 weeks. After 2 weeks the samples were taken based on changes in surface tension values.

### 3. Ten percent sacrificial batch experiment

The goals of this experiment were to observe degradation of oil, organism growth, biosurfactant production, compare the performance of native and introduced organisms, and quantify the attached waste oil.

In this experiment, 10 percent of the waste oil from a given source was mixed with deionized water and nutrients. The nutrients were added based on the projected organism growth of 7,200 mg/L COD for the waste oil #1 mixture and 15,600 mg/L COD for the waste oil #2 mixture. Fifty-four milliliters of a given mixture were dispensed into a 120-ml bottle. Half of the bottles for each of the waste oil source received 6 ml of pure culture organism mixture, while the other half received the same amount of dilution water.

The bottles were closed and mixed on a shaker table. Each bottle was opened twice daily for the first week and daily for the rest of the experiment to allow for aeration. The first set of bottles was analyzed after 5 hours of mixing. Each set included two subsets with waste oil #1 with and without introduced organisms and two subsets with waste oil #2 with and without introduced organisms. The sampling process of reactor suspension, supernatant and attached oil is diagrammed in Figure 3. The suspension and supernatant were sampled

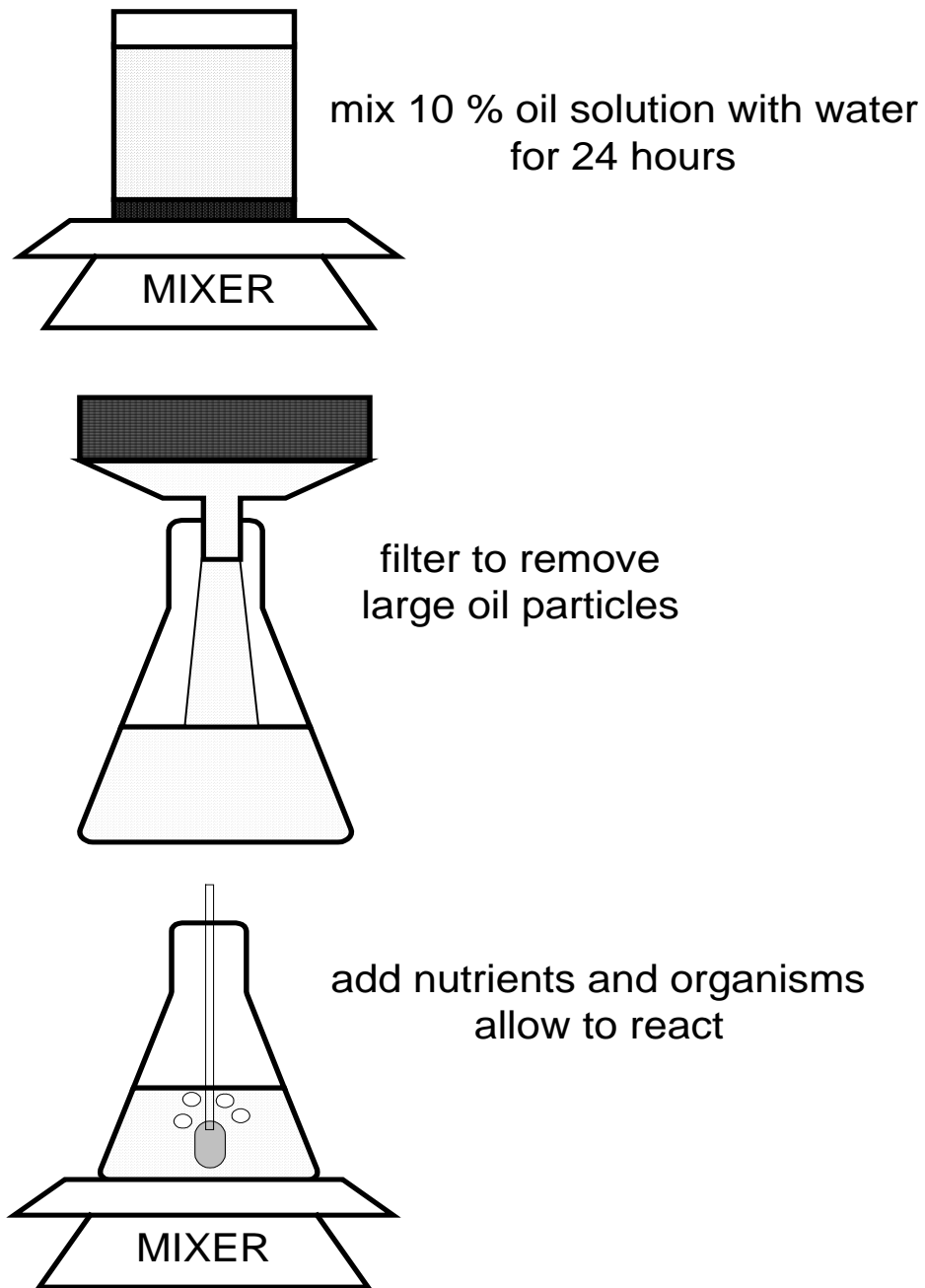


Figure 2. Experimental setup and reactor preparation for the filtered oil reactor experiment.

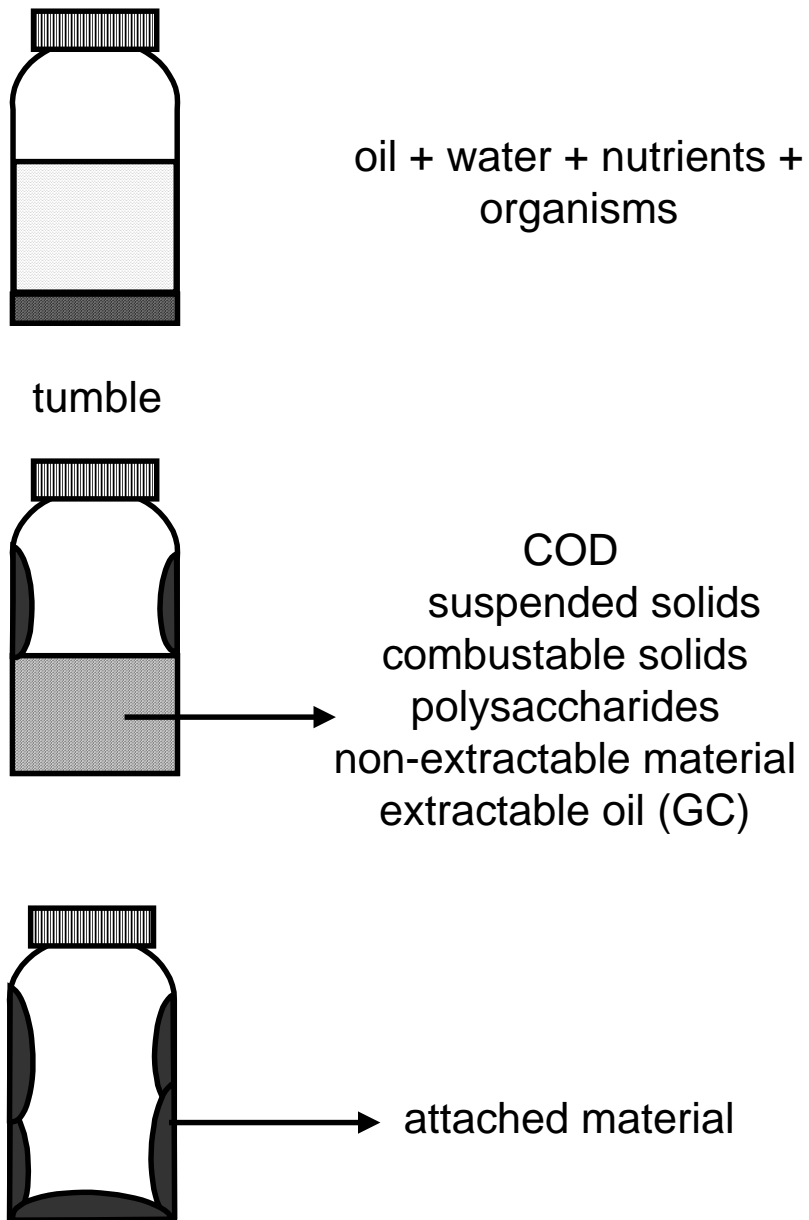


Figure 3. Experimental and measurement setup for sacrificial batch reactor experiment.

and analyzed to evaluate oil degradation and organisms growth. The attached oil was extracted using methylene chloride and evaluated in terms of mass.

#### 4. Twenty percent sacrificial batch experiment

The goals of this experiment were to observe degradation of oil, organism growth, biosurfactant production, compare the performance of native and introduced organisms, and quantify the attached oil at a higher oil concentration.

The experimental setup and procedure for this experiment was the same as the 10 percent waste oil in bottle reactor experiment described in the previous section. However, in this experiment, each of the reactors in the six subsets was 100-ml bottles with 60 ml of reaction mixture. Waste oil #1 set had a subset of reactors with and without introduced organisms. The same was true for waste oil #2 set and the 50/50 mixture of waste oil #1 and #2 set.

#### 5. Dewatering experiment

The goals of this experiment were to observe the changes in dewatering properties of the oily sludge over time and to determine the relationship between oil degradation, organism growth, and dewatering.

In this experiment, 10 percent of the waste oil from a given source was mixed with deionized water, nutrients, and introduced organisms in a 3 liter reactor. The nutrients were added based on a projected organism growth of 7200 mg/L COD for the waste oil #1 reactor and 15600 mg/L COD for the waste oil #2 reactor. Also, 160 milliliters of pure culture organism mixture were added to both reactors. The reactors were mixed with a paddle mixer propelled by a 60-rm. motor and aerated. Samples of the liquid portion of the reaction mixture were taken approximately every 2 weeks and both filtered and unfiltered samples were analyzed.

## **F. Analytical Methods**

### 1. COD measurements

The COD test was performed to measure the mass of organic material in the samples including waste oil and organisms. A decrease in COD was interpreted as a loss of waste oil. The COD test was performed according to the procedure described in Standard Methods (APHA 1995).

Five milliliter of diluted sample was combined with three milliliters of chromium digestion reagent and seven milliliters of sulfuric acid and  $\text{AgSO}_4$  in a glass tube. The tubes were placed on a heating block and cooked at  $150^\circ\text{C}$  for 2 hours. All of the samples were tested in triplicate. Each time the COD was measured an additional sample was prepared to test the recovery of a potassium hydrogen phthalate standard. The standard recovery test was performed for the aerated batch reactors and sacrificial batch reactors with a randomly selected sample.

During the initial experiments, problems with the COD test of the waste oil solutions were discovered. A potassium hydrogen phthalate standard was used to evaluate the COD tests effectiveness. The standard's recovery was 10 percent or lower due to incomplete digestion of oil. In addition the effectiveness was influenced by test conditions. The measured COD value increased with an increase in dilution of the sample and/or digestion time.

To minimize the variability in the measurements, the samples were diluted to obtain values within the test range and the same dilution was used for the entire experiment. The hot and cold blanks contained deionized water and nutrient solutions as they appeared in the reactor at the beginning of the experiment.

In an attempt to obtain the true COD values of the waste oil solutions, the "correct" COD values were measured through serial dilution oil. A two- percent mixture of each waste oil source was made by distilled water dilution. The solutions were then serially diluted from 1 to 10 up to 1 to 1250 for waste oil #1 and from 1 to 10 to 1 to 3125 for waste oil #2.

The resulting COD values were plotted against the dilution used to obtain the “correct” COD value. The “correct” COD were determined to be the values where the COD was approximately constant over a range of dilution. The process is shown in Figure 4 and the data can be found in the Appendix A. The “correct” or calculated values were considered to be the true COD values for the purpose of this study.

The findings in the “correct” COD value calculations suggested that the incomplete digestion in the experimental COD values was due to insufficient dilution of the waste oil samples. The experimental COD values corresponded to the shaded dilution region in Figure 4.

## 2. Suspended solids measurements

The suspended solids test was performed to measure the amount of waste oil and organisms in the samples. A decrease in suspended solids was interpreted as a loss of waste oil and an increase in suspended solids was interpreted as an increase in biomass or oil concentration. The measurements were performed in duplicate and were not subject to interference from the oil.

Suspended solids were performed in accordance with the procedure described in Standard Methods (APHA 1995). A known amount of sample was filtered through a previously weighed 1.5  $\mu\text{m}$  glass fiber filter (Whatman 934-AH). The filter was then dried for at least an hour in a 100°C drying oven and weighed again. The mass of the residual on the filter was the mass of the suspended solids in the filtered volume.

## 3. Methylene chloride extraction

An extraction process was used to prepare samples for GC analysis. Three different solvents were tested for extraction efficiency: hexane, methylene chloride, and 50/50 mixture of methylene chloride/methanol. The methylene chloride extraction resulted in a largest GC response and it was used throughout the study.

The sample and methylene chloride at a 1 to 1 weight ratio were placed in a 40 ml centrifuge tubes and tumbled for 24 hours. The samples were then centrifuged in a Beckman J-21C centrifuge for 10 minutes at about 500 g to separate the water and methylene chloride layers. The sample to methylene chloride weight ratio was modified to 1 to 2 for the 20

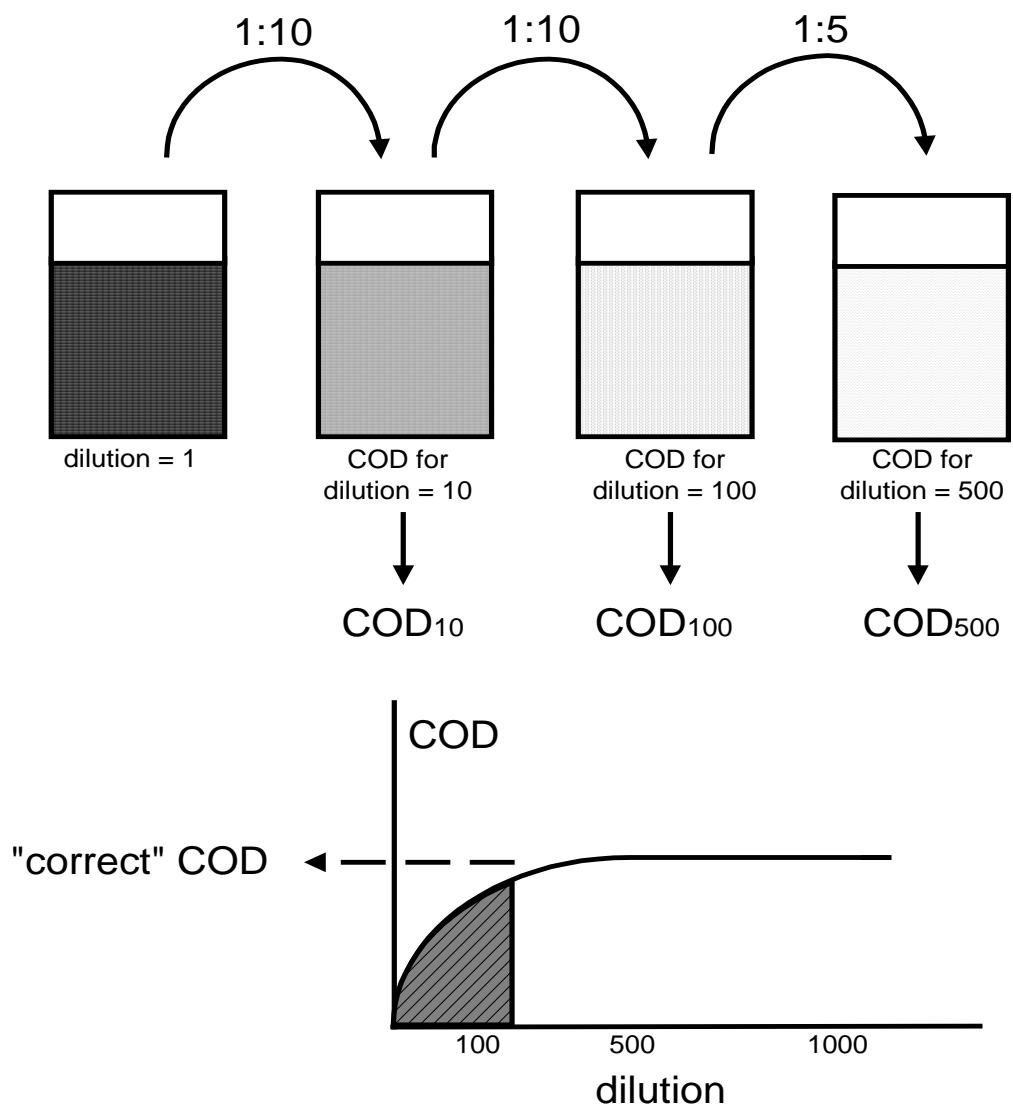


Figure 4. Schematic of "correct" COD values determination process. The shaded area indicates the dilution range for experimental COD values.

percent oil experiment and to 4 to 1 for the filtered oil experiment.

The water and associated non-extractable material layer were collected from the top of the centrifuged sample. This non-extractable material was removed, dried, and quantified in terms of nonextractable mass per total sample mass. The methylene chloride and extracted oil layer were collected from the bottom of the centrifuged sample. This material was analyzed on the GC. The extraction process is diagrammed in Figure 5.

#### 4. GC analysis

The GC analysis was performed to quantify changes in hydrocarbon concentration over time. The methylene chloride extracted oil was analyzed using a Hewlett Packard 5890A series chromatographer with a flame ionization detector (FID). The column was a 30-m long J&W Scientific DB-5 MS fused silica capillary column with an internal diameter of 0.25 mm with a 0.25  $\mu\text{m}$  film thickness.

A splitless injection of 1 $\mu\text{L}$  and helium carrier gas flow of 1.05 ml/min to 1.08 ml/min was used. For the filtered oil experiment, the samples were concentrated by evaporation to 0.5 ml and 2  $\mu\text{L}$  injection was used, due to the low oil concentration in samples. The injection port and detector were maintained at 300°C. The temperature program started at 70°C for 4 min, and then the temperature was increased to 300°C at rate of 7°/min where it was held for 3 min. The temperature was farther increased to 325°C at rate of 10°C/min for 5 min for a total analysis time of 47.37 min.

The known extractable oil concentration samples were prepared using known amounts of methylene chloride and extractable oil from both waste oil sources and analyzed on the GC. A calibration curve of concentration versus total GC response curve was created. The equations for each of the waste oil sources were:

$$\text{concentration}(mg / gr) = \frac{\text{response}}{1148974}$$

for waste oil #1, with  $R^2 = 0.78$

$$\text{concentration}(mg / gr) = \frac{\text{response}}{1454170}$$

for waste oil #2, with  $R^2 = 0.90$

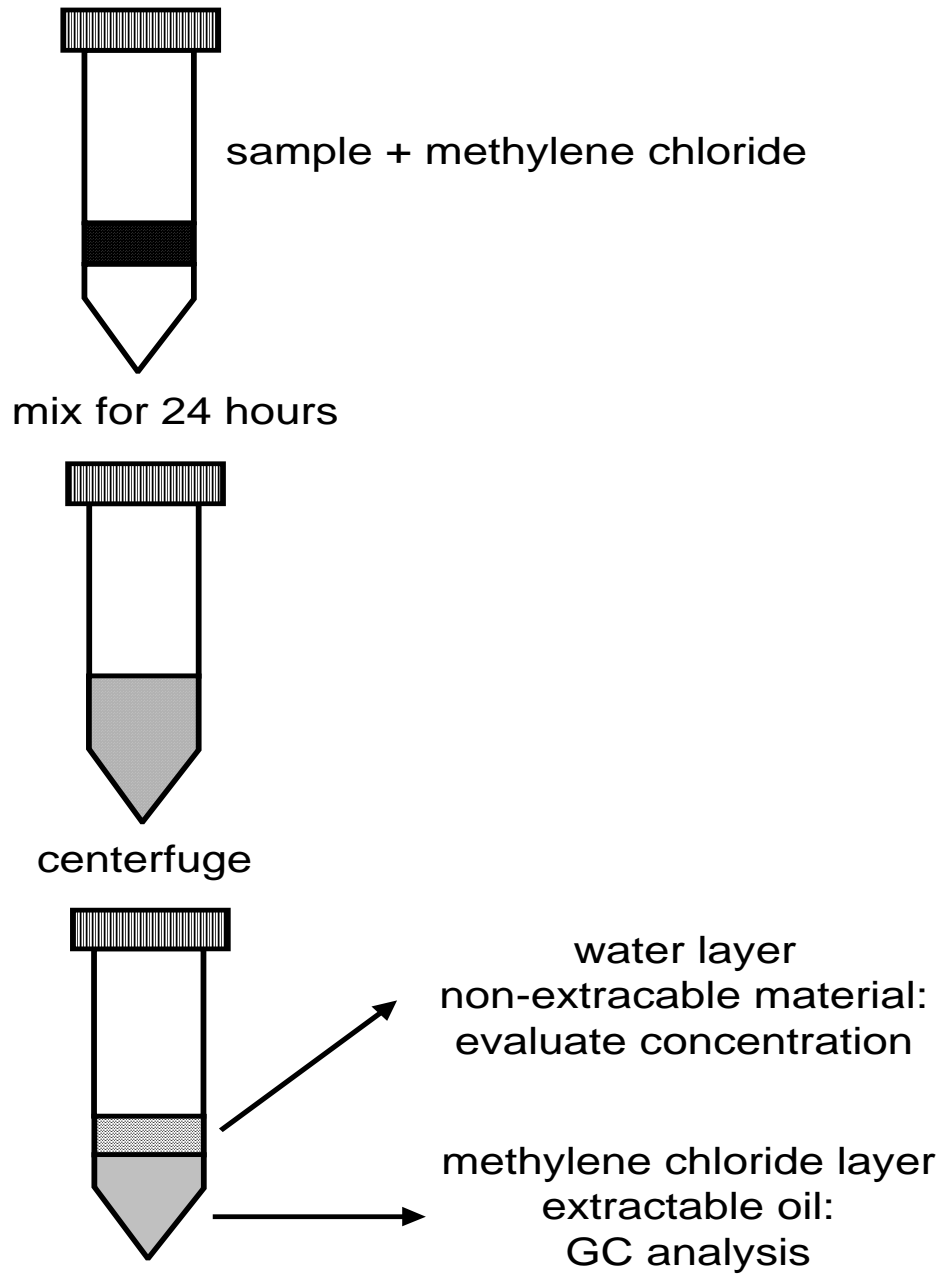


Figure 5. Methylene chloride extraction process and extractable and nonextractable oil definitions.

$$\text{concentration}(mg / gr) = \frac{\text{response}}{1271897}$$

for waste oil #1 and #2, with  $R^2 = 0.96$

## 5. Surface tension measurements

The surface tension measurements were performed to observe the biosurfactant production. The surface tension was measured in triplicate with a Fisher Surface Tensionmeter Model 20. The tensionmeter was calibrated once a month over the entire measurement range and the calibration was checked with distilled water each surface tension of samples was measured.

The measurements were performed in a 45-mm diameter flat bottom container with approximately 40-ml of sample. The platinum-iridium ring used for the measurements was cleaned with benzene and chromic acid followed by a distilled water rinse after each sample.

The measured surface tension values are referred to as apparent values. These values were obtained by multiplying a correction factor to obtain true surface tension values. The true surface tension values were calculated by multiplying the apparent values by a correction factor. The correction factor was dependent on diameter and thickness of the ring, temperature, density, and viscosity changes of sample and surrounding air. The true values were used in the discussion of the experiments.

## 6. Polysaccharide measurements

The polysaccharide measurements were used to evaluate the growth of organisms and production of biosurfactants. The polysaccharides were measured in duplicates using a colorimetric method developed by Dubios (1956).

The 1-ml samples were reacted with phenol for 10 min. Sulfuric acid was added and the mixture reacted for another 10 min. The mixture was then mixed vigorously and allowed to react for an additional 20 min. This reaction sequence gave rise to an orange-yellow color, which was measured by a Bausch and Lomb Spectronic 20.

No interferences due to the nutrient salts or oil were observed in this measurement. The samples were diluted to give a response within 0 to 100 mg/L. A glucose based calibration curve was obtained for every test.

#### 7. Capillary suction time measurements

CST was measured in order to evaluate the dewatering properties of the waste oil sludge (APHA 1995). The CST test is an index of the time needed for dewatering.

The CST is the time required for water in a sample to travel through a filter a specified distance between two points. CST was measured in triplicate and the average value was used for analysis.

#### 8. Specific resistance measurements

Specific resistance measurements were taken to evaluate the dewatering properties of the waste oil sludge (APHA 1995). The test was developed by Coakley (Tay and Jeyaseelan 1993,1997) and based on the Darcy's law of flow through porous media.

A hundred milliliters of the sample was filtered through an 8 µm filter (Whatman #2). A vacuum created by a 15-psi pump was applied to a Buchner funnel. The volume of the filtrate was measured as a function of time. The specific resistance was evaluated using the slope of a linear plot of filtrate volume (V) versus time per filtrate volume (t/V). The equation is:

$$\frac{t}{V} = \frac{u \times r \times w \times V}{2 \times P \times A^2} + \frac{u \times R}{P \times A}$$

where u is the filtrate viscosity (assumed to be the viscosity of water), r is the specific resistance, w weight of cake per volume of filtrate, A area of the filter, P pressure, R inverse of permeability. These measurements were performed in triplicate.

## IV. RESULTS AND DISCUSSION

As described in the material and methods section, four different experiments were conducted to study the biodegradation and dewatering properties of waste oil. These experiments were: a) aerated oil batch reactor experiment, b) filtered oil experiment, c) ten and twenty percent oil sacrificial batch experiments, and d) dewatering experiment. The data and observations from each of the experiments will be discussed individually.

### A. Ten Percent Oil Batch Aerated Reactors

The goals of this experiment were to determine the rate and extent of oil degradation, to compare the performance of native and introduced organisms, and to study the biosurfactant production in a batch waste oil reactor.

In this experiment, changes in COD, suspended solids, and extractable oil concentration were used to evaluate oil degradation. Changes in suspended solids and combustible solids were also used to evaluate organism growth, and changes in surface tension were used to evaluate biosurfactant production.

All of the parameters were evaluated for the liquid portion of the reactor mixture, which included dissolved and suspended oil and excluded attached oil as pictured in Figure 2. The COD values discussed below are the experimental COD values used for comparative purposes only. These values are, on average, 15 percent of the "correct" values and they were measured using a 1:50 dilution.

There were four types of reactors in this experiment: a reactor with native organisms, a reactor with introduced soil organisms, reactors with introduced mix of pure culture organisms, and reactors with introduced *Rhodococcus* organisms. The general trends and mechanisms were the same for all reactors and the differences in performance were due to differences in initial concentrations and organisms. Only one of the reactors will be discussed in detail and the data from the remaining reactors can be found in the Appendix B. The reactor performance will be compared based on the extent of oil removal as measured by the above mentioned parameters.

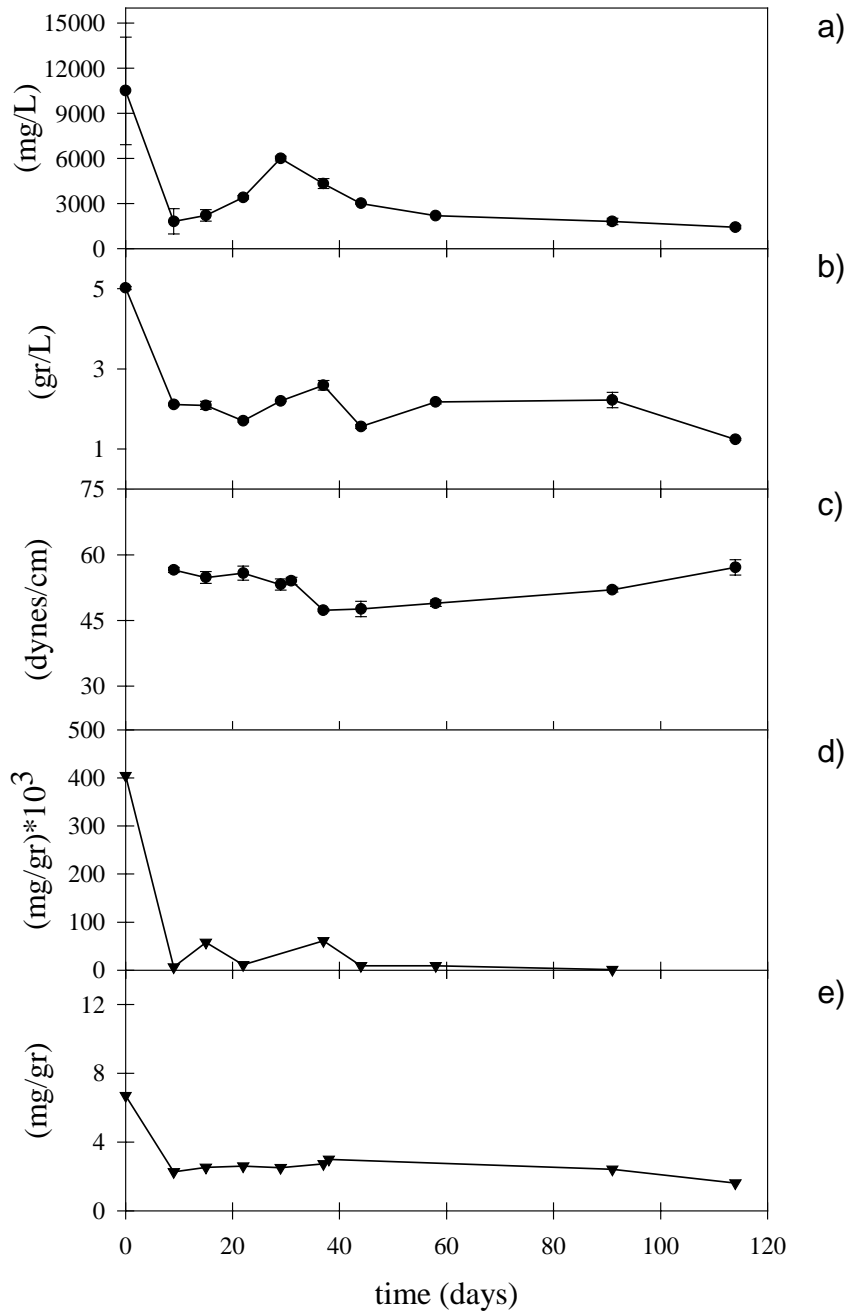


Figure 6. Results for the introduced mixed pure cultures 10 percent aerated batch reactor: a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Error bars are included in plots a) - c).

## 1. Trends and mechanisms for the mixed pure cultures reactor

Results for the 10 percent total waste oil reactor, which received a mix of pure culture organisms, are plotted in Figure 6. After 7 days, the COD values decreased by 83 percent. Over the next 21 days the COD increased, after which it again decreased.

The suspended solids, which measured the combination of oil and organisms decreased by 58 percent over the first 7 days. In days following the low value, the suspended solids increased to a peak 7 days after the COD peak, on day 37. After the peak, the suspended solids decreased again and then remained fairly constant. The increase in the above parameters implied that the solid oil from the reactor surfaces entered the liquid phase or that the organism population increased.

Surface tension was determined by two factors: oil concentration and biosurfactant concentration. The surface tension measurements taken in this experiment were obtained approximately 1 hour after the samples were removed from the reactor and after reaching room temperature of approximately 18°C. Therefore, the measurements were affected by flocculation and settling in the samples. By day 37 the surface tension decreased to 15 percent below the original value, which indicated the presence of biosurfactant in the suspension. The values presented here probably indicate a decrease in surface tension after the organisms released the surfactant into the solution. Following the low values, the surface tension increased to approximately the original value, indicating that oil and surfactants were removed from the suspension.

The decrease in COD, suspended solids, and combustible solids was interpreted as biodegradation of waste oil from the suspension. This was confirmed by the GC measurement which showed a 98 percent decrease in extractable oil over the first 7 days of the study. The changes in extractable oil are best seen in the chromatographs presented in Figure 7. The extractable oil compounds, which degraded in the first days of the experiment, were thought to be soluble and easily degradable. These compounds did not require biosurfactants to degrade.

After day 20, the surface tension decreased indicating biosurfactant presence in the suspension. As the surfactants were produced, the waste oil solubility increased and more

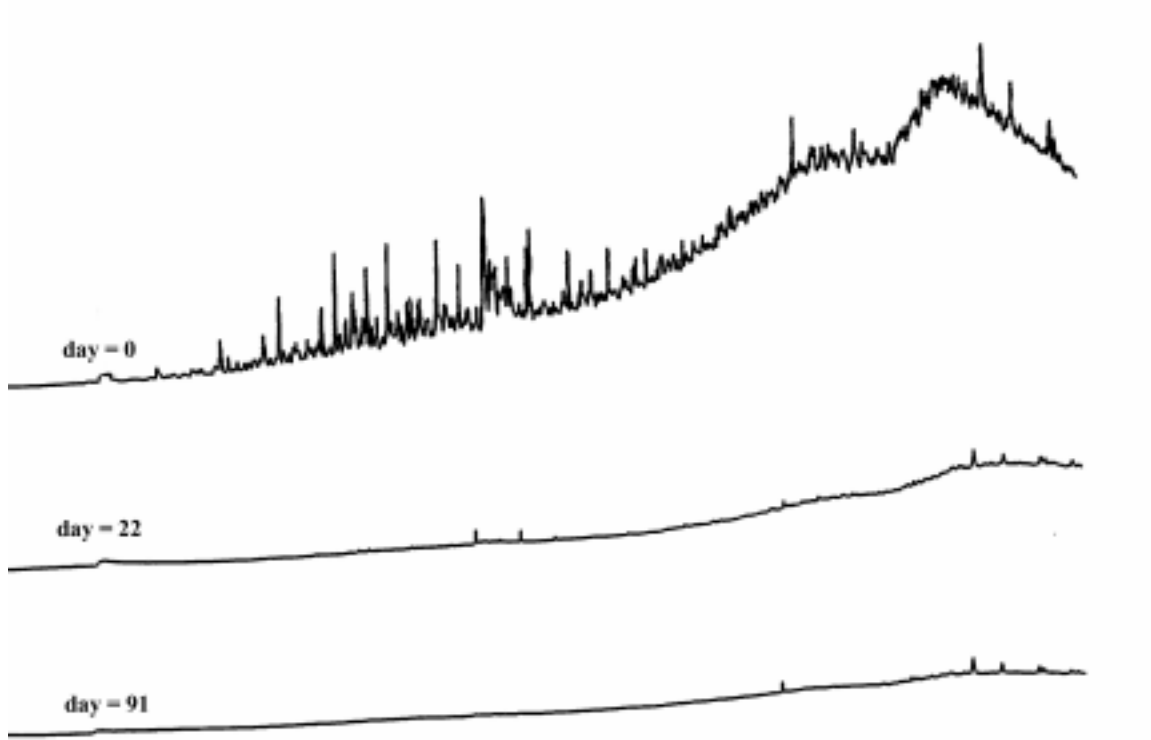


Figure 7. Selected chromatographs for introduced mixed pure cultures 10 percent aerated batch reactor showing degradation of extractable oil.

oil entered the liquid portion of the reactor. The increased amount of dispersed oil resulted in an increase in COD and extractable oil, which peaked on day 29. The suspended solids measured both oil and organisms and peaked 7 days after the COD peak suggesting that increased oil concentration stimulated organism growth. The compounds, which degraded in the later days of the experiment, were thought to be relatively insoluble, since they required biosurfactant for degradation.

Sixty percent of the non-extractable material was degraded in the first 7 days and its concentration remained approximately constant for the rest of the experiment. These observations indicated that methylene chloride extractable materials are degradable while the nonextractable materials are only slightly degradable. This suggested that methylene chloride extractable fraction may be used as an indicator of biodegradability.

In summary, the easily degradable compounds were degraded first. The difficult to degrade compounds were degraded after surfactants were produced to increase their solubility. After the oil was degraded, the biosurfactants became ineffective and surface tension of the suspension increased.

## 2. Performance comparison of the native versus introduced organisms

Native organisms were compared to introduced organisms with regard to their ability to degrade oil sludge based on percent removal of COD, change in suspended solids, change in extractable or GC oil, and change in non-extractable material. All of the reactors showed the same pattern of waste oil degradation and biosurfactant production. Depending on the type of organisms present and the composition of the mixture, the magnitude and time of onset of biosurfactant production varied. This caused a variation in the magnitude and time of the secondary peaks in COD, suspended solids, and combustible solids values.

The reactor performance was compared at two times: day 22 and day 91 and it is summarized in Figures 8. On day 22 the native culture reactor removed the least amount of oil, as measured by COD, suspended solids, and extractable oil. Next was the soil culture reactor, followed by the *Rhodococcus* amended reactor. The mixed pure culture reactor had the highest percentage removal. This reactor contained a mixture of seven pure cultures grown on diesel fuel, anthracene, and naphthalene, as indicated in Table 3. On day 91, at the

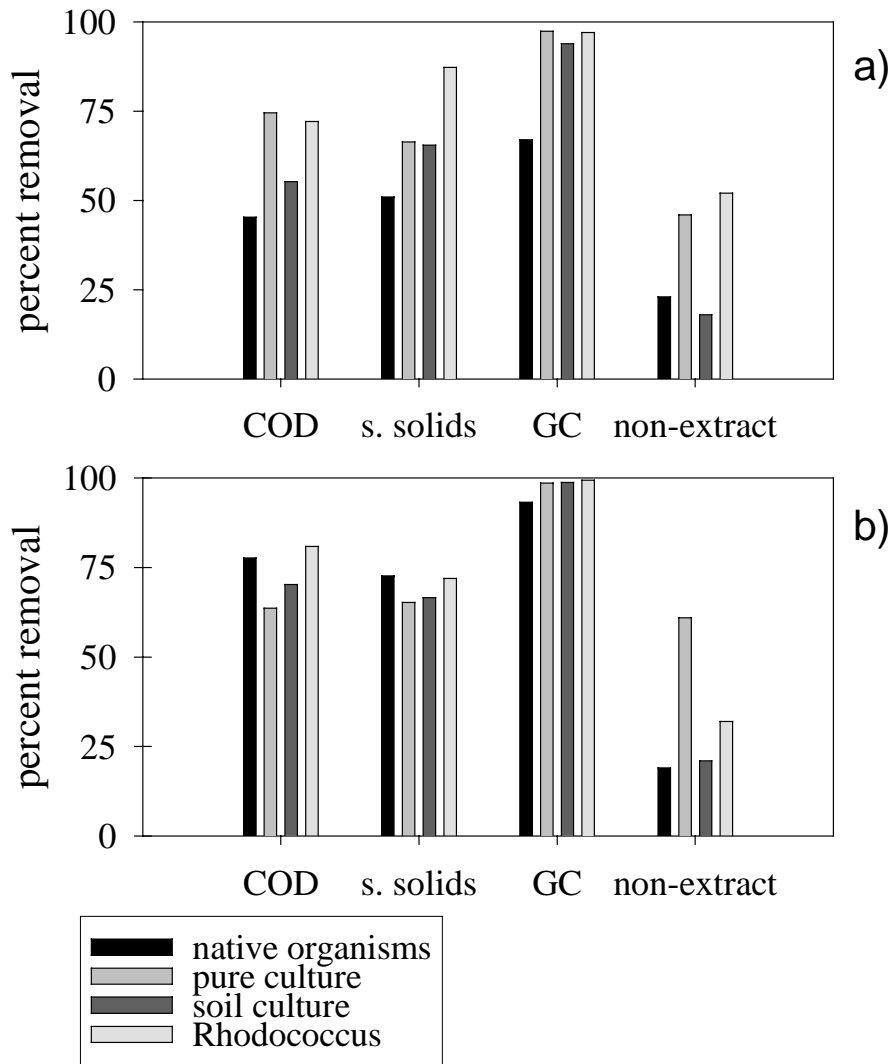


Figure 8. Performance comparison of 10 percent aerated batch reactors: a) day 22, b) day 91. Percent removal values are based single observations on days 0, 22, and 91

end of the experiment, the removal percentage values for the reactors were approximately the same (within 10 percent of each other) for all reactor types.

The non-extractable solids did not follow the pattern described above. The majority of removal took place in the first 22 days of the experiment and the removal percentages did not change substantially over time.

In a short-term comparison, the introduced organisms removed more material than the native organisms. The shorter lag period in the introduced organism reactor was attributed to a higher number and higher diversity of organisms due to an organism addition. In conclusion, the addition of organisms increased the extent of degradation for short contact periods but it was not beneficial in longer-term studies.

## **B. Filtered Oil Experiments**

The goal of these experiments was to quantify oil degradation, organism growth, and biosurfactant production without the interference of suspended oil particles. In the previous experiment, the presence of globular and attached oil added to experimental parameter variability and made it difficult to draw conclusions about biosurfactant production. The filtered oil experiment was performed twice. An initial trial was conducted then a second trial was used to obtain additional data, especially for surfactant production.

The parameters used to evaluate oil degradation were the loss of COD and extractable oil concentration. Changes in suspended solids and polysaccharides were assumed to be due to microbial activity. Finally, changes in surface tension and polysaccharides concentration were used to evaluate biosurfactant production.

In this experiment, the total oil concentration was very low relative to the other experiments in this study and the experimental COD values were not influenced by the dilution used. Both total and filtrate COD and polysaccharides were measured. The filtered samples were produced by filtering samples through 1.5  $\mu\text{m}$  filters. The particulate polysaccharide values were obtained by taking the difference between total and filtrate measurements.

## 1. Filtered oil - trial one

The experimental data for trial one are shown in Figure 9. The total COD did not change over the first two days of the experiment. Over the next 19 days, the total COD value decreased by 42 percent. These measurements are not indicative of the oil amount removed since the COD loss by degradation was offset somewhat by the COD gain in organisms. The filtrate COD value decreased sharply in the first 2 days from its initial value of 167 mg/L to 56 mg/L. The changes in suspended solids were indicative of changes in the organism mass. The suspended solids increased by 142 percent in the first 9 days, then decreased somewhat, but remained well above of the initial value.

The concentration of particulate polysaccharides decreased slightly, then on day 6 reached a peak of 23.5 mg/L, which corresponded with the appearance of foam in the reactor. After day 6, the particulate polysaccharide concentration in the reactor decreased.

The initial decrease in polysaccharide concentration suggested that small quantities of organics in the oil, measured by filtrate polysaccharides and filtrate COD, were consumed by the organisms before the more complex oil was degraded. The extractable oil concentration decreased quickly by 80 percent. Since polysaccharides are a part of some biosurfactant molecules, the polysaccharide peak suggested that the organisms produced biosurfactant, which remained attached to the organism membrane, as observed in other studies (Koch et al. 1991, Phale et al. 1995).

During the biosurfactant production period (day 2 to day 6) no observed organism growth was measured by suspended solids, but after the peak on day 6 the organism degraded the emulsified oil quickly and grew as measured by the COD and suspended solids increase.

The final decrease in particulate polysaccharides and increase in both filtrate polysaccharides and filtrate COD suggested a release of biosurfactant from the cell membrane into the solution occurred after a majority of the oil was degraded. The surfactants were then rendered inactive and later the organisms turned to endogenous growth, causing a decrease in both COD and suspended solids. The surface tension measurements, in Figure 9, were taken 1 hour after the sample was removed from the reactor and reached a room temperature of 18°C. These measurements were affected by flocculation

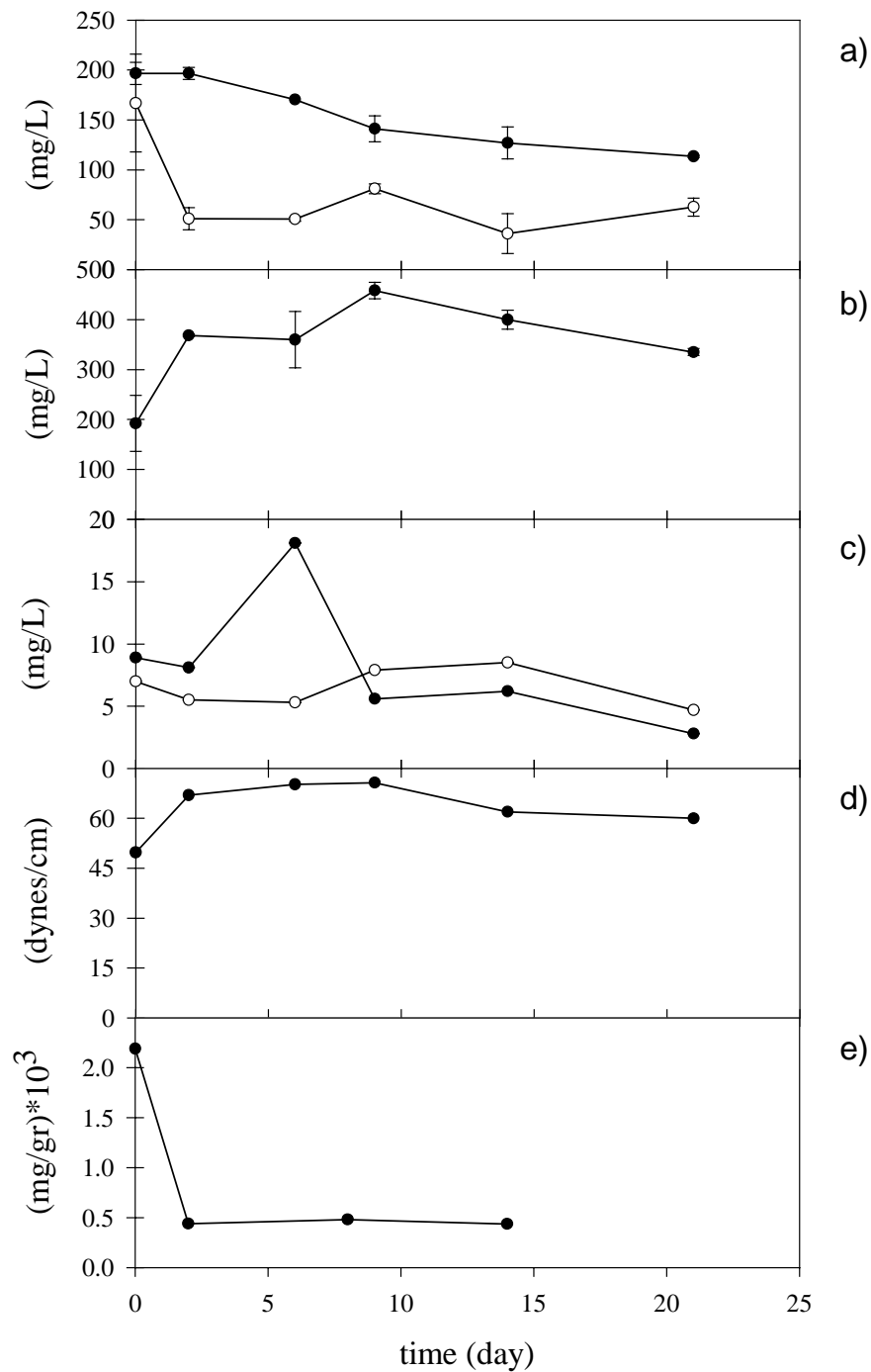


Figure 9. Results for trial 1 of filtered oil reactor experiment: a) ● total COD, ○ filtrate COD b) suspended solids, c) ● particulate polysaccharides, ○ filtrate polysaccharides d) surface tension, e) extractable oil. Error bars are included in plots a) - c).

and settling of the organisms and the surface tension did not decrease until the polysaccharide measurements indicated a release of biosurfactants into the solution. It was later observed that surface tension values increased from the time of sample removal to the time of the measurement. Therefore, this experiment was repeated (trial two) to gain further insight into surface tension changes.

## 2. Filtered oil - trial two

The reactor in this trial was set up using the same procedure as in trial one with one exception. The introduced organisms were not the original mixture of pure cultures, but were the organisms obtained from the trial one experiment. The data from this trial is shown in Figure 10.

The initial total COD was lower than in the first trial at 177 mg/L. The total and filtrate COD decreased, generally followed a parabolic decay pattern ( $R^2 = .9916$ ) with a 60 and 48 percent removal in the first 9 days of the experiment, respectively. The suspended solids increased throughout the experiment, indicating the growth of organisms. The initial growth rate was slow, but it increased to a peak value between day 5 and day 15.

The filtrate polysaccharides in this experiment were 1.7 times higher than in the previous trial. In both trials, the initial filtrate polysaccharides decreased in the first days of the experiment. The degradation of simple organic compounds including filtrate polysaccharides resulted in a minor organism growth measured as suspended solids. Since the polysaccharides were degraded preferentially over oil, the oil concentration remained approximately constant.

The particulate polysaccharides reached a peak value on day 8, followed by a decrease, as seen in the previous trial. The peak indicated maximum surfactant production, 48 percent extractable oil degradation, and increased rate of organism growth. The surface tension values plotted in Figure 10 are the values obtained immediately after the sample was removed from the reactor and were representative of the reactor conditions. The surface tension, as in trial 1, increased slightly over the first 5 days of the experiment when the filtrate polysaccharides were degraded. Low values were reached at the time of the particulate polysaccharide peak, on day 8, corresponding to increased rates of organism

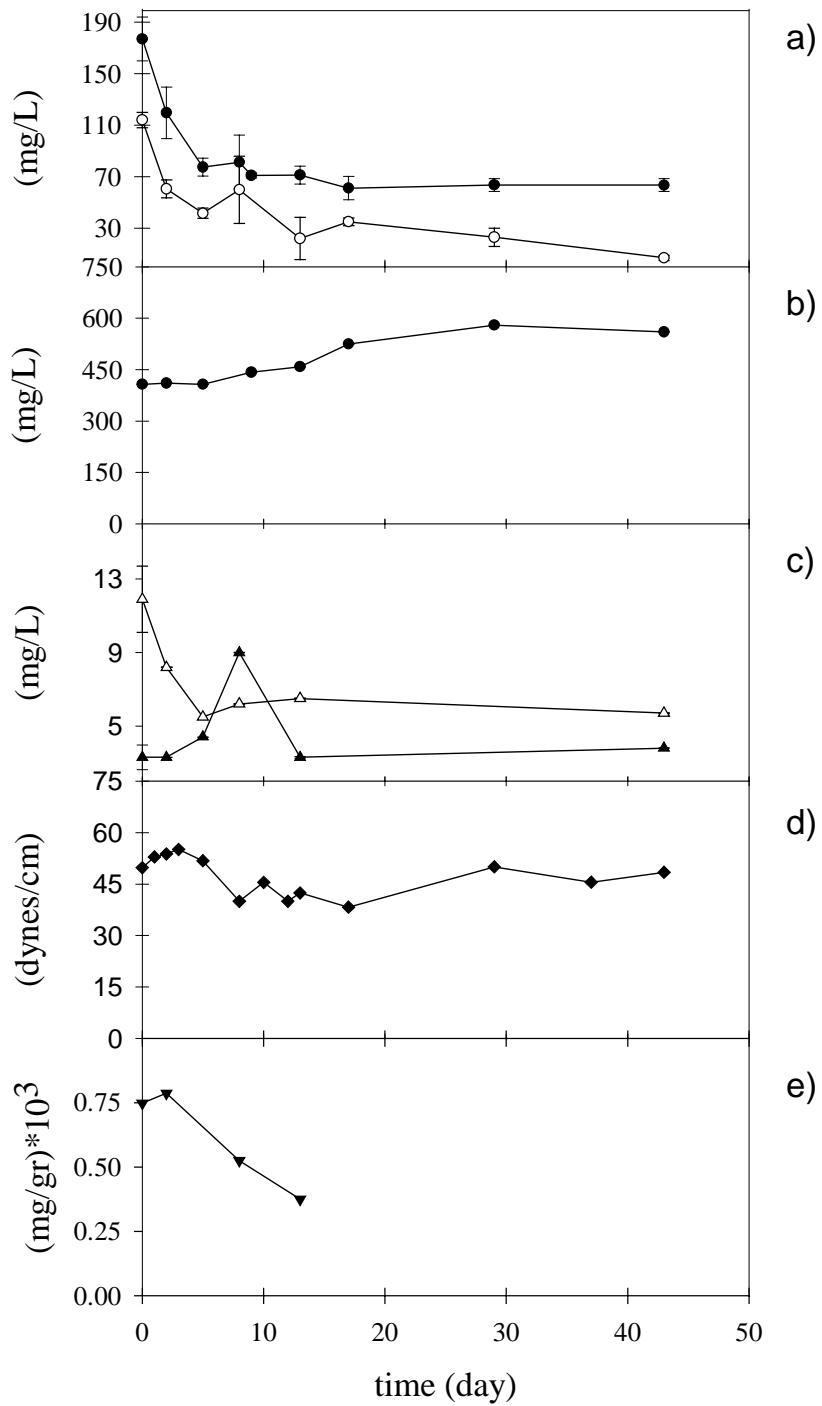


Figure 10. Results for trial 2 of filtered oil reactor experiment: a) ● total COD, ○ filtrate COD b) suspended solids, c) ● particulate polysaccharides, ○ filtrate polysaccharides d) surface tension, e) extractable oil. Error bars are included in plots a) - c).

growth, and oil degradation. After day 8 the surface tension slowly climbed up to approximately 48 dynes/cm by the end of the experiment. The 4°C variation in temperature over this time period likely accounted for some of the variability in surface tension.

Figure 11 shows how surface tension of a sample from a given day changed from the time of removal from the reactor to the time of measurement. These changes reinforced the hypothesis that the surfactant molecules were most likely attached to the organisms. As the organisms settled in the measuring vessel, the surfactants were removed from the solution and the surface tension increased. The time for settling of a particle, such as bacterial cell, is related to its size and mass by a second-degree polynomial. The changes in experimental surface tension over time can be fitted with the same type of function.

On day 17 the majority of oil was degraded and biosurfactants were no longer needed. The biosurfactants were released into the solution resulting in approximately constant surface tension over time, Figure 11, increased filtrate polysaccharides and filtrate COD, Figure 10. After day 17, the surfactants were no longer active increasing surface tension to values slightly higher than the initial values.

### 3. Summary of filtered oil experiments

From the two filtered oil experiment trials, a degradation mechanism was proposed. In both experiments the easily degradable materials, some of which was measured as filtrate polysaccharides, appeared to be degraded before the heavy oil. The surfactants were produced and remained attached to the cellular membranes of the producing organisms as long as their presence was beneficial to growth. The presence of biosurfactant was dependent on the composition of the substrate and organism type. It was thought that after their release into the solution the surfactants became inactive. It is possible that the biosurfactants were degraded as it was suggested by Bruheim et al. (1997) and Oberbremer and Muller-Hurtig (1989).

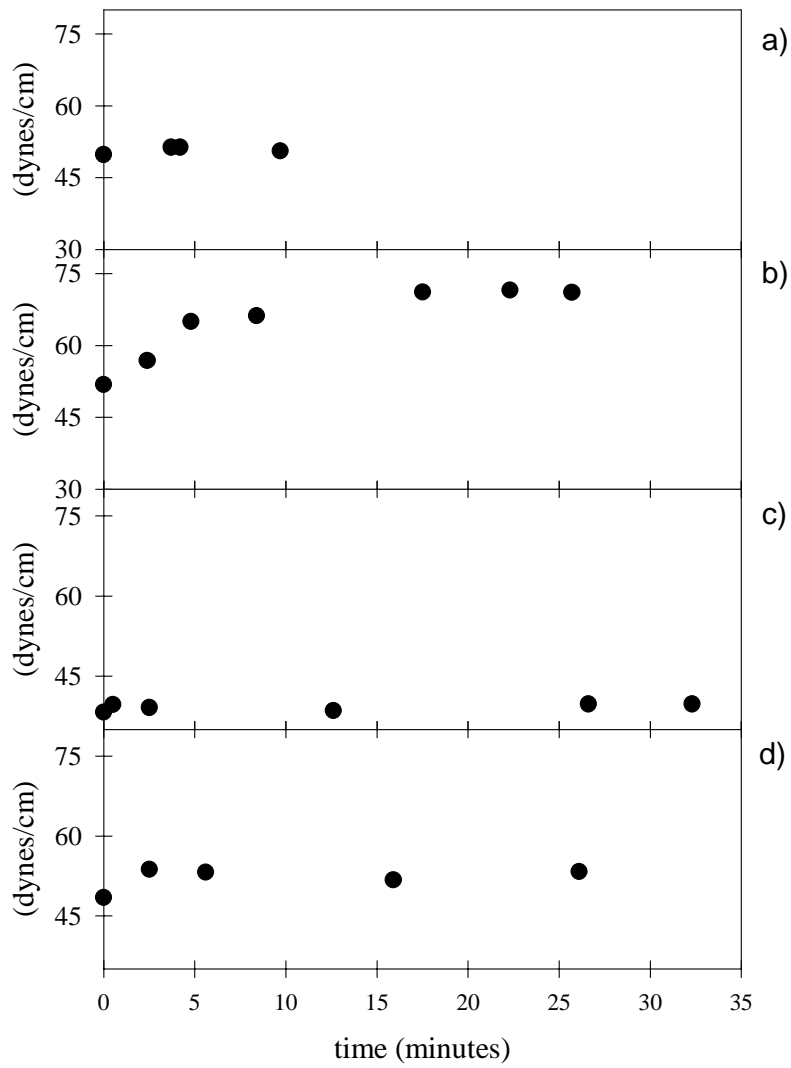


Figure 11. Effect of measurement time on surface tension in samples from trial 2 of filtered oil reactor experiment.

### **C. Sacrificial Batch Reactors with 10 Percent Oil**

The goal of this experiment was to more clearly define the degree of oil degradation by quantifying oil attached to the surfaces of the reactor. The presence of the attached oil caused variations in the sampled parameters and difficulty in evaluating oil degradation. This experiment was performed in a series of closed vessels where changes in the attached, suspended, and dissolved oil were measured by completely sacrificing each of the reactor vessels at sampling time.

The reactors were sampled for COD and extractable oil to monitor oil degradation. The extractable oil was characterized by gas chromatography and representative chromatographs for the reactors with introduced organisms are shown in Appendix C. The reactors were sampled for suspended solids to monitor organism growth and oil degradation. They were also sampled for polysaccharides to monitor organism growth and biosurfactant production. All of the above parameters were evaluated for the total oil suspension in the reactor and for the supernatant in a settled reactor. The attached oil and non-extractable material were weighed and normalized based on the initial sample size.

The COD values discussed below are the experimental COD values. These values were on average, 28 percent of the calculated values for waste oil #1, 13 percent for waste oil #2, and 19 percent for 50/50 mixture of waste oil #1 and #2.

Each of the subsets, described in the Materials and Methods section, behaved differently due to differences in the substrate. The waste oil #1 reactors will be discussed in detail. The waste oil #2 reactor set and combination waste oil #1 and #2 reactor set will be discussed briefly to show differences between the reactor sets. The performance of the three reactors will be compared based on the percent removal of the experimental parameters.

#### **1. Waste oil #1 sacrificial reactors**

The experimental data for the waste oil #1 reactor set is plotted in Figure 12. The overall decrease in total COD was 33 percent for the reactor with added organisms and 12 percent for the reactor with native organisms over 44 days of the experiment. The supernatant COD followed the same pattern for both reactors and decreased by an average of

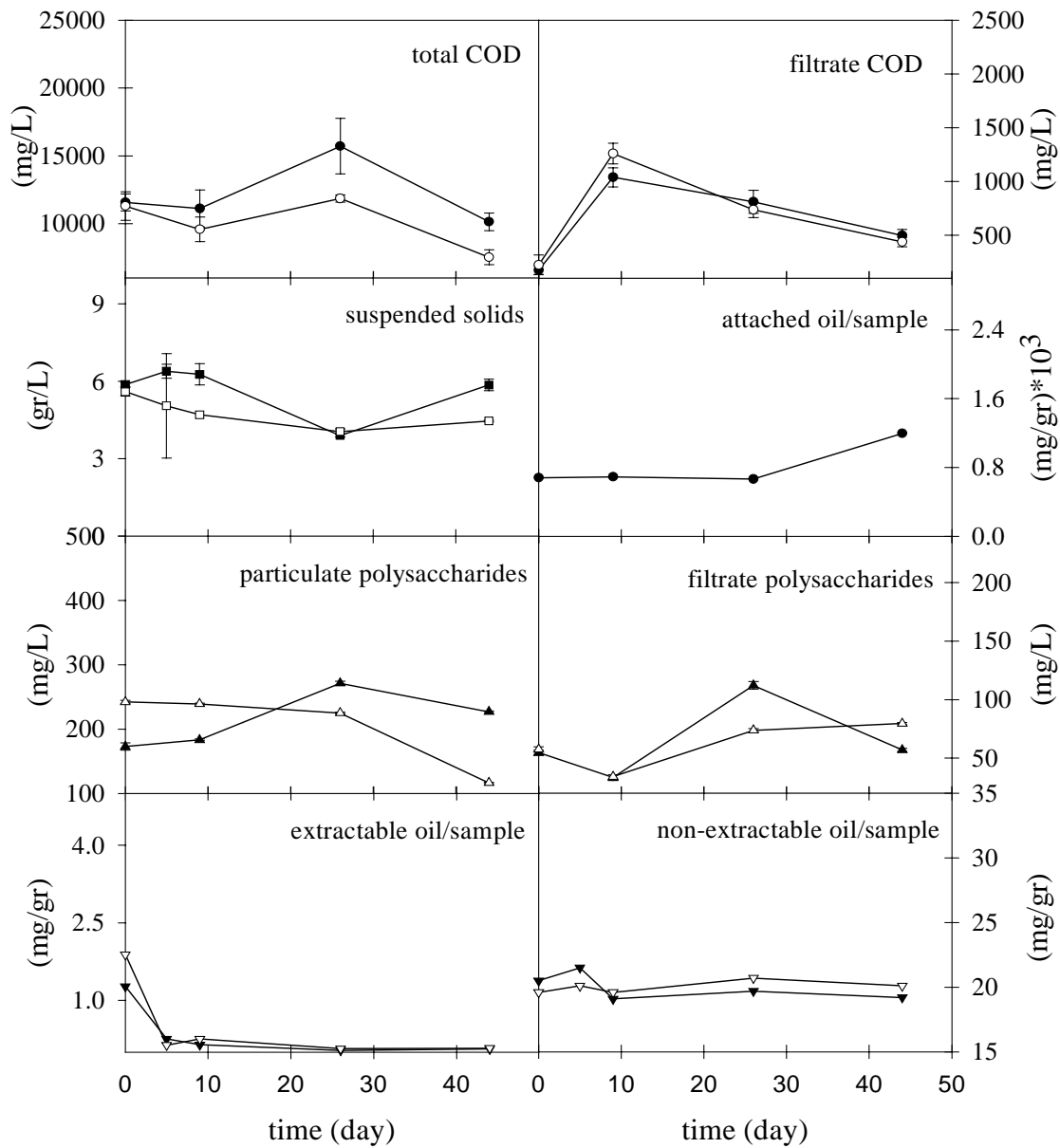


Figure 12. Results for waste oil #1 for 10 percent sacrificial batch reactor experiment. (○ introduced organisms, ● native organisms) Error bars are included for total COD, filtrate COD, suspended solids, particulate polysaccharides, and filtrate polysaccharides.

59 percent. The initial increase was attributed to emulsifying biomass activity and the following decrease was attributed to oil degradation.

The suspended solids in the introduced organism reactors decreased by 20 percent over the span of the experiment. The suspended solids in the native organism reactors decreased by 34 percent only to increase to approximately the original value on day 44. The combustible solids for both reactors followed the same pattern of increases and decreases as suspended solids.

The data appeared to follow the same trends as was seen in the 10 percent aerated batch reactor experiment. The COD decreased until day 26, when the values increased due to oil entrance into the liquid suspension in the reactor. The suspended solids decreased until day 26 as the oil was degraded, then increased as the organisms grew on the solubilized oil and decreased the COD values.

The polysaccharide measurements were consistent with the above observations. The particulate polysaccharides in the introduced organism reactor slowly decreased for 44 days of the experiment to 52 percent of the original value. The particulate polysaccharides for the native organisms increased with time for the first 26 days by 57 percent and decreased for the next 18 days to 31 percent above the original value. Changes in these parameters were attributed to the changes in organism population. The polysaccharides of the supernatant for both native and introduced organism reactors decreased in the first 9 days of the experiment. The initial decrease in polysaccharides was consistent with observations in the filtered oil experiment.

It was thought that initially the organisms grew on simple organic compounds in the reactor before degrading the heavy oil. The increase in the supernatant polysaccharides for the native organisms was attributed to the presence of biosurfactants. This peak occurred at the same time as the increase in COD and corresponded with the later increase in suspended solids indicating organism growth.

An average of 96 percent of the extractable oil was removed. The majority of the extractable oil was removed in the first 5 days of the experiment for both reactors. The non-extractable material remained approximately constant during the experiment. Again, this suggests that methylene chloride extraction can be used to predict degradability. The

majority of the waste oil #1 consisted of nonextractable material, so that the total reduction in COD and suspended solids were smaller than ones noted in the 10 percent aerobic batch reactor experiment where a combination of waste oil #1 and #2 was used. The polysaccharide measurements indicated that native organisms were able to grow on this substrate while the introduced organisms did not grow well. Since these reactors were not actively aerated, the possibility of oxygen limitations is strong and therefore no conclusions about organism performance can be drawn.

## 2. Waste oil #2 sacrificial reactors

The experimental data for waste oil #2 reactors is plotted in Figure 13. The COD and suspended solids behaved differently than in the 10 percent aerated batch reactors. In the first days of the experiment, the organism presence increased the COD and suspended solids values, probably by improving the emulsification of oil. The decrease, which followed, occurred first in the introduced organism reactors then in the native organism reactors. This difference was most likely due to the presence of greater number of organisms in the introduced organism reactors. The peak seen in the 10 percent aerated batch reactors on day 29 was not observed in this experiment, most likely due to the higher initial concentration of oil in the reactor.

The polysaccharides also behaved differently. The initial concentration of supernatant polysaccharides was low and the degradation of simple organic compounds measured as filtrate polysaccharides seen in the waste oil #1 did not occur in this source. The organisms grew on oil. The particulate polysaccharides of introduced organism peaked on day 9 indicating both organism growth and biosurfactant production. The decrease in particulate polysaccharides corresponded to an increase in supernatant polysaccharides, a possible indication of growth for the first 26 days. A peak in the supernatant on day 26, indicated a possible biosurfactant release into the solution. The last 18 days were

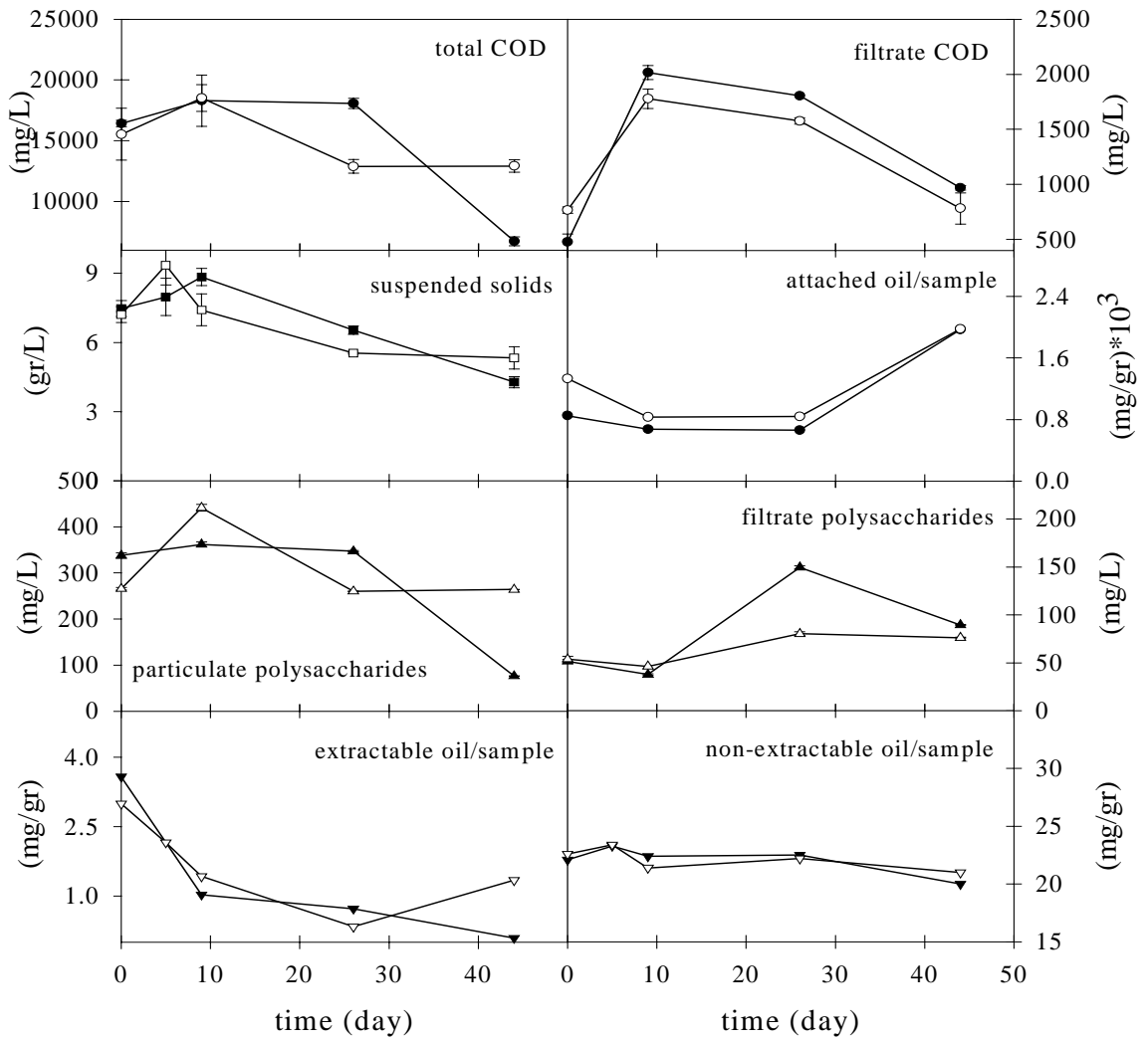


Figure 13. Result for waste oil #2 for 10 percent sacrificial batch reactor experiment. (○ introduced organisms, ● native organisms). Error bars are included for total COD, filtrate COD, suspended solids, particulate polysaccharides, and filtrate polysaccharides.

characterized by a sharp decrease in surfactants and increase in organism attachment to the reactor vessel for both reactors.

The extractable oil for this reactor set decreased at a slower rate than in the waste oil #1 reactor set, most likely due to a higher initial concentration. The introduced organisms removed 89 percent of the initial concentration, while the native organisms removed 80 percent of the initial concentration in 26 days. The non-extractable material remained approximately constant for this waste oil source.

The waste oil #2 reactors had a larger fraction of methylene chloride extractable oil and this oil fraction was effectively removed in the 44 days of the experiment with rapid biosurfactant production. Both native and introduced organisms were able to grow on this substrate and their numbers decreased when the extractable oil was removed from the solution as measured by the polysaccharides. As with waste oil #1 reactors, the introduced organisms removed larger amounts of oil. Again, this phenomenon was attributed to the fact that the introduced organism reactor had a larger number of organisms due to the addition of pure culture mix and the addition of easily degradable materials used as growth substrate for the pure cultures.

### 3. Waste oil #1 and #2 mixture sacrificial reactors

The experimental data for waste oil #1 and #2 reactors is plotted in Figure 14. The introduced organisms showed COD and suspended solids patterns similar to the waste oil #1 reactor set. The native organisms exhibited a constant decrease in the COD and small changes in suspended solids.

The particulate polysaccharides in the introduced organism reactors increased in the first 26 days. This peak corresponded to an increase in COD and suspended solids and was most likely due to biosurfactant production, since the surfactant molecules remained attached to the cellular membrane of the organism. The supernatant polysaccharides decreased initially as they were degraded and then they increased substantially suggesting surfactant presence in the solution. The particulate polysaccharides in the native organism reactors increased slightly in the first 9 days and then decreased by 64 percent. The final

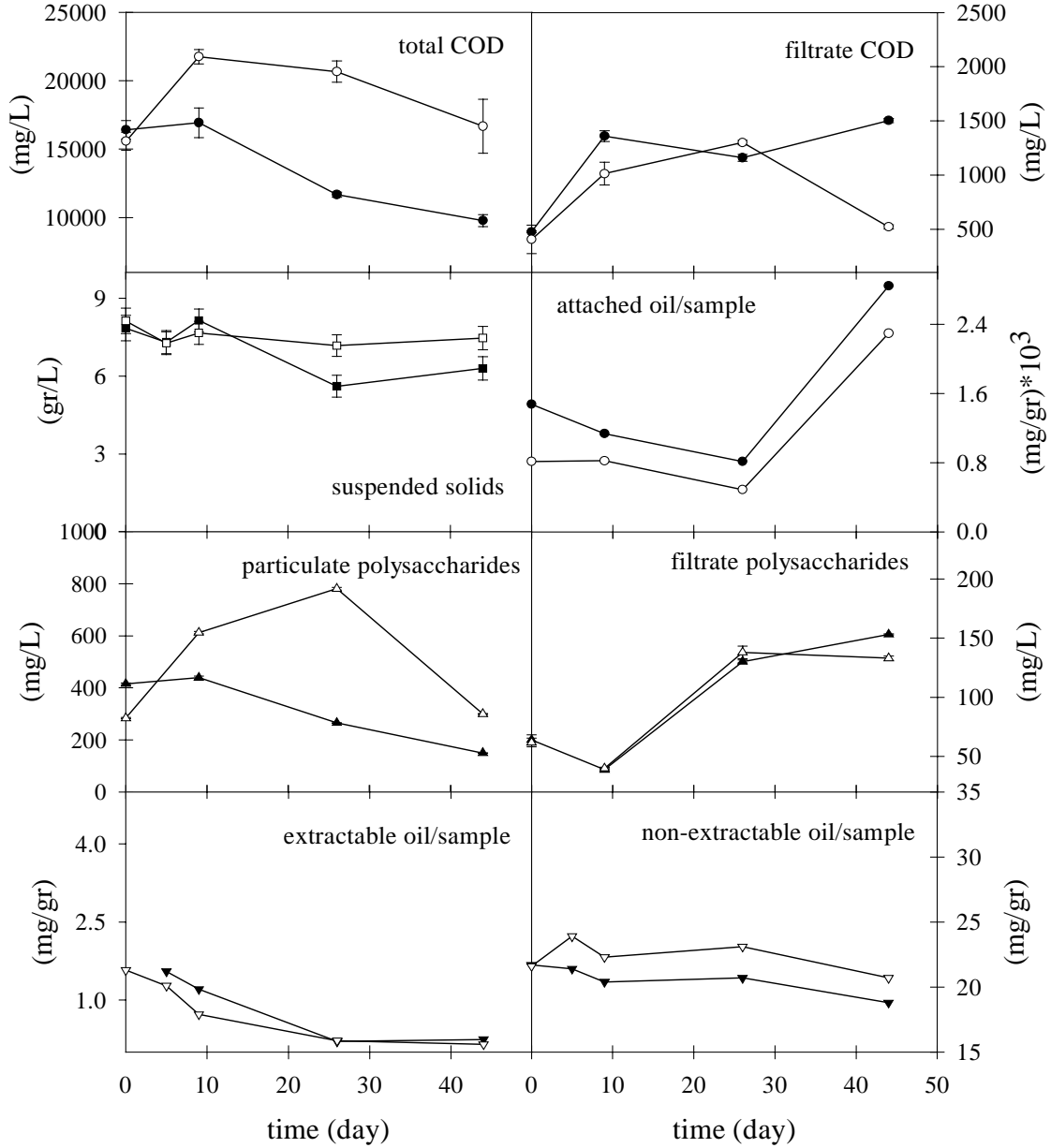


Figure 14. Results for waste oil #1 and #2 for 10 percent sacrificial batch reactor experiment. (○ introduced organisms, ● native organisms) Error bars are included for total COD, filtrate COD, suspended solids, particulate polysaccharides, and filtrate polysaccharides.

values were higher than the final values in the waste oil #2 reactor set. Even though the particulate polysaccharides decreased over time, the supernatant polysaccharides increased indicating surfactant production. It seems that the native organisms followed a pattern which was the sum of responses from the two previous reactor sets.

The added organisms removed 90 percent of the extractable oil in the 44-days of the experiment. The initial concentration of the extractable oil in the native organism reactor was not known. The native organisms removed 84 percent of the extracted oil from day 5 to day 44 at the end of the experiment. The non-extractable material decreased by 4 percent for the introduced organism reactors and by 13 percent in the native organism reactor.

In this experiment the native organisms removed a larger percentage of COD, solids, and non-extractable material than the introduced organisms. It seems that the combination of organisms from the two waste oil sources resulted in diverse population able to degrade heavy oil.

#### 4. Summary of trends in sacrificial batch reactors - 10 percent

The performance of the introduced and native organisms is summarized in Figure 15. Overall, the removal in the introduced organism reactors was smaller than for the native organisms. The removal of extractable oil was about 90 percent in both types of reactors, which reinforces a previous conclusion that the oil extracted by methylene chloride is a readily degradable material. The non-extractable material was about 20 mg per gram of sample for all reactors.

#### **D. Sacrificial Batch Reactors with 20 Percent Oil**

The goal of this experiment was to study biodegradation of waste oil at a higher concentration. The reactors were set up and sampled in the same fashion as the reactors in the sacrificial batch reactors with 10 percent waste oil.

The COD values discussed below were on average 23 percent of the calculated values for waste oil #1 and 7 percent for waste oil #2. This large difference was due to interferences and incomplete digestion in the COD test. The recovery of a standard in this experiment was 5 to 15 percent and below depending on the reactor. The higher oil

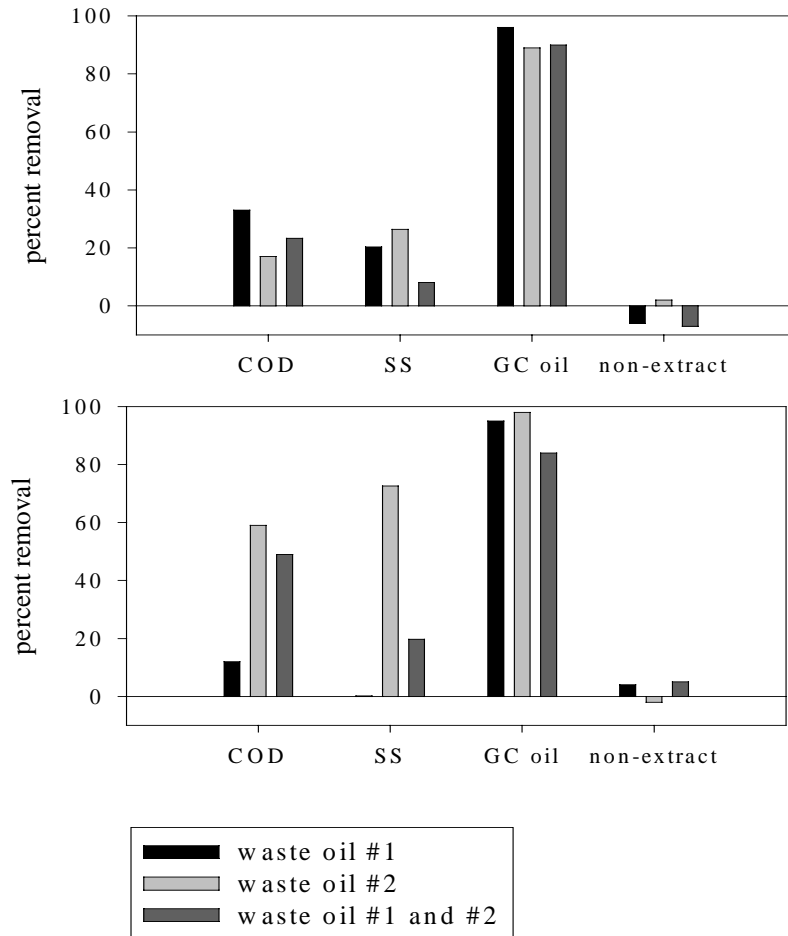


Figure 15. Comparison of oil percent removal by for sacrificial batch reactor experiment: a) introduced organisms, b) native organisms. Percent removal values are based single observations on days 0 and 44.

concentration and the lack of active aeration devices raised a strong possibility of oxygen limitations in this experiment. The data is shown in Appendix D.

The higher concentration of oil and short experimental time resulted in smaller percent removals of COD and suspended solids in the reactors. The polysaccharide measurements indicated that the organisms were able to grow at higher oil concentrations. The data and chromatographs enclosed in Appendix D show that they were able to degrade the extractable oil quickly and effectively. The non-extractable oil did not degrade substantially, suggesting again that methylene chloride extraction can be used as a guide to degradability of waste oil.

The introduced organisms were not effective in increasing the extent of removal since they needed more time to adjust to the new environment. The native organisms grew slowly and did not show a distinct biosurfactant production, but they were very effective in removal of oil.

#### **E. Ten Percent Waste Oil Dewatering Experiment**

In this experiment the two waste oil sources were studied separately to observe changes in dewatering properties and their relationship to oil degradation. Dewatering properties were measured using CST and specific resistance, and oil degradation was measured using COD and suspended solids.

All of the oil degradation measurements were performed twice: once before and once after filtration through an 8  $\mu\text{m}$  filter (Whatman #2) used to evaluate the specific resistance. The pair of before and after values was used to estimate the properties of the biodegraded waste and clarifier outflow, respectively.

The calculated COD of a 10 percent solution was 41,050 for waste oil #1 and 127,500 for waste oil source #2. These values were 80 percent and 88 percent above the obtained experimental values. The experimental values were used for comparison purposes only.

## 1. Waste oil #1 degradation and dewatering

The waste oil #1 results are plotted in Figure 16. The total COD decreased by 34 percent in 2 stages over 79 days of the experiment. The COD did not change between days 14 and 42. The filtrate COD did not change over the first 14 days; it dropped 80 percent between days 14 and 42, and increased slightly over the remaining 37 days. The suspended solids decreased slowly in an exponential pattern with a 43 percent decrease in 42 days and a 9 percent decrease in the remaining 37 days.

The CST doubled over the first 14 days and the specific resistance increased by a factor of 10. After day 42, the CST decreased to a value 15 percent above the original value. The specific resistance decreased to a value 19 percent above the original value.

In the previous experiments the total COD plateau and filtrate COD decreased indicated the production of biosurfactants. The presence of biosurfactants increased the solubility and dispersion of oil. This combination contributed to an increase in degradation of oil, and deterioration in dewatering properties by increasing the mass of dispersed solids. As the oil was degraded, the dewatering properties improve, biosurfactant production stops, and dispersed solids are removed.

## 2. Waste oil #2 degradation and dewatering

The waste oil #2 results are plotted in Figure 17. The COD decreased by 73 percent in 2 stages with no change between days 14 and 42. The same pattern was observed in the filtrate COD. The filtrate COD decreased by 3 percent in 14 days, and then it increased by 16 percent, and then decreased 48 percent in the remaining 37 days.

The suspended solids decreased slowly in a linear pattern. The filtrate suspended solids remained approximately constant for 42 days then they decreased by 28 percent. As observed in waste oil #1 reactor, the total COD filtrate suspended plateau corresponded to deterioration in dewatering properties.

The CST increased by 240 percent in 14 days and the specific resistance increased by a factor of a 100. After day 42, the CST declined to 34 percent over the initial value. The specific resistance remained high for 28 days and then decreased.

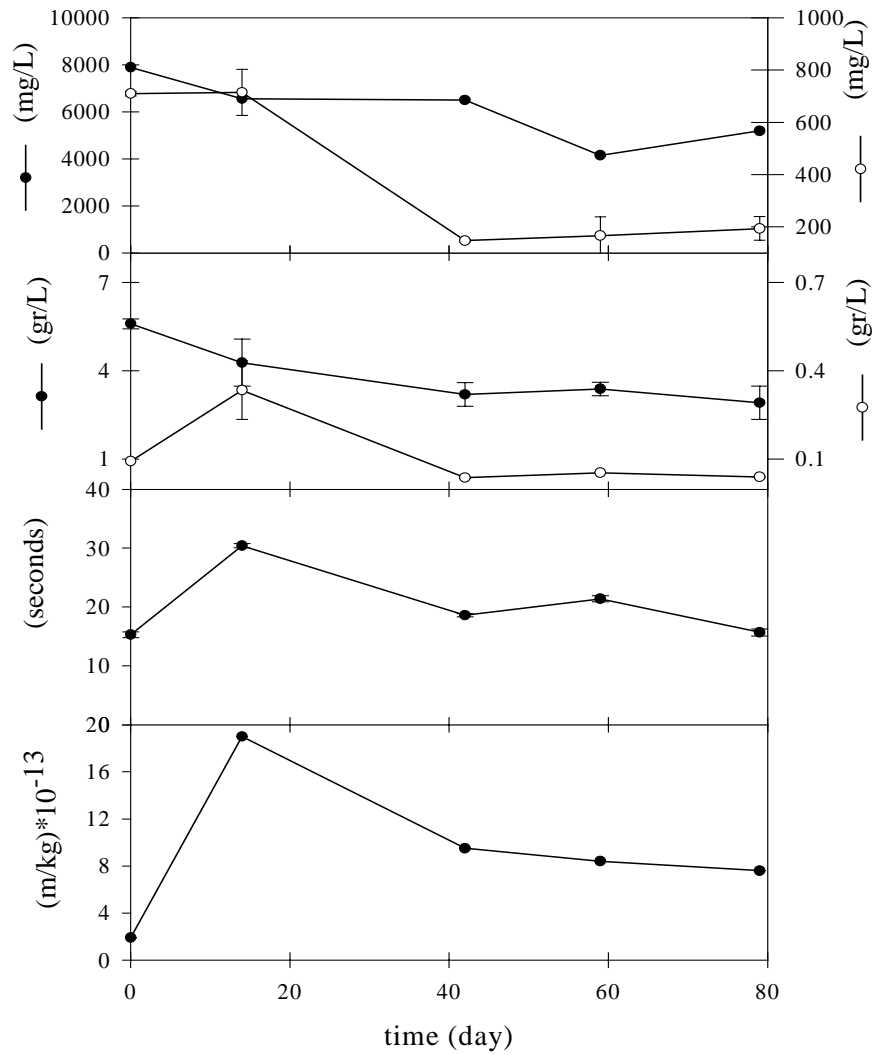


Figure 16. Results for waste oil #1 for the dewatering experiment: a) ● total COD, ○ 8 μm filter filtrate COD, b) ● total suspended solids, ○ 8 μm filter filtrate suspended solids, c) CST, d) specific resistance. Error bars are included in plots a) - d).

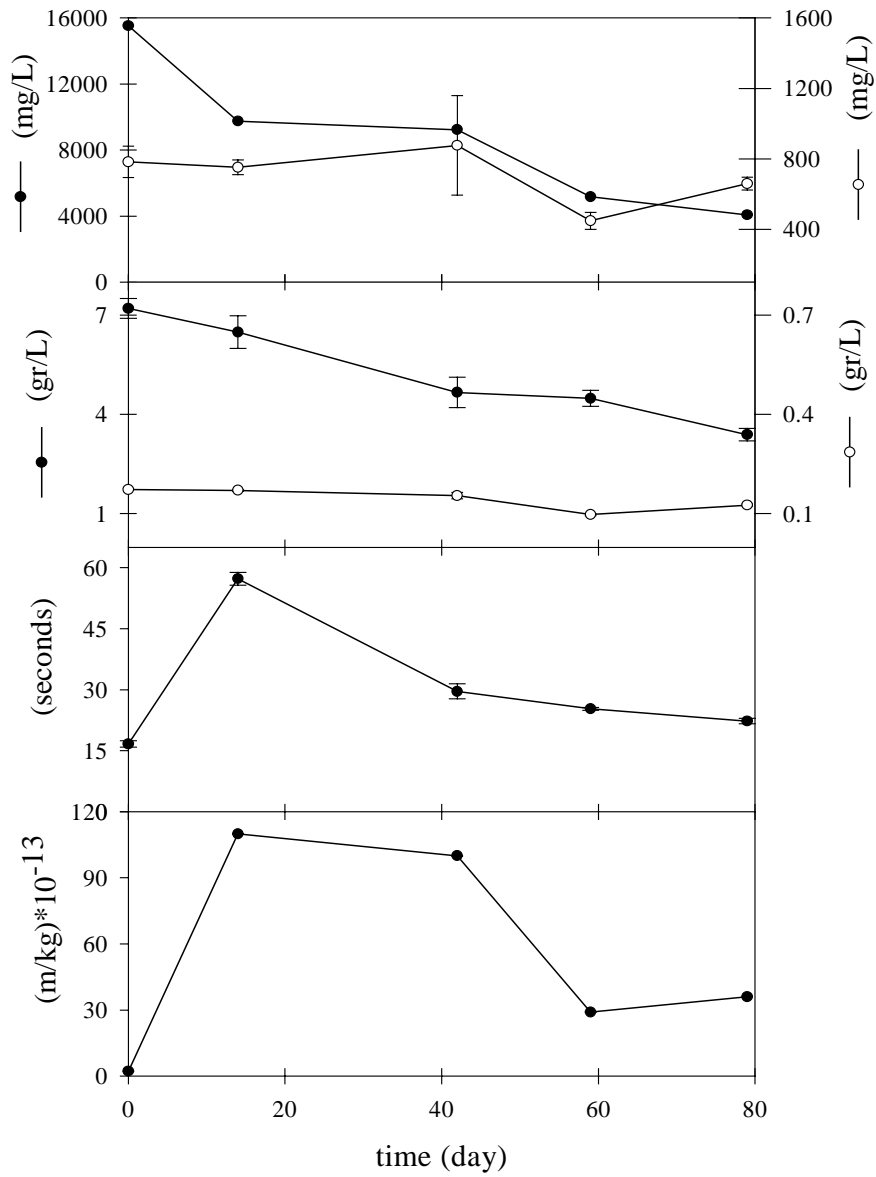


Figure 17. Results for waste oil #2 for the dewatering experiment: a) ● total COD, ○ 8 μm filter filtrate COD, b) ● total suspended solids, ○ 8 μm filter filtrate suspended solids, c) CST, d) specific resistance. Error bars are included in plots a) - d).

Again, the total COD plateau and suspended solids plateau was attributed to the production of biosurfactants. The presence of biosurfactants increases dispersion of oil and deterioration of dewatering properties. This waste oil source contained a larger fraction of degradable material and the time of biosurfactant production and oil degradation was longer than for waste oil source #1. Therefore, the dewatering properties improved slowly and to a lesser extent.

### 3. Performance comparison of waste oil dewatering

The two waste oil sources showed the same general patterns of degradation. The easily degradable material was degraded first. This first stage of degradation resulted in the largest percent removal of material. The recalcitrant material was degraded with the help of the biosurfactants. This second stage of degradation resulted in a small percent removal of material.

The production of biosurfactants at the end of the first degradation stage caused a temporary deterioration of dewatering properties. The dewatering properties improved with the disappearance of biosurfactants and lowering of oil concentration in the reactor.

The dewatering parameters are summarized in Table 6. The dewatering properties in waste oil #2 reactor, which contained a larger amount of degradable material, increased to much higher values than the same properties in the waste oil #1 reactor. Also, the waste oil #2 reactor properties did not decrease to the original values within the time of the experiment.

Table 6. Summary of dewatering characteristics

	Waste oil #1	Waste oil #2
Initial CST (sec)	15.3	16.7
Maximum CST (sec)	30.4	57.3
Day 60 CST (sec)	21.4	25.3
Initial specific resistance (m/kg)	$1.9 \times 10^{13}$	$2.2 \times 10^{13}$
Maximum specific resistance (m/kg)	$1.9 \times 10^{14}$	$1.1 \times 10^{15}$
Day 60 specific resistance (m/kg)	$8.4 \times 10^{13}$	$2.9 \times 10^{14}$

Overall, the oil from waste oil source #2, which had a larger extractable oil concentration, was more biodegradable and degraded faster and to a greater extent than waste oil source #1 as shown in Figure 18 a). The COD and combustible solids show a large difference between the two sources while the suspended solids are approximately the same for the two sources. One possible explanation could be due to the heterogeneous nature of the waste.

A large fraction of material was removed in the filtration process and the average values are summarized in Figure 18 b). The amount of solids removed was approximately the same for both of the waste oil sources.

The COD percent removal by filtration was very low, 11 percent for waste oil #1 and 20 percent for waste oil #2. These values do not correlate positively to the large percent removal for the solids in the reactors. This suggests that the majority of the experimental COD value is contributed by the soluble fraction of the oil not removed by the filtration process. The globules of oil are not digested completely during the COD test resulting in the 80 to 87 percent differences between the calculated and experimental values in COD.

From this experiment, it was concluded that the reactor residence time should be chosen based on the dewatering properties not on the extent of oil degradation.

## **F. Summary and Engineering Significance**

This series of experiments showed that both waste oil #1 and #2 were biologically degradable. The time and extent of biodegradation were dependent on the original concentration of oil and conditions of the experiment. The percent of removed oil in terms of suspended solids for all the experiments is summarized in Table 7.

In all experiments the oil degraded by the same proposed biodegradation mechanism, which consisted of four stages. First, the easily degradable oil components were degraded. Second, the biosurfactants were produced and the waste oil was emulsified. During this time the dewatering properties of the oil sludge declined substantially. Next, the more difficult to degrade compounds were degraded. Finally after the majority of oil was

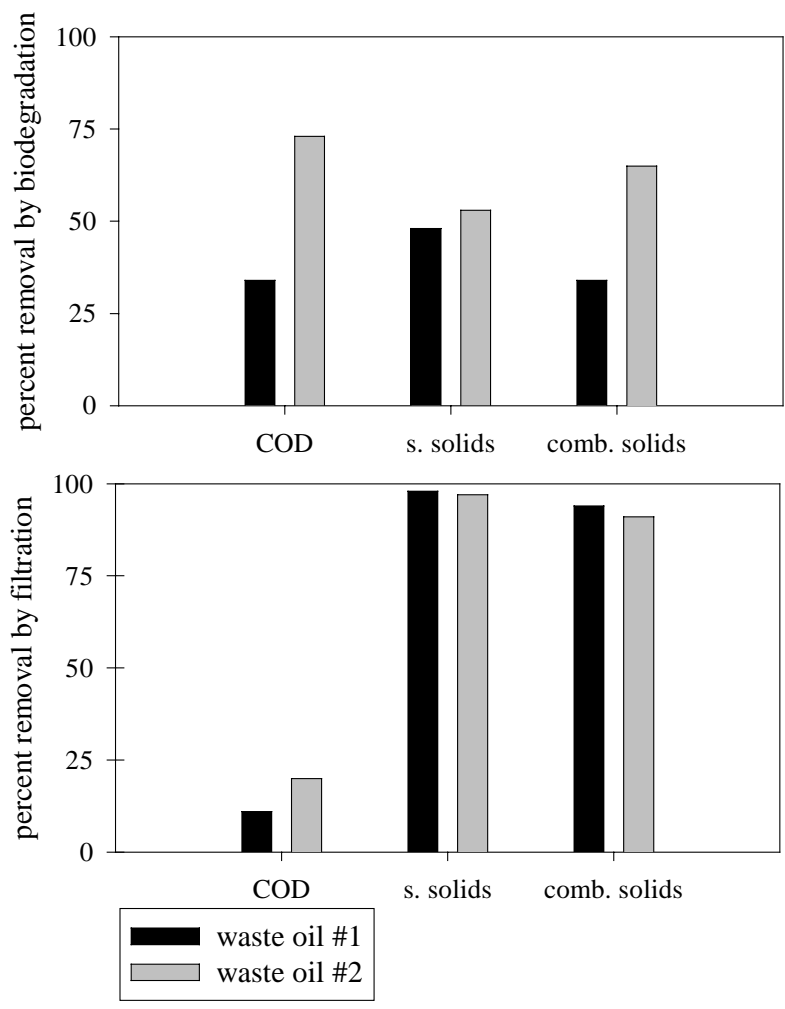


Figure 18. Comparison of percent removal for the dewatering experiment: a) biodegradation (percent removal values are based on single observations on days 0 and 79), b) 8  $\mu\text{m}$  filter filtration (percent removal values are based on the average of single observation).

degraded, the organisms turned to endogenous growth and the biosurfactants became inactive. The dewatering properties improved with the removal of biosurfactants and dispersed oil.

Table 7. Summary of oil removal represented by percent removal of suspended solids.

Experiment	Initial oil as total solids (mg/gr)	Length of experiment (days)	Average suspended solids removal
Filtered oil	0.023	29	53 (COD removal)
Aerated batch	10	91	80
10 % sacrificial batch	11	44	20
20 % sacrificial batch	22	48	17
Dewatering	33	80	50

The dewatering properties were directly related to the amount of biodegradable material in the reactor. The waste oil #2 reactor, which contained more biodegradable material, showed a larger deterioration in the dewatering properties and the final dewatering properties were well above the initial values.

In all experiments, a large percentage of the methylene chloride extracted oil was removed quickly, while the non-extractable oil remained in the solution. These observations suggest that methylene chloride extraction is a good indicator of biodegradability of oil mixtures.

Due to the step-wise nature of the degradation process, a batch system will likely be most effective for oil degradation. In a continuous system where fresh substrate is continuously supplied to the organisms, the easily degradable waste oil components would be preferentially degraded and biosurfactant production might be inhibited. Even if biosurfactants were produced, optimization of their production would likely lead to a very poorly dewatering residual. In addition control of biosurfactant production would be difficult in the case of feed oil composition changes.

In all experiments the addition of organisms did not produce substantial enhancement of oil degradation to justify their cost. An addition of synthetic surfactant was not

recommended since the organisms were very successful in producing biosurfactant when needed.

## **G. Recommendations**

Biodegradation has been shown to be effective in the removal of hydrocarbons from the waste oil solution in this laboratory study and several recommendations on the future system design can be made. In this study the microorganisms easily degraded methylene chloride extractable oil, while the non-extractable oil was not easily degraded. Therefore, methylene chloride extraction can be used to obtain the first estimate of waste oil susceptibility to biodegradation.

It is recommended that the waste oil be diluted prior to biodegradation to reduce potential toxic effects to the biomass and reduce the oxygen demand. In order to facilitate biodegradation of waste oil, addition of nutrients along with oxygen is recommended. Addition of commercial microorganisms produced minimal benefit over the indigenous microbial culture. The indigenous microorganisms are acclimated to waste oil and will result in removal of hydrocarbons when provided favorable growth conditions. For fresh oil that has not had the opportunity for natural seeding, organism addition may be beneficial. For our system where oil was stored prior to treatment microbial amendments were not beneficial.

The recommended reactor configuration includes a plug flow reactor or a series of continuously stirred reactors. The continuously stirred reactor series could consist of four reactors. In the first reactor of the series, easily degradable material would be removed. In the second reactor, biosurfactants would be produced. In third, the emulsified compounds would be removed, and in the fourth the biosurfactant would be inactivated to produce a more easily dewatering sludge.

## V. CONCLUSIONS

Biodegradation and dewatering properties of oily sludge were studied in order to reduce volume of hydrocarbon waste sludge. Biodegradation was measured using COD, suspended solids, GC measurements of extractable material, and nonextractable material concentration. The role of biosurfactants in the degradation process was observed using surface tension and polysaccharide measurements. Based on the results obtained during this study the following conclusions can be made:

- 1) The extent of hydrocarbon waste oil biodegradation was dependent on the initial concentration and composition of the oily sludge.
- 2) The addition of organisms did not have a substantial positive impact on the biodegradation.
- 3) The hydrocarbon waste oil contained two distinct oil fractions: methylene chloride extractable oil and methylene chloride non-extractable oil. The non-extractable oil did not biodegrade during the experimental time, while the extractable oil degraded well. This suggests that methylene chloride extraction could be used for a rapid estimate of waste oil degradability.
- 4) The presence of biosurfactant caused dispersion of oil and deterioration in dewatering properties.
- 5) Biosurfactants were produced when needed and initially are attached to the cellular membranes of the producing organisms. The biosurfactants were released and rendered ineffective when they were no longer required for organism growth.

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## APPENDIX A

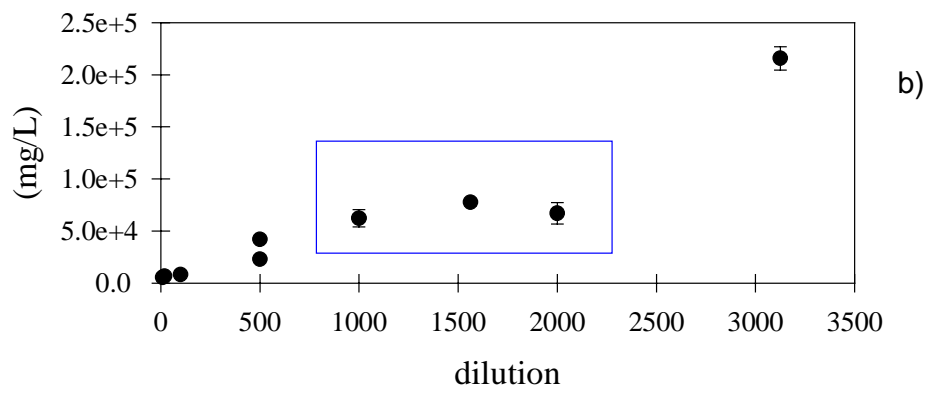
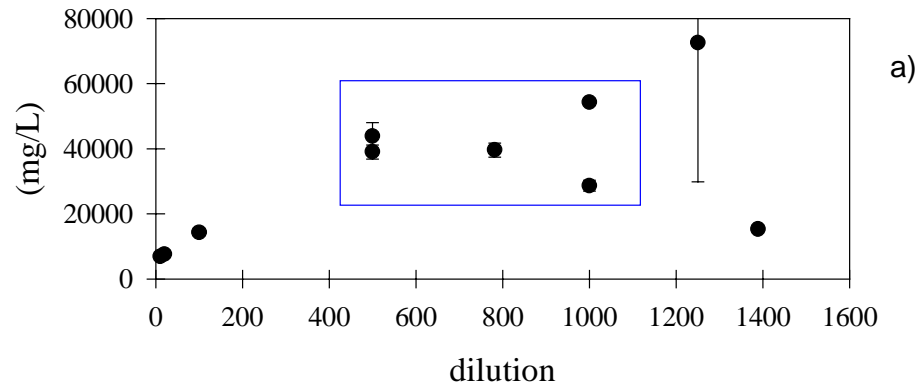


Figure A1. Data for calculations of “correct” COD: a) waste oil #1, b) waste oil #2.

## APPENDIX B

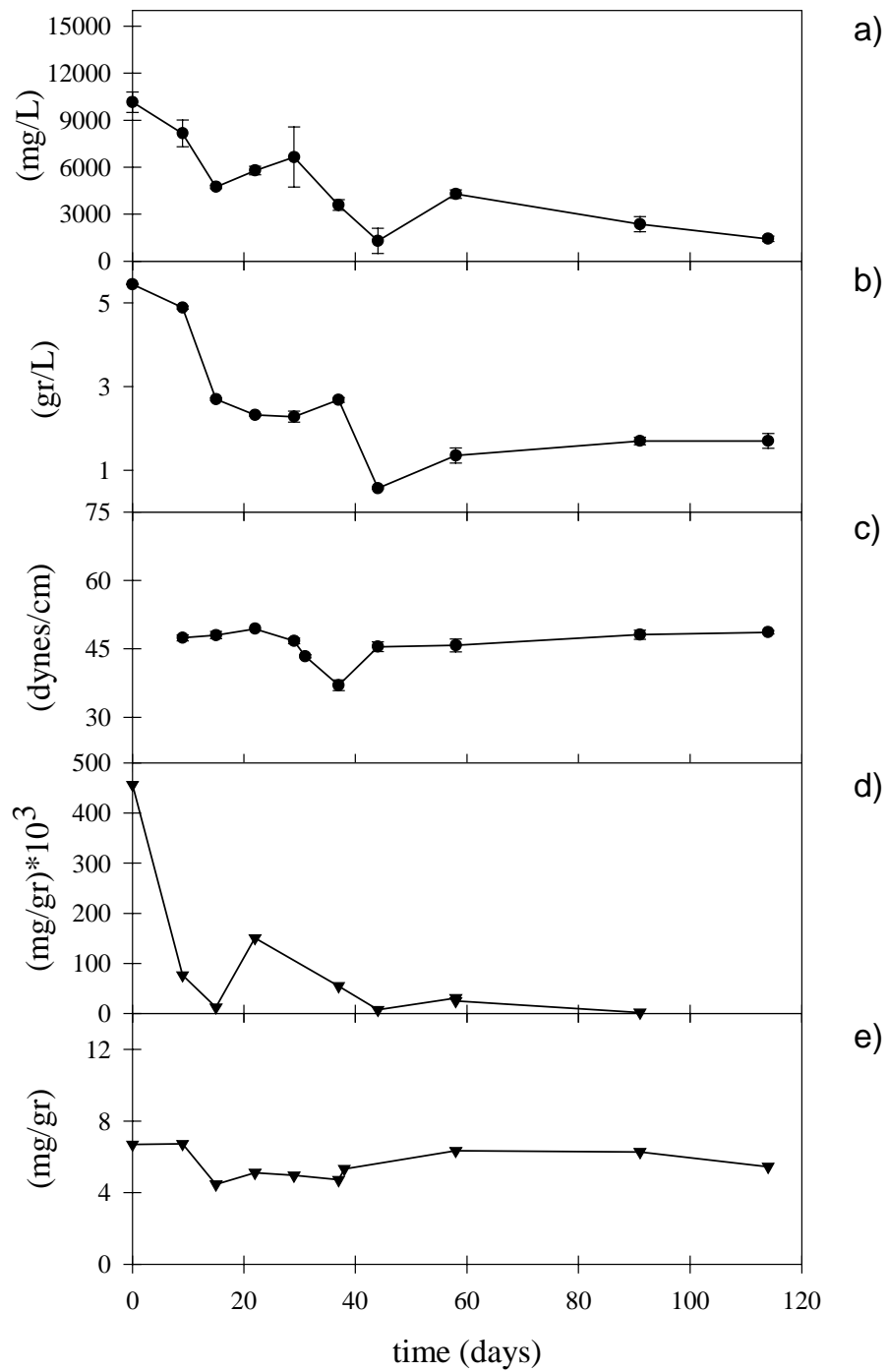


Figure B1. Results for native culture 10 percent aerated batch reactor (C): a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Plots a) - c) include error bars.

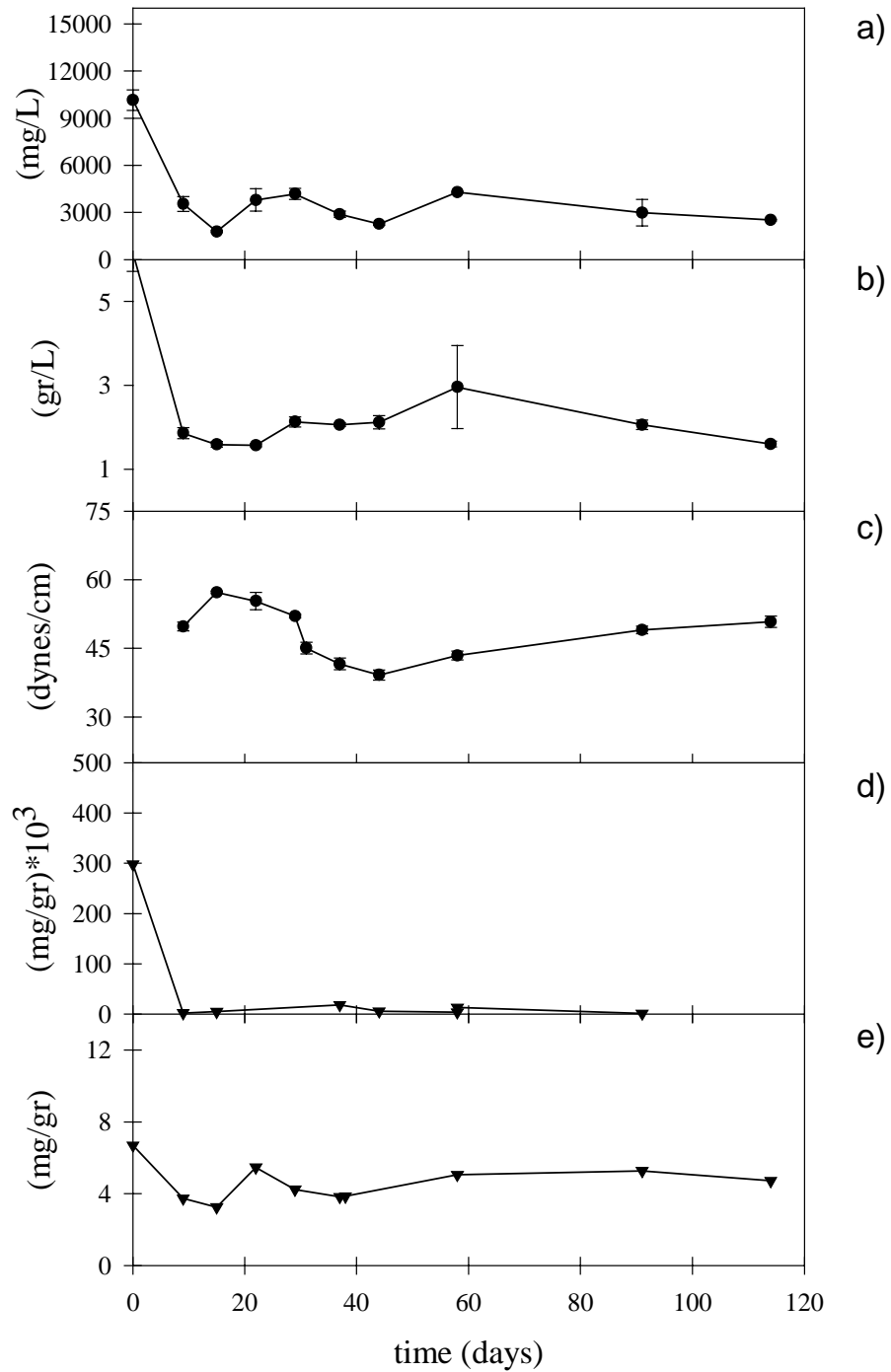


Figure B2. Results for introduced soil culture 10 percent aerated batch reactor (W): a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Plots a) - c) include error bars.

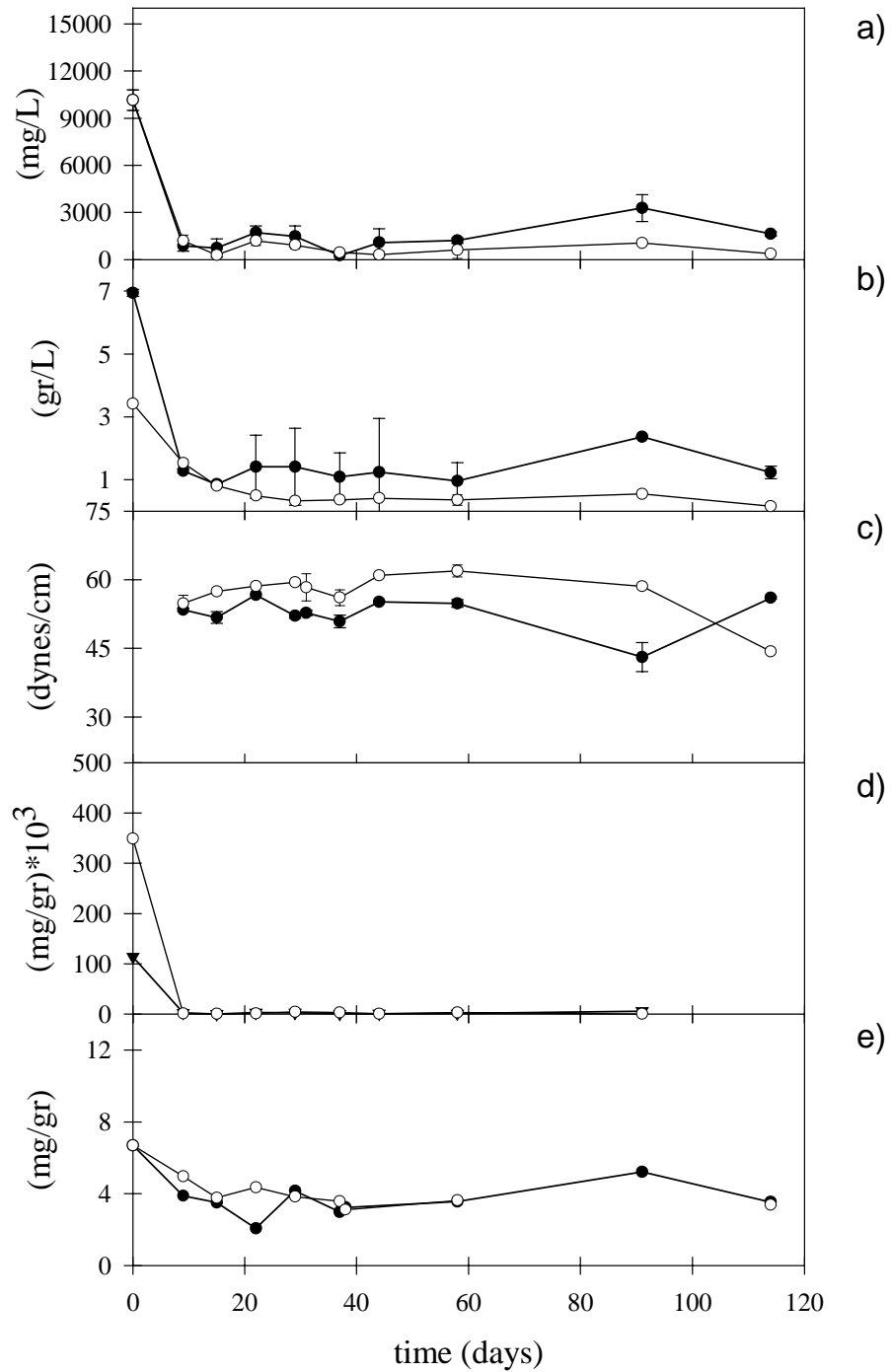


Figure B3. Results for introduced *Rhodococcus* culture 10 percent aerated batch reactor: a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. (● trial 1 data (Rh1) , ○ trial 2 data (Rh2)). Plots a) - c) include error bars.

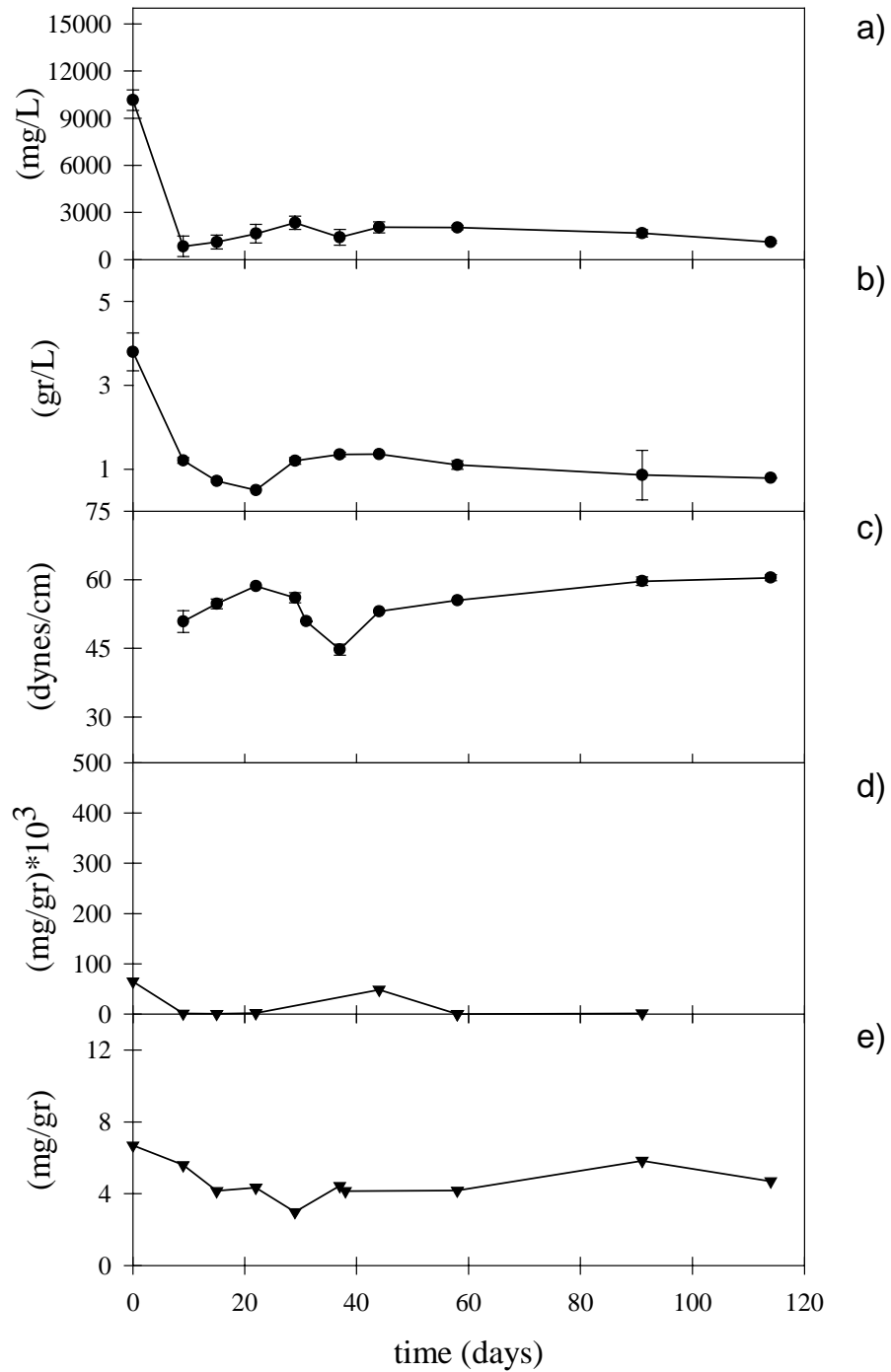


Figure B4. Results for trial 2 for introduced pure culture mixture 10 percent aerated batch reactor (M): a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Plots a) - c) include error bars.

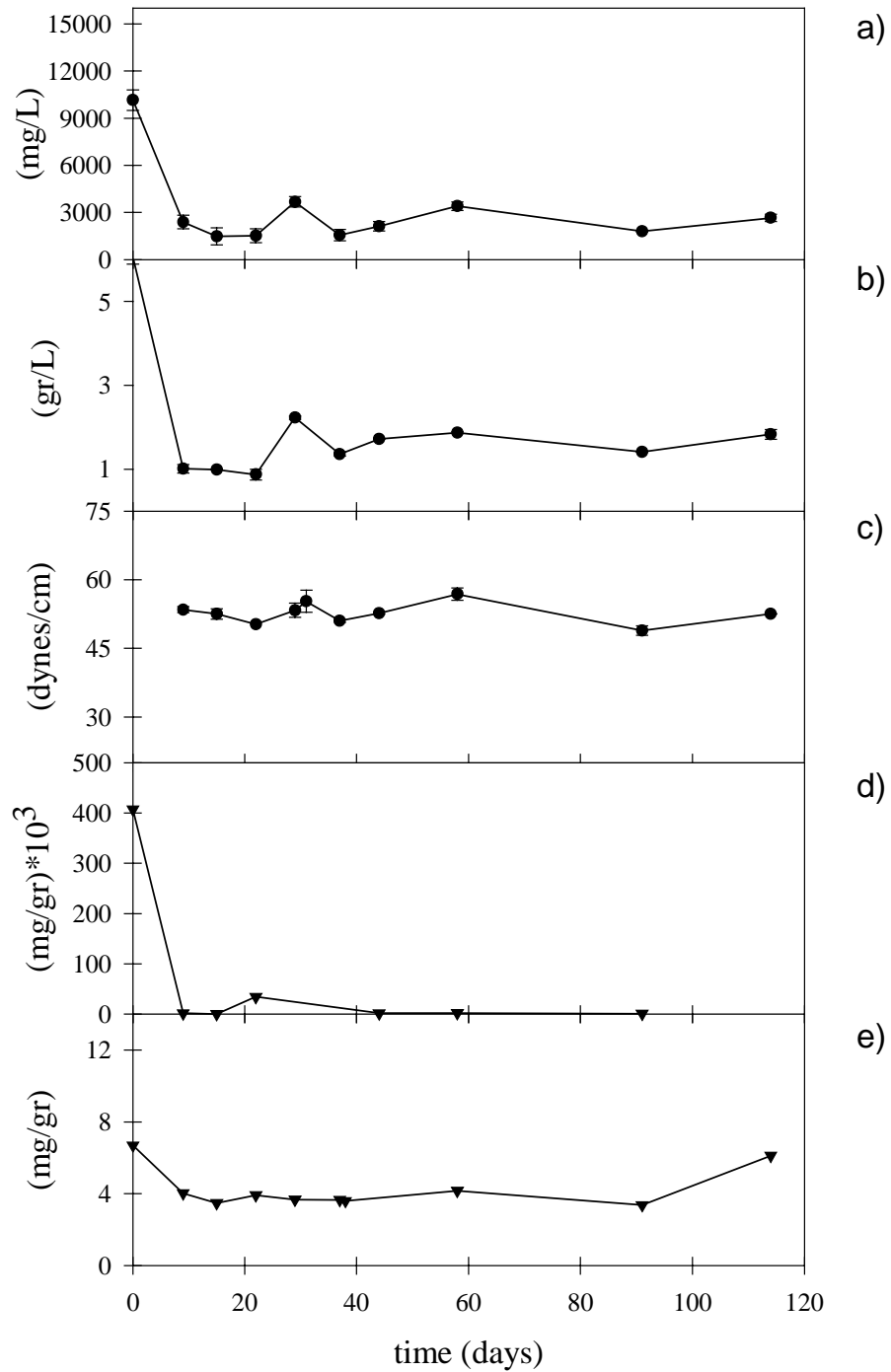


Figure B5. Results for trial 3 for introduced pure culture mixture 10 percent aerated batch reactor (M1): a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Plots a) - c) include error bars.

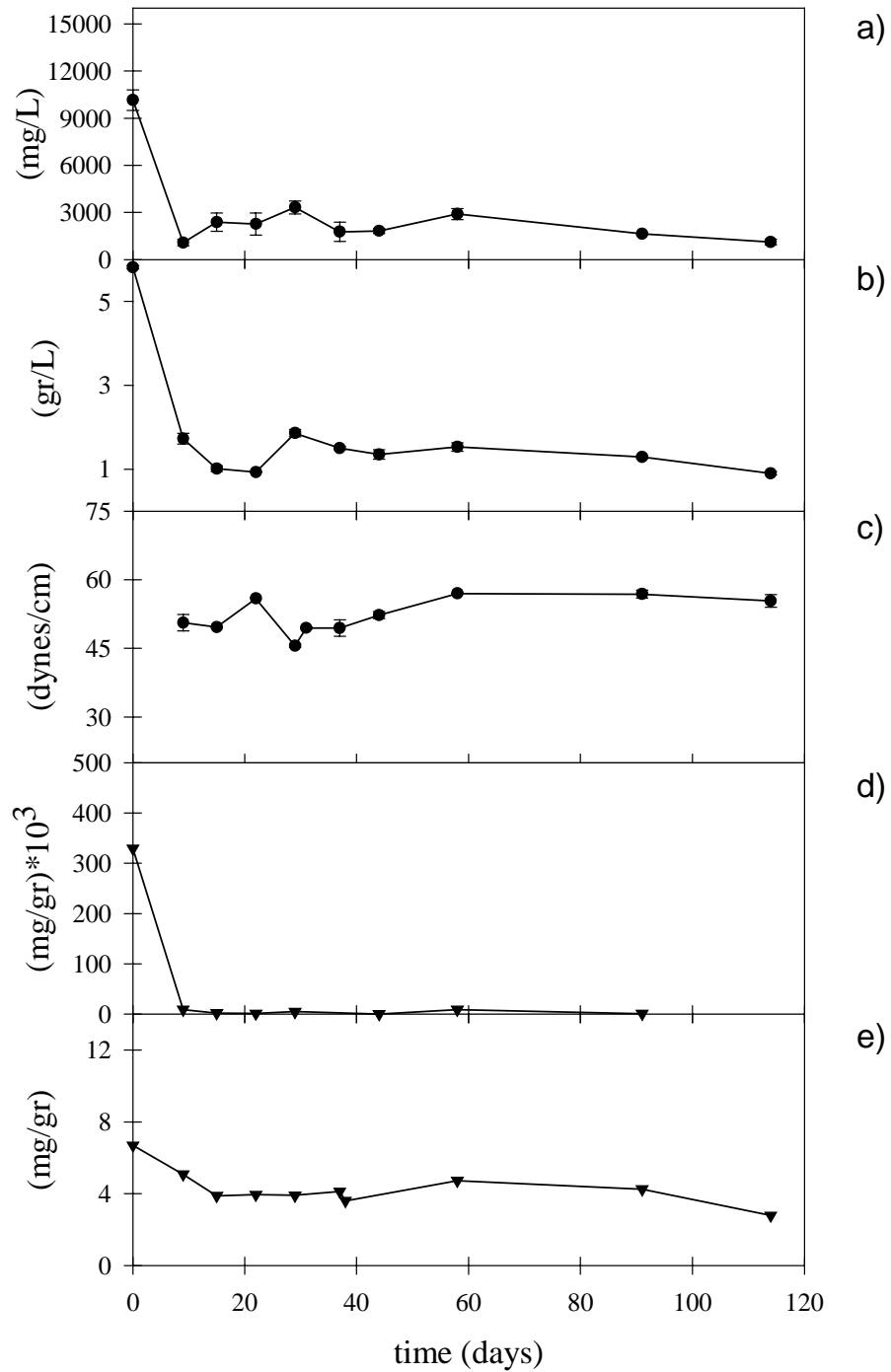


Figure B6. Results for trial 4 for introduced pure culture mixture 10 percent aerated batch reactor (M2): a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Plots a) - c) include error bars.

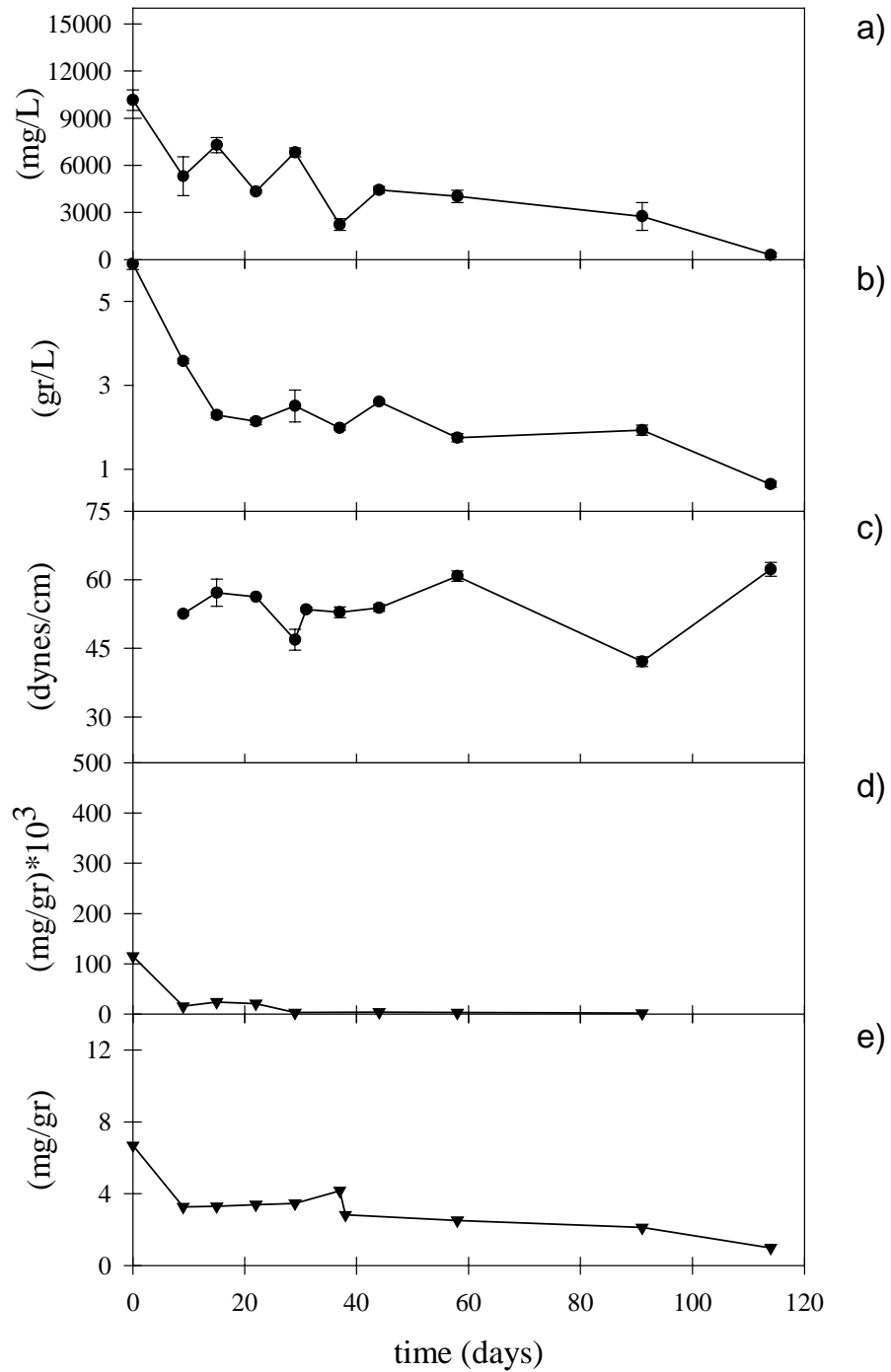


Figure B7. Results for trial 2 for introduced pure culture mixture 10 percent aerated batch reactor (M3): a) COD, b) suspended solids, c) surface tension, d) extractable oil, e) non-extractable oil. Plots a) - c) include error bars.

## **APPENDIX C**

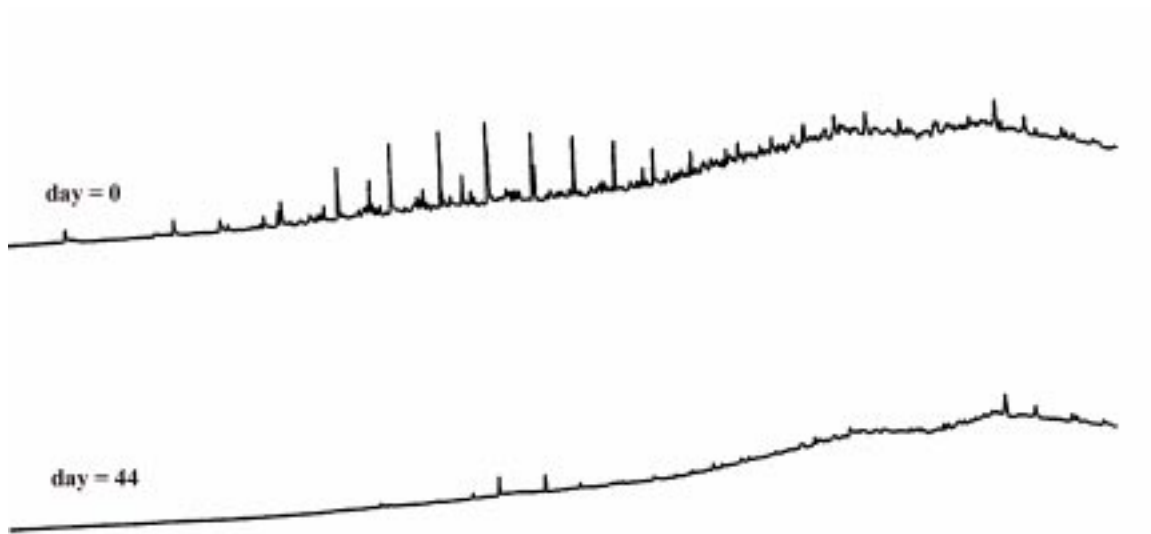


Figure C1. Selected chromatographs for waste oil #1 introduced organisms 10% sacrificial batch reactors.

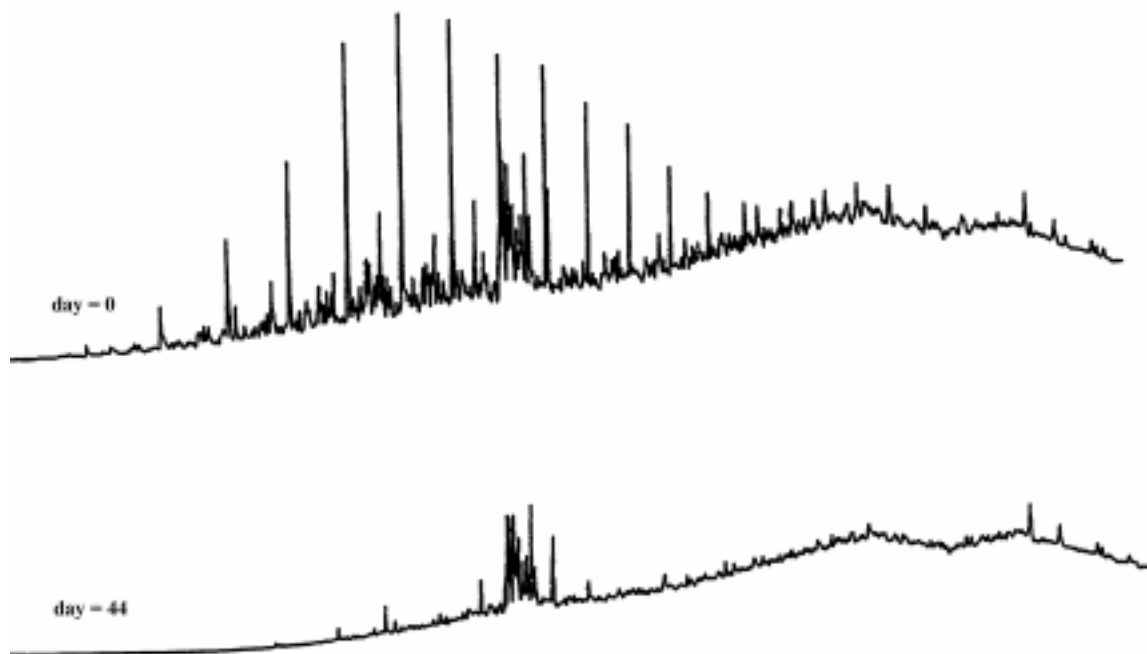


Figure C2. Selected chromatographs for waste oil #2 introduced organisms 10% sacrificial batch reactors.

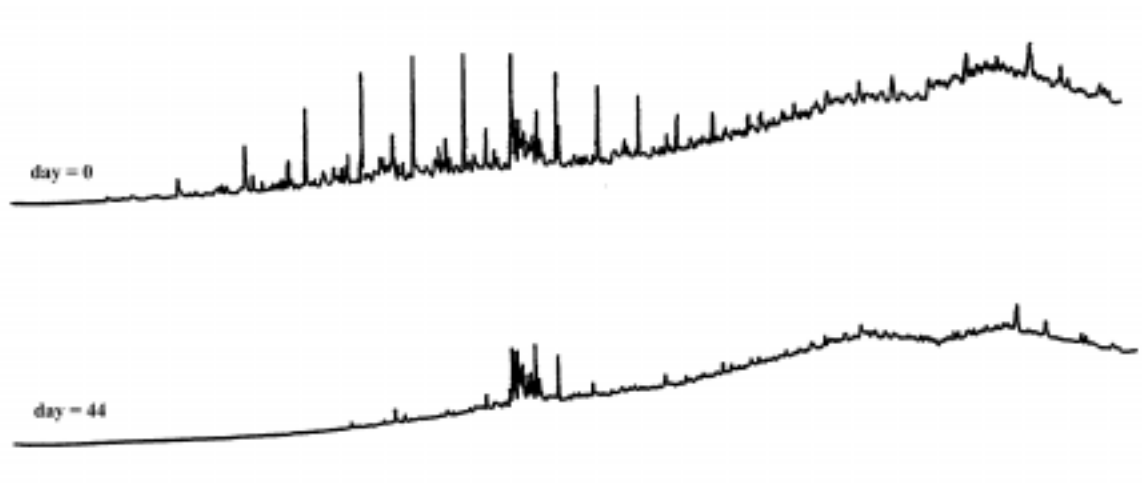


Figure C3. Selected chromatographs for mixture waste oil #1 and #2 introduced organisms 10% sacrificial batch reactors.

## **APPENDIX D**

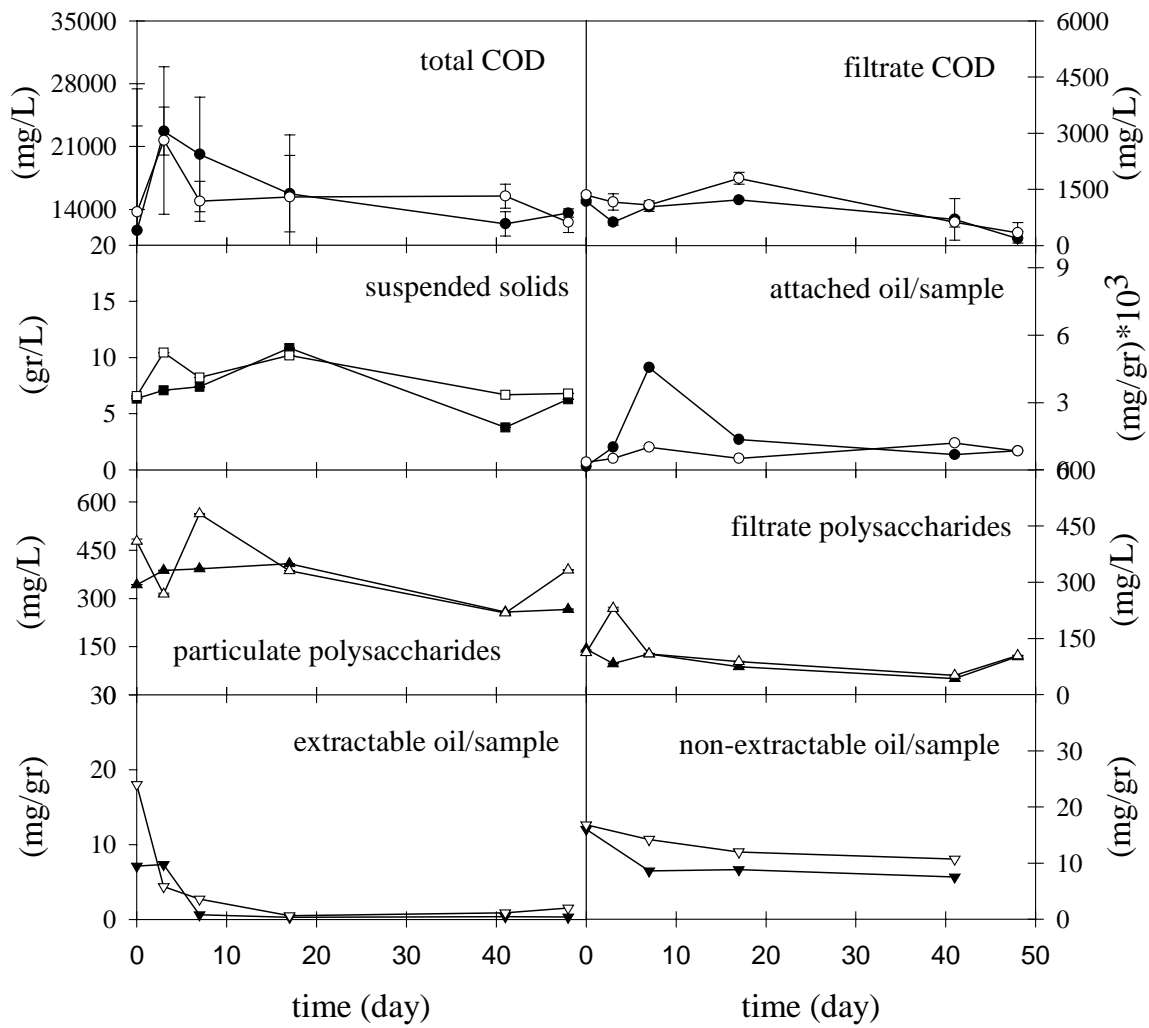


Figure D1. Results for waste oil #1 20 percent sacrificial batch reactor experiment. (● native organisms, ○ introduced organisms). Error bars are included for total COD, filtrate COD, suspended solids, particulate polysaccharides, and filtrate polysaccharides.

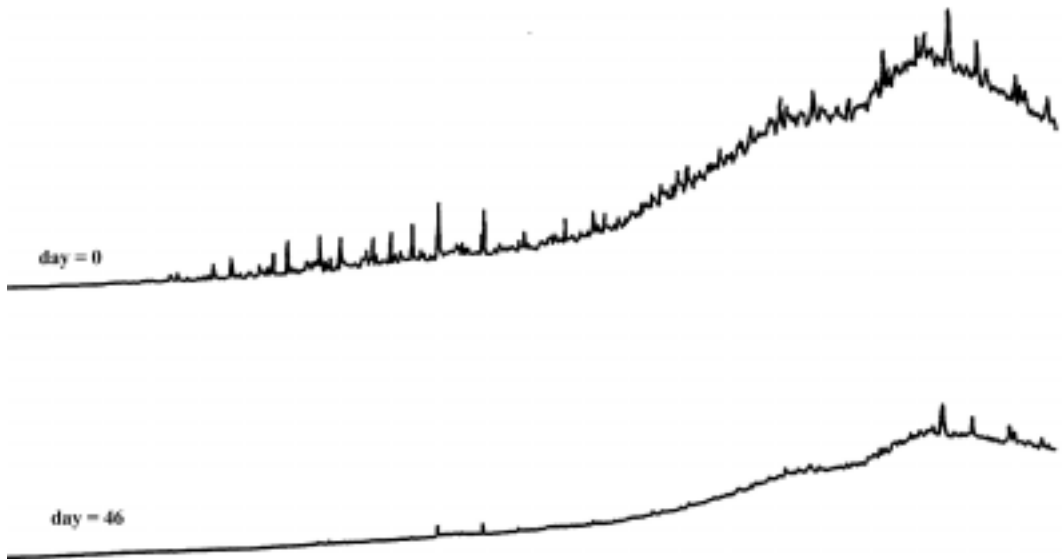


Figure D2. Selected chromatograph for waste oil #1 introduced organisms 20% sacrificial batch reactors.

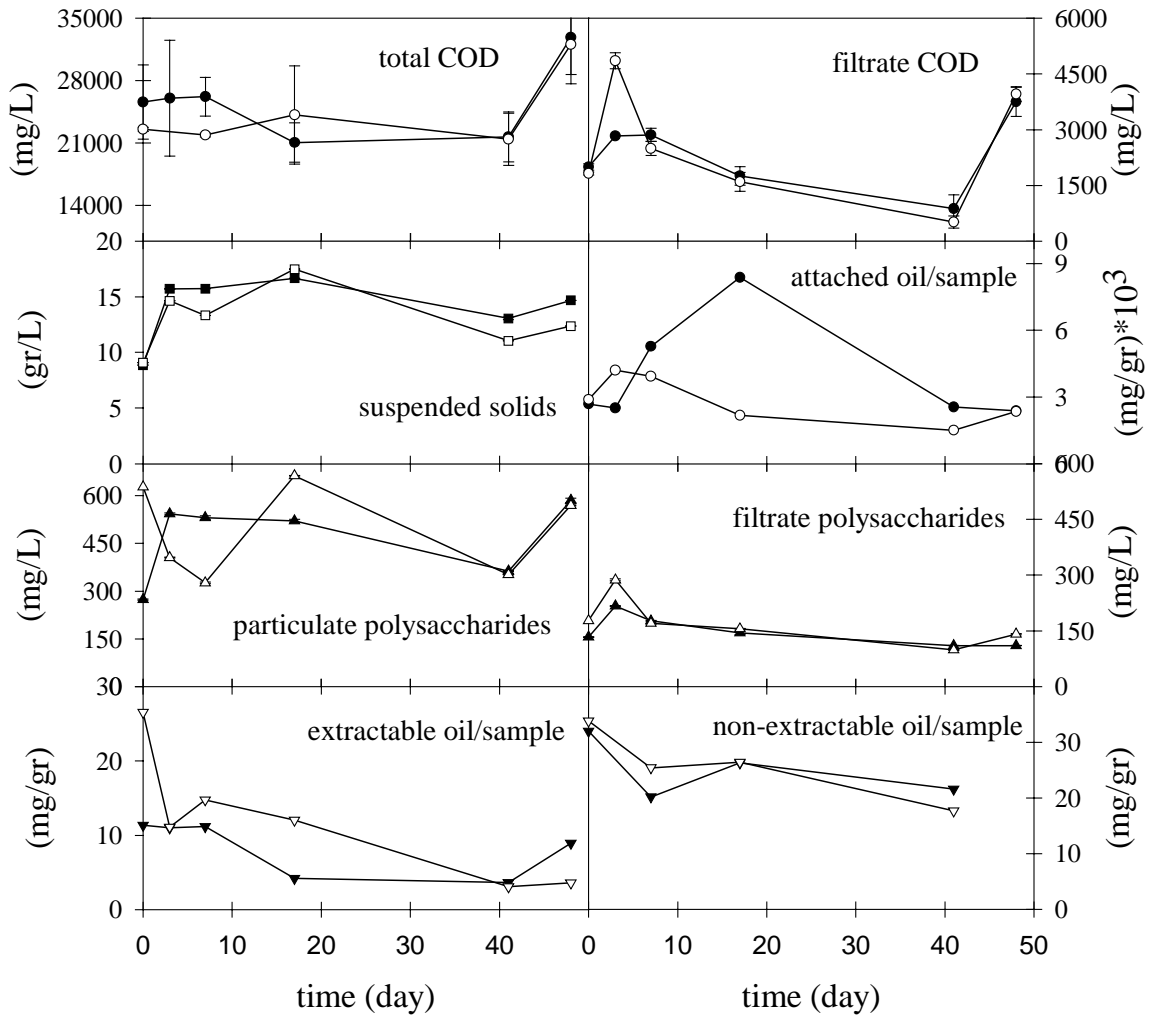


Figure D3. Results for waste oil #2 20 percent sacrificial batch reactor experiment. (● native organisms, ○ introduced organisms). Error bars are included for total COD, filtrate COD, suspended solids, particulate polysaccharides, and filtrate polysaccharides.

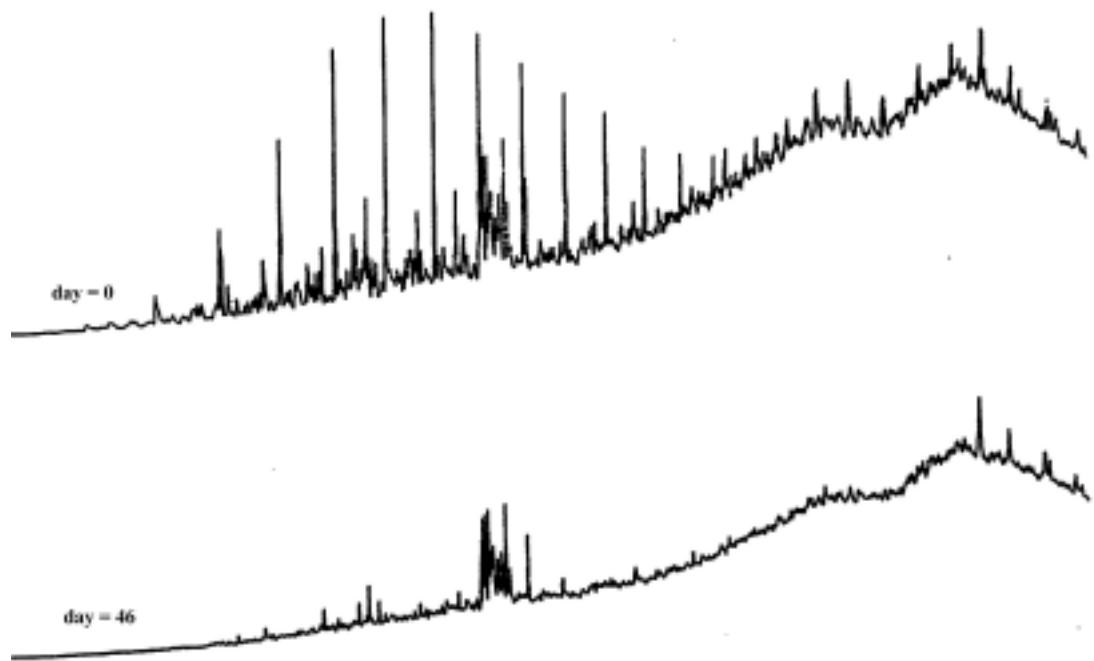


Figure D4. Selected chromatograph for waste oil #2 introduced organisms 20% sacrificial batch reactors.

## **APPENDIX E**

Table E1. Experimental COD concentration values for aerated batch reactor experiments.

	date time	31-Mar 0	9-Apr 9	15-Apr 15	22-Apr 22	29-Apr 29	7-May 37	14-May 44	28-May 58	1-Jul 91	23-Jul 114
reactor C	(mg/L)	10149 ± 653	8157 ± 846	4745 ± 107	5791 ± 272	4740 ± 1921	3634 ± 342	1208 ± 804	2039 ± 272	2361 ± 479	2201 ± 176
reactor P	(mg/L)	10149 ± 653	1814 ± 842	2203 ± 380	3410 ± 118	6000 ± 164	4329 ± 333	3008 ± 45	2196 ± 0	1810 ± 208	1433 ± 119
reactor W	(mg/L)	10149 ± 653	3550 ± 469	1785 ± 54	3795 ± 711	4181 ± 355	2887 ± 208	2270 ± 79	4288 ± 45	2990 ± 833	2520 ± 48
reactor Rh1	(mg/L)	10149 ± 653	875 ± 348	2848 ± 2417	1718 ± 424	1471 ± 661	270 ± 0	1093 ± 863	1216 ± 166	3279 ± 849	1631 ± 133
reactor M	(mg/L)	10149 ± 653	849 ± 655	1481 ± 709	1654 ± 598	2348 ± 426	1424 ± 490	2062 ± 355	2039 ± 136	1679 ± 227	1124 ± 74
reactor Rh2	(mg/L)	10149 ± 653	1196 ± 362	304 ± 107	1205 ± 311	929 ± 219	457 ± 0	307 ± 223	627 ± 555	1062 ± 0	370 ± 86
reactor M1	(mg/L)	10149 ± 653	2392 ± 437	747 ± 581	1526 ± 446	3677 ± 328	1566 ± 354	2128 ± 298	3399 ± 275	1797 ± 23	2656 ± 235
reactor M2	(mg/L)	10149 ± 653	1080 ± 204	2392 ± 483	2269 ± 707	3329 ± 410	1769 ± 610	1826 ± 157	2902 ± 359	1639 ± 60	1124 ± 182
reactor M3	(mg/L)	10149 ± 653	5305 ± 1228	7291 ± 380	4327 ± 82	6813 ± 279	2229 ± 373	4438 ± 208	4026 ± 387	2754 ± 887	293 ± 141

Table E2. Suspended solids concentration values for aerated batch reactor experiments.

	date time	31-Mar 0	9-Apr 9	15-Apr 15	22-Apr 22	29-Apr 29	7-May 37	14-May 44	28-May 58	1-Jul 91	23-Jul 114
reactor C	(gr/L)	5.45 ± 0.01	4.89 ± 0.04	2.70 ± 0.03	2.32 ± 0.03	2.28 ± 0.13	2.68 ± 0.06	0.57 ± 0.02	1.35 ± 0.18	1.70 ± 0.09	1.70 ± 0.18
reactor P	(gr/L)	5.02 ± 0.04	2.11 ± 0.03	2.09 ± 0.10	1.88 ± 0.03	2.20 ± 0.02	2.30 ± 0.12	1.66 ± 0.05	2.17 ± 0.04	2.22 ± 0.19	1.24 ± 0.02
reactor W	(gr/L)	6.17 ± 0.45	1.86 ± 0.13	1.59 ± 0.07	1.57 ± 0.04	2.13 ± 0.12	2.06 ± 0.04	2.12 ± 0.16	2.96 ± 0.99	2.06 ± 0.11	1.60 ± 0.07
reactor Rh1	(gr/L)	6.94 ± 0.11	1.28 ± 0.06	0.85 ± 0.07	1.41 ± 1.00	1.41 ± 1.22	1.09 ± 0.76	1.24 ± 1.70	0.96 ± 0.58	2.36 ± 0.01	1.23 ± 0.20
reactor M	(gr/L)	3.80 ± 0.45	1.21 ± 0.07	0.72 ± 0.03	0.50 ± 0.00	1.20 ± 0.08	1.35 ± 0.05	1.36 ± 0.03	1.10 ± 0.10	0.86 ± 0.59	0.79 ± 0.01
reactor Rh2	(gr/L)	3.42 ± 0.00	1.53 ± 0.10	0.81 ± 0.07	0.49 ± 0.01	0.32 ± 0.05	0.36 ± 0.02	0.41 ± 0.01	0.35 ± 0.17	0.54 ± 0.00	0.15 ± 0.04
reactor M1	(gr/L)	6.09 ± 0.20	1.01 ± 0.10	0.99 ± 0.01	0.87 ± 0.13	2.23 ± 0.04	1.36 ± 0.05	1.72 ± 0.01	1.87 ± 0.02	1.41 ± 0.01	1.83 ± 0.12
reactor M2	(gr/L)	5.83 ± 0.04	1.73 ± 0.13	1.01 ± 0.07	0.93 ± 0.01	1.86 ± 0.09	1.50 ± 0.04	1.35 ± 0.11	1.53 ± 0.10	1.29 ± 0.04	0.90 ± 0.04
reactor M3	(gr/L)	5.90 ± 0.13	3.58 ± 0.06	2.29 ± 0.07	2.14 ± 0.08	2.51 ± 0.38	1.98 ± 0.05	2.61 ± 0.03	1.75 ± 0.10	1.93 ± 0.12	0.64 ± 0.07

Table E3. Surface tension values for aerated batch reactor experiments.

	date time	31-Mar 0	9-Apr 9	15-Apr 15	22-Apr 22	29-Apr 29	7-May 37	14-May 44	28-May 58	1-Jul 91	23-Jul 114
reactor C	(dynes/cm)	47.41 ± 0.64	47.99 ± 0.78	49.40	46.73 ± 0.64	43.35 ± 0.35	37.03 ± 1.17	45.47 ± 1.06	45.75 ± 1.39	48.10 ± 0.97	48.64 ± 0.41
reactor P	(dynes/cm)	56.50 ± 0.64	54.83 ± 1.34	55.81 ± 1.63	53.21 ± 1.27	53.99 ± 0.85	47.30 ± 0.31	47.59 ± 1.77	48.95 ± 0.74	51.97 ± 0.55	57.11 ± 1.79
reactor W	(dynes/cm)	49.79 ± 0.99	57.24 ± 0.28	55.32 ± 1.91	52.03 ± 0.42	45.04 ± 1.27	41.58 ± 1.27	39.19 ± 1.71	43.43 ± 0.97	49.01 ± 0.85	50.79 ± 1.25
reactor Rh1	(dynes/cm)	53.45 ± 0.35	51.74 ± 1.27	56.65	52.13 ± 0.57	52.72 ± 0.57	50.89 ± 1.40	55.17 ± 0.10	54.81 ± 0.80	43.08 ± 3.16	56.03 ± 0.25
reactor M	(dynes/cm)	50.86 ± 2.40	54.73 ± 1.06	58.63	56.06 ± 1.13	50.91 ± 0.07	44.72 ± 1.19	53.08 ± 0.21	55.50 ± 0.12	59.68 ± 0.93	60.44 ± 0.67
reactor Rh1	(dynes/cm)	54.83 ± 1.77	57.44 ± 0.28	58.63	59.42 ± 0.28	58.33 ± 2.97	56.09 ± 1.72	60.94 ± 0.47	61.90 ± 1.30	58.56 ± 0.35	44.33 ± 0.23
reactor M1	(dynes/cm)	53.45 ± 0.64	52.52 ± 1.13	50.28	53.31 ± 1.56	55.27 ± 2.40	50.99 ± 0.31	52.69 ± 0.23	56.85 ± 1.35	48.88 ± 1.00	52.56 ± 0.06
reactor M2	(dynes/cm)	50.62 ± 1.77	49.64 ± 0.21	55.86	45.57 ± 0.35	49.45 ± 0.21	49.43 ± 1.81	52.26 ± 0.76	56.98 ± 0.12	56.85 ± 0.85	55.37 ± 1.35
reactor M3	(dynes/cm)	52.52 ± 0.28	57.14 ± 2.97	56.26	46.88 ± 2.26	53.50 ± 0.42	52.88 ± 1.16	53.86 ± 0.76	60.78 ± 1.12	42.09 ± 1.05	62.27 ± 1.48

Table E4. Extractable oil concentration for aerated batch reactor experiment.

	date time	31-Mar 0	9-Apr 9	15-Apr 15	22-Apr 22	29-Apr 29	7-May 37	14-May 44	28-May 58	1-Jul 91
reactor C	(mg/gr)	0.4564	0.0767	0.0127	0.1507	0.0549	0.0076	0.0311	0.0252	0.0019
reactor P	(mg/gr)	0.4044	0.0063	0.0576	0.0110		0.0610	0.0088	0.0092	0.0010
reactor W	(mg/gr)	0.2981	0.0023	0.0048		0.0182	0.0058	0.0038	0.0132	0.0012
reactor Rh1	(mg/gr)	0.1137	0.0024	0.0003	0.0031	0.0029	0.0007	0.0009	0.0012	0.0061
reactor M	(mg/gr)	0.0649	0.0012	0.0007	0.0022		0.0488	0.0004	0.0002	0.0014
reactor Rh2	(mg/gr)	0.3494	0.0013	0.0003	0.0015	0.0047	0.0031	0.0009	0.0030	0.0004
reactor M1	(mg/gr)	0.4071	0.0016	0.0005	0.0344		0.0017		0.0019	0.0008
reactor M2	(mg/gr)	0.3291	0.0092	0.0019	0.0011	0.0048		0.0004	0.0090	0.0010
reactor M3	(mg/gr)	0.1505	0.0155	0.0243	0.0209	0.0031		0.0042	0.0031	0.0016

Table E5. Nonextractable oil concentration for aerated batch reactor experiment.

	date time	31-Mar 0	9-Apr 9	15-Apr 15	22-Apr 22	29-Apr 29	7-May 37	14-May 44	28-May 58	1-Jul 91	23-Jul 114
reactor C	(mg/gr)	6.69	6.73	4.47	5.12	4.97	4.72	5.33	6.34	6.28	5.45
reactor P	(mg/gr)	6.69	2.26	2.53	2.60	2.50	2.73	2.99		2.41	1.61
reactor W	(mg/gr)	6.69	3.74	3.26	5.47	4.24	3.83	3.86	5.06	5.27	4.72
reactor Rh1	(mg/gr)	6.69	3.89	3.52	2.07	4.16	2.98	3.24	3.57	5.22	3.54
reactor M	(mg/gr)	6.69	5.60	4.16	4.35	2.98	4.44	4.15	4.18	5.86	4.70
reactor Rh2	(mg/gr)	6.69	4.97	3.77	4.36	3.84	3.58	3.12	3.63		3.39
reactor M1	(mg/gr)	6.69	4.03	3.49	3.92	3.68	3.66	3.60	4.17	3.37	6.12
reactor M2	(mg/gr)	6.69	5.08	3.89	3.95	3.91	4.13	3.61	4.74	4.26	2.79
reactor M3	(mg/gr)	6.69	3.27	3.31	3.39	3.47	4.17	2.83	2.52	2.12	0.99

Table E6. COD concentration values for trial 1 of filtered oil reactor experiment.

date time	3-Feb 0	5-Feb 2	9-Feb 6	12-Feb 9	17-Feb 14	24-Feb 21
total (mg/L)	196.7 ± 11	196.7 ± 6	170.3 ± 0	141.2 ± 13	127.0 ± 16	113.5 ± 1
filtered (mg/L)	166.8 ± 49	51.1 ± 11	50.7 ± 2	81.1 ± 5	36.2 ± 20	62.7 ± 9

Table E7. Suspended solids concentration values for trial 1 of filtered oil reactor experiment.

date time	3-Feb 0	5-Feb 2	9-Feb 6	12-Feb 9	17-Feb 14	24-Feb 21
total (mg/L)	192.00 ± 56.04	368.33 ± 2.36	360.00 ± 58.57	458.33 ± 16.50	400.00 ± 18.86	335.00 ± 7.07

Table E8. Polysaccharides concentration values for trial 1 of filtered oil reactor experiment.

date time	3-Feb 0	5-Feb 2	9-Feb 6	12-Feb 9	17-Feb 14	24-Feb 21
total (mg/L)	15.89 ± 0.04	13.56 ± 0.00	23.46 ± 0.01	13.51 ± 0.02	14.76 ± 0.05	7.44 ± 0.02
filtered (mg/L)	6.96 ± 0.02	5.47 ± 0.00	5.33	7.87 ± 0.01	8.52 ± 0.03	4.66 ± 0.02

Table E9. Surface tension values for trial 1 of filtered oil reactor experiment.

date time	3-Feb 0	5-Feb 2	9-Feb 6	12-Feb 9	17-Feb 14	24-Feb 21
(dynes/cm)	49.78 ± 0.28	67 ± 0.00	70.22 ± 0.21	70.72 ± 0.14	62 ± 0.14	60.01 ± 2.47

Table E10. Extractable oil concentration values for trial 1 of filtered oil reactor experiment

date time	3-Feb 0	9-Feb 6	12-Feb 9	17-Feb 14
(mg/gr)	0	0.000442	0.000482	0.000438

Table E11. COD concentration values for trial 2 of filtered oil reactor experiment.

date time	24-Feb 0	26-Feb 2	1-Mar 5	4-Mar 8	9-Mar 13	13-Mar 17	25-Mar 29	8-Apr 43
total (mg/L)	177.0 ± 17	119.6 ± 20	77.4 ± 7	71 ± 2	71.3 ± 7	61.2 ± 9	63.6 ± 5	63.6 ± 5
filtered (mg/L)	114.1 ± 6	60.6 ± 7	41.6 ± 4		22.0 ± 16.4	35 ± 3	23 ± 7	7 ± 2

Table E12. Suspended solids concentration values for trial 2 of filtered oil reactor experiment.

date time	24-Feb 0	26-Feb 2	1-Mar 5	5-Mar 9	9-Mar 13	13-Mar 17	25-Mar 29	8-Apr 43
(mg/L)	407.50 ± 0.02	411.00 ± 0.06	407.73 ± 0.02	442.50 ± 0.02	459.00 ± 0.04	525.00 ± 0.04	580.00 ± 0.12	560.00 ± 0.07

Table E13. Polysaccharides concentration values for trial 2 of filtered oil reactor experiment.

date time	24-Feb 0	26-Feb 2	1-Mar 5	4-Mar 8	8-Apr 43
total (mg/L)	15.22 ± 1.9	11.6 ± 0.3	9.9 ± 0.9	15.2 ± 1.2	9.5 ± 0.4
filtered (mg/L)	11.92 ± 1.8	8.2 ± 0.1	5.5 ± 0.4	6.2 ± 0.6	5.7 ± 0.0

Table E14. Surface tension values for trial 2 of filtered oil reactor experiment.

date time	24-Feb 0	25-Feb 1	26-Feb 2	27-Feb 3	1-Mar 5	4-Mar 8	6-Mar 10	8-Mar 12	9-Mar 13	13-Mar 17	25-Mar 29	2-Apr 37	8-Apr 43
initial (dynes/cm)	49.8	52.9	53.8	55.1	51.8	40.0	45.5	40.0	42.44	38.24	50.00	45.52	48.43
average (dynes/cm)	50.8	59.7	61.3	64.4	60.0	44.8	56.5	50.2	53.46	39.77	53.65	65.45	53.31

Table E15. Extractable oil concentration values for trial 2 of filtered oil reactor experiment.

date time	24-Feb 0	26-Feb 2	5-Mar 9	9-Mar 13
(mg/gr)	0.00142	0.00151	0.00101	0.00038

Table E16. Surface tension changes in time for trial 2 filtered oil experiment.

24-Feb day 0	(min) (dynes/cm)	0.00	3.67	4.17	9.70								
25-Feb day 1	(min) (dynes/cm)	0.00	3.33	7.20	13.73	19.15							
26-Feb day 2	(min) (dynes/cm)	0.00	1.83	3.50	6.22	9.87	15.38	20.10					
27-Feb day 3	(min) (dynes/cm)	0.00	3.17	8.83	13.78								
1-Mar day 5	(min) (dynes/cm)	0.00	2.43	4.82	8.35	17.53	22.27	25.70					
4-Mar day 8	(min) (dynes/cm)	0.00	1.32	4.12	9.15	14.85	21.30	32.42					
6-Mar day 10	(min) (dynes/cm)	0.00	1.40	3.50	6.17	8.33	11.87	14.67	20.05	23.17	27.95	34.75	35.87
8-Mar day 12	(min) (dynes/cm)	0.00	2.15	3.50	7.82	13.20	17.97	21.08	24.08				
9-Mar day 13	(min) (dynes/cm)	0.00	4.25	5.95	10.32	13.48	19.65	26.48	36.73	43.07			
13-Mar day 17	(min) (dynes/cm)	0.00	0.50	2.45	12.57	26.55	32.33						
25-Mar day 29	(min) (dynes/cm)	0.00	2.38	4.67	6.47	8.55	10.38	12.22	13.70	15.38	17.90	22.90	
2-Apr day 37	(min) (dynes/cm)	0.00	1.70	3.33	7.43	8.80	11.05	12.70	15.78				
8-Apr day 43	(min) (dynes/cm)	0.00	2.45	5.55	15.87	26.13							

Table E17. Total COD concentration values for 10 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/L)	11309 ± 1041	9589 ± 902	11863 ± 272	7534 ± 549
waste #1 (native org.)	(mg/L)	11570 ± 625	11123 ± 1369	15712 ± 2043	10149 ± 647
waste #2 (introduced org.)	(mg/L)	15531 ± 2134	18521 ± 1095	12884 ± 567	12919 ± 517
waste #2 (native org.)	(mg/L)	16417 ± 271	18301 ± 2114	18069 ± 416	6731 ± 359
waste #1and #2 (introduced org.)	(mg/L)	15583 ± 651	21753 ± 528	20661 ± 778	16673 ± 1967
waste #1and #2 (native org.)	(mg/L)	16417 ± 682	16932 ± 1078	11679 ± 181	8388 ± 439

Table E18. Supernatant COD concentration values for 10 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/L)	224 ± 92	1259 ± 95	737 ± 71	438 ± 44
waste #1 (native org.)	(mg/L)	175 ± 40	1039 ± 89	811 ± 106	498 ± 55
waste #2 (introduced org.)	(mg/L)	766 ± 28	1778 ± 89	1576 ± 29	782 ± 142
waste #2 (native org.)	(mg/L)	476 ± 73	2017 ± 64		969 ± 21
waste #1and #2 (introduced org.)	(mg/L)	405 ± 132	1012 ± 105	1299 ± 21	521 ± 20
waste #1and #2 (native org.)	(mg/L)	476 ± 9	1359 ± 51	1159 ± 32	1502 ± 19

Table E19. Suspended solids concentration values for 10 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	15-Dec 5	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(gr/L)	5.60 ± 0.17	5.05 ± 2.02	4.70 ± 0.00	4.05 ± 0.01	4.46 ± 0.04
waste #1 (native org.)	(gr/L)	5.87 ± 0.10	6.39 ± 0.27	6.27 ± 0.41	3.90 ± 0.06	5.86 ± 0.22
waste #2 (introduced org.)	(gr/L)	7.21 ± 0.35	9.35 ± 0.86	7.41 ± 0.69	5.53 ± 0.04	5.33 ± 0.48
waste #2 (native org.)	(gr/L)	7.47 ± 0.35	7.97 ± 0.81	8.84 ± 0.37	6.53 ± 0.18	4.28 ± 0.24
waste #1and #2 (introduced org.)	(gr/L)	8.13 ± 0.49	7.27 ± 0.44	7.67 ± 0.44	7.18 ± 0.42	7.47 ± 0.45
waste #1and #2 (native org.)	(gr/L)	7.85 ± 0.49	7.31 ± 0.44	8.15 ± 0.44	5.61 ± 0.42	6.30 ± 0.45

Table E20. Total polysaccharides concentration values for 10 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/L)	300.0 ± 0.3	273.2 ± 1.3	298.8 ± 1.0	195.9 ± 0.2
waste #1 (native org.)	(mg/L)	227.5 ± 6.0	216.6 ± 3.0	383.5 ± 1.1	284.0 ± 0.5
waste #2 (introduced org.)	(mg/L)	319.6 ± 1.1	487.8 ± 7.1	340.6 ± 1.9	340.3 ± 0.3
waste #2 (native org.)	(mg/L)	389.7 ± 6.0	400.2 ± 5.3	496.7 ± 0.3	165.8 ± 0.5
waste #1and #2 (introduced)	(mg/L)	345.2 ± 2.6	652.2 ± 5.1	919.0 ± 0.5	432.4 ± 0.6
waste #1and #2 (native)	(mg/L)	479.5 ± 2.3	477.5 ± 6.5	395.6 ± 1.4	302.6 ± 1.3

Table E21. Supernatant polysaccharides concentration values for 10 percent sacrificial batch reactor experiment.

date time		10-Dec 0	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/L)	57.6 ± 2.2	34.2 ± 0.0	73.7 ± 1.5	79.6 ± 0.9
waste #1 (native org.)	(mg/L)	54.6 ± 2.1	33.4 ± 2.5	112.2 ± 3.2	57.1 ± 1.3
waste #2 (introduced org.)	(mg/L)	53.9 ± 3.1	46.3 ± 0.0	80.4 ± 2.0	76.3 ± 0.5
waste #2 (native org.)	(mg/L)	51.7 ± 2.0	37.9 ± 0.8	149.7 ± 1.5	89.5 ± 0.5
waste #1and #2 (introduced)	(mg/L)	61.7 ± 3.6	39.9 ± 0.4	137.9 ± 5.4	133.0 ± 1.8
waste #1and #2 (native)	(mg/L)	63.9 ± 4.3	38.8 ± 2.0	130.2 ± 1.3	153.1 ± 0.5

Table E22. Extractable oil concentration values for 10 percent sacrificial batch reactor experiment.

date time		10-Dec 0	15-Dec 5	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/gr)	1.886	0.133	0.254	0.065	0.073
waste #1 (native org.)	(mg/gr)	1.270	0.253	0.142	0.033	0.059
waste #2 (introduced org.)	(mg/gr)	2.996	2.158	1.423	0.331	1.338
waste #2 (native org.)	(mg/gr)	3.579		1.027	0.722	0.085
waste #1and #2 (introduced)	(mg/gr)	1.574	1.274	0.724	0.222	0.151
waste #1and #2 (native org.)	(mg/gr)	9.207	1.550	1.212	0.207	0.245

Table E23. Nonextractable oil concentration values for 10 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	15-Dec 5	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/gr)	19.6	20.1	19.6	20.7	20.1
waste #1 (native org.)	(mg/gr)	20.5	21.5	19.1	19.7	19.2
waste #2 (introduced org.)	(mg/gr)	22.6	23.4	21.4	22.2	21.0
waste #2 (native org.)	(mg/gr)	22.1	23.3	22.4	22.5	20.0
waste #1and #2 (introduced org)	(mg/gr)	21.6	23.9	22.3	23.1	20.7
waste #1and #2 (native org.)	(mg/gr)	21.7	21.4	20.4	20.7	18.8

Table E24. Attached oil concentration values for 10 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	19-Dec 9	5-Jan 26	23-Jan 44
waste #1 (introduced org.)	(mg/gr)	0.00033	0.00130		0.00220
waste #1 (native org.)	(mg/gr)	0.00068	0.00069	0.00066	0.00119
waste #2 (introduced org.)	(mg/gr)	0.00133	0.00083	0.00084	0.00198
waste #2 (native org.)	(mg/gr)	0.00085	0.00067	0.00066	0.00197
waste #1and #2 (introduced)	(mg/gr)	0.00081	0.00082	0.00049	0.00230
waste #1and #2 (native org.)	(mg/gr)	0.00148	0.00114	0.00081	0.00285

Table E25. Total COD concentration values for 20 percent sacrificial batch reactor experiment.

	date time	26-Sep 0	29-Sep 3	3-Oct 7	13-Oct 17	6-Nov 41	12-Nov 48
waste #1 (native org.)	(mg/L)	11650	22710 ± 2682	20131 ± 6388	15742 ± 4264	12387 ± 1341	13595 ± 453
waste #2 (native org.)	(mg/L)	25600 ± 4157	26019 ± 6493	26189 ± 2176	21039 ± 2204	21677 ± 2791	32834 ± 4137
waste #1 (introduced org.)	(mg/L)	13722 ± 2373	21677 ± 8212	14902 ± 2218	15371 ± 6944	15484 ± 1341	12549 ± 1109
waste #2 (introduced org.)	(mg/L)	22533 ± 231		21889 ± 2211	24156 ± 5510	21419 ± 2931	32052 ± 4422

Table E25. Supernatant COD concentration values for 20 percent sacrificial batch reactor experiment.

	date time	26-Sep 0	29-Sep 3	3-Oct 7	13-Oct 17	6-Nov 41	12-Nov 48
waste #1 (native org.)	(mg/L)	1179 ± 43	619 ± 77	1032 ± 118	1218 ± 22	697 ± 558	182 ± 197
waste #2 (native org.)	(mg/L)	1997 ± 86		2863 ± 180	1757 ± 253	877 ± 366	3752 ± 391
waste #1 (introduced org.)	(mg/L)	1349 ± 55	1161 ± 219	1084 ± 109	1795 ± 160	619 ± 134	339 ± 275
waste #2 (introduced org.)	(mg/L)	1824 ± 39	4857 ± 216	2497 ± 185	1600 ± 253	516 ± 161	3961 ± 197

Table E26. Suspended solids concentration values for 20 percent sacrificial batch reactor experiment.

	date time	26-Sep 0	29-Sep 3	3-Oct 7	13-Oct 17	6-Nov 41	12-Nov 48
waste #1 (native org.)	(gr/L)	6.34 ± 0.004	7.08 ± 0.005	7.39 ± 0.006	10.84 ± 0.005	3.79 ± 0.002	6.29 ± 0.003
waste #2 (native org.)	(gr/L)	8.86 ± 0.003	15.71 ± 0.003	15.73 ± 0.007	16.66 ± 0.006	13.05 ± 0.107	14.68 ± 0.003
waste #1 (introduced org.)	(gr/L)	6.58 ± 0.007	10.45 ± 0.011	8.24 ± 0.000	10.19 ± 0.005	6.69 ± 0.003	6.81 ± 0.005
waste #2 (introduced org.)	(gr/L)	9.08 ± 0.004	14.63 ± 0.001	13.32 ± 0.001	17.48 ± 0.005	11.05 ± 0.007	12.36 ± 0.006

Table E27. Total polysaccharides concentration values for 20 percent sacrificial batch reactor experiment.

	date time	26-Sep 0	29-Sep 3	3-Oct 7	13-Oct 17	6-Nov 41	12-Nov 48
waste #1 (native org.)	(mg/L)	464.46 ± 0.30	469.75 ± 1.92	500.92 ± 1.72	482.84 ± 0.58	300.90 ± 2.28	367.19 ± 0.98
waste #2 (native org.)	(mg/L)	590.51 ± 1.01	544.15 ± 0.68	671.93 ± 2.34	474.73 ± 0.57	306.90 ± 0.92	492.93 ± 1.12
waste #1 (introduced org.)	(mg/L)	406.73 ± 2.00	759.50 ± 2.83	707.43 ± 5.61	665.34 ± 0.35	473.22 ± 0.27	695.02 ± 6.59
waste #2 (introduced org.)	(mg/L)	804.69 ± 0.00	692.37 ± 1.92	496.41 ± 2.74	817.83 ± 0.80	450.62 ± 1.87	710.16 ± 0.35

Table E28. Supernatant polysaccharides concentration values for 20 percent sacrificial batch reactor experiment.

	date time	26-Sep 0	29-Sep 3	3-Oct 7	13-Oct 17	6-Nov 41	12-Nov 48
waste #1 (native org.)	(mg/L)	121.69 ± 0.00	82.89 ± 1.00	108.63 ± 1.11	74.70 ± 0.21	42.76 ± 0.18	101.52 ± 2.05
waste #2 (native org.)	(mg/L)	112.53 ± 6.48	230.48 ± 2.54	108.69 ± 1.67	88.08 ± 0.85	51.87 ± 1.11	104.29 ± 1.24
waste #1 (introduced org.)	(mg/L)	133.00 ± 1.01	216.89 ± 0.62	176.83 ± 0.92	144.61 ± 4.51	110.12 ± 0.84	110.12 ± 0.84
waste #2 (introduced org.)	(mg/L)	177.50 ± 0.00	286.97 ± 2.79	170.32 ± 0.61	155.49 ± 0.49	98.41 ± 0.41	140.78 ± 1.79

Table E29. Extractable oil concentration values for 20 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	15-Dec 3	19-Dec 7	5-Jan 17	23-Jan 41
waste #1 (introduced org.)	(mg/gr)	7.115	7.340	0.598	0.287	0.371
waste #1 (native org.)	(mg/gr)	11.364	10.991	11.176	4.202	3.656
waste #2 (introduced org.)	(mg/gr)	18.013	4.361	2.703	0.478	0.861
waste #2 (native org.)	(mg/gr)	26.537	11.103	14.762	12.044	3.080

Table E30. Nonextractable oil concentration values for 20 percent sacrificial batch reactor experiment.

	date time	26-Sep 0	3-Oct 7	13-Oct 17	6-Nov 41
waste #1 (native org.)	(mg/gr)	16.0	8.6	8.8	7.5
waste #2 (native org.)	(mg/gr)	32.0	20.2	26.3	21.6
waste #1 (introduced org.)	(mg/gr)	16.8	14.2	12.0	10.7
waste #2 (introduced org.)	(mg/gr)	33.8	25.4	26.4	17.7

Table E31. Attached oil concentration values for 20 percent sacrificial batch reactor experiment.

	date time	10-Dec 0	15-Dec 3	19-Dec 7	5-Jan 17	23-Jan 41
waste #1 (introduced org.)	(mg/gr)	0.000	0.001	0.005	0.001	0.001
waste #1 (native org.)	(mg/gr)	0.003	0.003	0.005	0.008	0.003
waste #2 (introduced org.)	(mg/gr)	0.000	0.001	0.001	0.001	0.001
waste #2 (native org.)	(mg/gr)	0.003	0.004	0.004	0.002	0.002

Table E32. Total solids for the dewatering experiment.

	date time	9-Dec 0	23-Dec 14	20-Jan 42	6-Feb 59	26-Feb 79
waste #1 total	(mg/gr)	25.82	19.91	18.69	17.90	17.14
8 mm filtrate	(mg/gr)	22.28	18.61	16.55	16.39	14.72
waste #2 total	(mg/gr)	39.92	27.22	24.93	22.48	21.83
8 mm filtrate	(mg/gr)	20.26	22.43	21.00	21.79	18.91

Table E33. Suspended solids for the dewatering experiment.

	date time	9-Dec 0	23-Dec 14	20-Jan 42	6-Feb 59	26-Feb 79
waste #1 total	(mg/L)	5.600 ± 0.170	4.275 ± 0.800	3.194 ± 0.400	3.380 ± 0.230	2.911 ± 0.570
8 mm filtrate	(mg/L)	0.092 ± 0.000	0.334 ± 0.100	0.037 ± 0.004	0.053 ± 0.002	0.039 ± 0.001
waste #2 total	(mg/L)	7.210 ± 0.300	6.492 ± 0.500	4.664 ± 0.460	4.487 ± 0.240	3.385 ± 0.190
8 mm filtrate	(mg/L)	0.173 ± 0.000	0.170 ± 0.003	0.154 ± 0.010	0.097 ± 0.003	0.126 ± 0.004

Table E33. Volatile suspended solids for the dewatering experiment.

	date time	9-Dec 0	23-Dec 14	20-Jan 42	6-Feb 59	26-Feb 79
waste #1 total	(mg/L)	3.63 ± 0.13	2.59 ± 0.20	2.14 ± 0.03	2.06 ± 0.01	1.94 ± 0.12
8 mm filtrate	(mg/L)	0.03 ± 0.00	0.20 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00
waste #2 total	(mg/L)	4.93 ± 0.30	3.75 ± 0.04	2.96 ± 0.35	2.51 ± 0.09	1.72 ± 0.01
8 mm filtrate	(mg/L)	0.03 ± 0.00	0.13 ± 0.00	0.11 ± 0.00	0.05 ± 0.00	0.08 ± 0.00

Table E34. COD solids for the dewatering experiment.

date time		9-Dec 0	23-Dec 14	20-Jan 42	6-Feb 59	26-Feb 79
waste #1 total	(mg/L)	7895 ± 1044	6557 ± 596	6505 ± 1282	4157 ± 954	5192 ± 1560
8 mm filtrate	(mg/L)	710 ± 8	715 ± 88	147 ± 5	167 ± 72	194 ± 45
waste #2 total	(mg/L)	15531 ± 2134	9757 ± 273	9233 ± 1726	5176 ± 490	4079 ± 904
8 mm filtrate	(mg/L)	783 ± 89	752 ± 42	876 ± 282	448 ± 48	659 ± 37

Table E35. CST for the dewatering experiment.

date time		9-Dec 0	23-Dec 14	20-Jan 42	6-Feb 59	26-Feb 79
waste #1	(sec)	15.3 ± 0.5	30.4 ± 0.4	18.6 ± 0.3	21.4 ± 0.5	15.7 ± 0.6
waste #2	(sec)	16.7 ± 0.8	57.3 ± 1.6	29.6 ± 1.8	25.3 ± 0.4	22.3 ± 0.7

Table E36. Specific resistance for the dewatering experiment.

date time		9-Dec 0	23-Dec 14	20-Jan 42	6-Feb 59	26-Feb 79
waste #1	(m/kg)	1.9E+14 ± 1.0E+09	1.9E+15 ± 1.7E+09	9.5E+14 ± 2.3E+09	8.4E+14 ± 8.8E+08	7.6E+14 ± 9.9E+07
waste #2 total	(m/kg)	2.2E+14 ± 2.6E+08	1.1E+16 ± 2.8E+10	1.0E+16 ± 2.7E+10	2.9E+15 ± 7.8E+09	3.6E+15 ± 6.6E+09

## **VITA**

Agata Magdalena Fallon was born in Gliwice, Poland in 1972. In 1994 she graduated with honors from Virginia Polytechnic Institute and State University with a bachelor degree in Chemical Engineering.

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