DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ENHANCED BIOREMEDIATION OF CONTAMINANTS IN SOIL

by
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September, 2005
İZMİR

ENHANCED BIOREMEDIATION OF CONTAMINANTS IN SOIL

A Thesis Submitted to the
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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"ENHANCED BIOREMEDIATION OF CONTAMINANTS IN SOIL"** completed by **Soner YAVUZ** under supervision of **Prof. Dr. Ayşen TÜRKMAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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ABSTRACT

Contaminated soil has an important influence not only on the fertility of soil and functions of ecosystem but also on the health of animals and human beings via food chains. Contaminated soils are increasingly becoming a global problem with development of various industries day by day. The use of microorganisms or microbial processes to destroy or reduce the concentration of contaminants in a contaminated soil is used in many countries in recent years. Biodegradation process is supported by microorganisms which use the organic carbon from the pollutants as energy source and cells building blocks. One of the soil treatment method is enhanced biodegradation. In this method, the biodegradation of pollutants(oil, petroleum products, etc.) is enhanced by adding nutrients and/or microorganisms.

Different aspects of bacterial degradation of organic contaminants in soil, and how to improve the efficiency is studied in this thesis. There are a number of different procedures that have been tested to improve reliability, cost efficiency and speed of bioremediation. Bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur.

In this thesis engine oil spiked soils and a soil from Raman, which is exposed to petroleum contamination has been studied, by adding compost, activated sludge and biosurfactant to enhance the degradation rate. The results of the experimental studies show that the degradation rate increases in the presence of enhancing materials.

Keywords: Biodegradation, Bioremediation, Contaminated Soil, In-situ.

KİRLENMİŞ TOPRAK ORTAMINDA HIZLANDIRILMIŞ AYRIŞMA

ÖZET

Toprak kirliliğinin sadece toprak verimliliği ve ekosistem fonksiyonları üzerinde değil aynı zamanda besin zinciri yoluyla hayvan ve insan sağlığı üzerinde de önemli etkileri vardır. Toprak kirliliği çeşitli endüstrilerin gelişmesiyle günden güne global bir problem halini almaktadır. Mikroorganizmalarla veya mikroorganizmaların kullanıldığı proseslerle kirlenmiş topraktaki kirliliğin ayrıştırılması son yıllarda pek çok uygulama alanı bulmuştur. Biyolojik ayrışma; kirleticinin organik karbonunu kendi hücre büyümesini sağlamak için enerji kaynağı olarak kullanan mikroorganizmalarca yapılır. Toprak arıtım metotlarından birisi; hızlandırılmış ayrıştırma yöntemidir. Bu yöntemde toprakta besi maddesi yada mikroorganizma ilavesi ile ayrışma hızlandırılarak, topraktaki kirliliğin (yağ, petrol ürünleri vs.) arıtılması sağlanır.

Topraktaki organik kirliliğin bakteriyel ayrışmasında görülen değişiklikler ve bu ayrışmanın veriminin nasıl artırabileceği bu tez çalışmasında incelenecektir. Biyolojik ayrışma hızını, yöntemin güvenirliğini, verimini geliştirebilmek için çok sayıda prosedürler vardır. Biyolojik arıtma metotlarıyla ayrışmanın meydana gelebilmesi; uygun çevre koşullarında, uygun yerde, uygun mikroorganizma ile yöntemin uygulanabilmesine bağlıdır.

Bu çalışmada makine yağı ile kirletilmiş toprak ve Raman'da petrolle kirlenmeye maruz kalmış toprak örneklerinde ayrışma hızını artırmak amacıyla kompost, aktif çamur ve biyosurfaktant eklenerek denemeler yapılmıştır. Deneysel çalışma sonuçları ayrışma hızının arttığını ortaya koymaktadır.

Anahtar Kelimeler: Biyolojik Ayrışma, Biyolojik Arıtım, Kirlenmiş Toprak, Yerinde.

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CHAPTER ONE
INTRODUCTION

Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is one of the major problems threatening the industrialized world today. All the industrialized countries face contaminated soil problem in addition to water and air pollution. Especially the toxic organics which are difficult to biodegrade remain in soil media for a long time and reach human being via water and food. The national priority list currently contains over 1200 sites, with potential sites numbering over 32 000 in USA (Baker and Herson, 1994). The need to remediate these sites has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal.

Bioremediation, the use of microorganisms or microbial processes to degrade environmental contaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up of ground water, soils, lagoons, sludge's, and process-waste streams.

Bioremediation frequently must address multiphasic, heterogonous environments, such as soils in which the contaminant is present in association with the soil particles, dissolved in soil liquids, and in the soil atmosphere. Because of these complexities, successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, and chemistry.

Soil contamination from leaking underground storage tanks (LUSTs) and its adverse effects are major problems that we are facing today because LUSTs are ubiquitously distributed. Stored fuels consist of various petroleum hydrocarbons having potential toxic effect to human beings (Wang and Bartha, 1994).

Polycyclic aromatic hydrocarbons (PAHs) are one of the most prevalent contaminants found in soil. The origin of PAH contaminated soils include anthropogenic sources such as abandoned manufactured gas sites, leaking underground storage tanks, wood treatment sites, and current industrial processes. (Atlas, 1995)

Polycyclic aromatic hydrocarbons (PAHs) are pollutants of great environmental concern because of their toxic, mutagenic, and carcinogenic properties. (Pothuluri and Cerniglia, 1998; Van Agteren et al., 1998).

Bioremediation is emerging as the most cost-effective treatment method for hydrocarbon-contaminated soil, especially when the contaminants are medium distillate fuel (Wang and Bartha, 1994). Of several forms of treatment for excavated soils, composting has advantages over other types of technologies. Advantages include relatively low capital and operating costs, simplicity of operation and design, and relatively high treatment efficiency (Freeman and Harris, 1995; USEPA, 1996).

This study has been performed in the reactors that contained soil artificially contaminated by engine oil. Experiments have been conducted in three series.

The samples were taken weekly and analyzed for water content, organic matter content, water soluble chemical oxygen demand, total petroleum hydrocarbon and water soluble total organic carbon. Additionally, pH value of the soil was measured.

1.1 Soil Pollution Problems in Turkey

Hydrocarbon pollution of the subsurface, especially in unsaturated soils, has become a big problem with the development of the petrochemical industry and installation of numerous petrol stations and underground pipes. Physical, chemical and biological technologies have been developed to remove hydrocarbon pollutants from soils and restore environmental quality. However, costs are high, and many techniques are difficult to use for in-situ remediation. It still remains necessary to

study the natural attenuation of hydrocarbons in soil and to develop simple cost-effective techniques for enhanced remediation. (Atlas,1991; Edward, 1993; Leahy and Colwell, 1990; Robert, 1992).

To humans and most terrestrial organisms, soil is the most important part of the geosphere. Though only a tissue-thin layer compared to the Earth's total diameter, soil is the medium that produces most of the food required by living things. In addition to being the site of most food production, soil is the receptor of large quantities of pollutants, such as particulate matter from power plant smokestacks. Fertilizers and some other materials applied to soil often contribute to water and air pollution. Therefore, soil is a key component of environmental chemical cycles.

Accidental contamination of soil with petroleum hydrocarbons results from oil production and shipping. Oil spilled on soil is an environmental concern because contaminated soils may be unsuitable for agricultural, industrial, or recreational use and are potential sources for surface and ground water contamination. Cleanup technologies that involve excavation for incineration or burial in a landfill are costly. On-site remediation technologies offer an economical alternative that is effective at removing pollutants from soil (Bartha, 1986).

Petroleum leakage and spill are most important in soil media above an aquifer used for drinking water supply. The potential deleterious effects of oily substances upon domestic water supplies may be grouped into the following categories:

a) Hazards to the health of consumers,
b) The production of tastes and odors,
c) The presence of turbidity, films or iridescence,
d) The increased difficulty of water treatment.

Floating or emulsified oil in raw-water supplies will complicate the coagulation, flocculation and sedimentation processes at the treatment plant. Oil-coated floe may not settle properly. If free or emulsified oil reaches sand filters or ion-exchange beds it will coat. The grains, decrease the effectiveness of filtration, and interfere with back washing. Taste and odor-producing compounds will require the greater use of activated carbon or heavy chlorination. Again, however, the taste-and-odor factor will control the threshold or limiting concentration of oily material acceptable in a domestic water supply. The main sources of oil in soil are :

a) Waste discharges
b) Accidental spill
c) Leakages

In the case of soil the activity of site inventory is defined to include mainly identification, location, characterization, preliminary assessment, and registration.

In addition to the contamination of old waste sites, soils are being contaminated by the handling of environmentally dangerous substances in trade and industry or by public institutions as well as through leaking pipe work and sewer pipes (Thoenes, 1990).

Turkey has contaminated soil problems although they do not yet have high priority among other environmental problems. The causes of contaminated soil problems in Turkey may be summarized as follows:

1) Industrialization: In Turkey, industrialization has started in about 1930's. For a long time, no environmental considerations were taken into account. In 1970's environmental pollution control studies have been introduced especially with respect to water and air pollution. Even today, water and air pollution problems are discussed in more detail and soil contamination to a lesser extent. Industrial wastes causing soil contamination also include oil pollution in many cases.

2) Leaking tanks and pipes: In Turkey 40 to 60% of the sewage pipe works needs renewal, in some towns even more than 60% of sewage pipe work is defective. The defective sewage pipes constitute a danger to the subsoil and to the groundwater. Since Turkey is located between Europe and Middle East, transportation of goods is very important. A considerable amount of oil is transported. Thousands of filling stations use buried underground tanks. Many kilometers of underground pipelines carry petroleum products. Although there are no studies about the leakage from these tanks and pipelines, it is known that they start to leak after some time.

3) Accidental spills: 90% of transportation is performed by motorway in Turkey. The number of buses and trucks is equal to total of them in European countries. Accidental spill is an important contamination source for oil because of the occasional tanker trucks accidents**.**

4) Midnight dumping: During to the application of removal of cesspool contents in some areas, sometimes the wastewaters are discharged to uninhabited areas.

1.2 Contaminated Sites in Turkey

Same examples of the identified contaminated sites and major soil and groundwater problems associated with these sites in Turkey are as follows:

Beykan Oil Field Site: At this site, petroleum hydrocarbon pollution of surface soils, surface and groundwater caused by oil production activities of the Beykan Oil Field is of concern. The Beykan Oil Field is enclosed by the watershed of a medium size dam constructed during early-sixties for irrigation purposes. Due to recent increases in domestic water supply demand, the dam was considered as a potential resource to meet the increasing water demand in the area. A total of 38 oil producing wells are placed within the various protection zones surrounding the dam's reservoir; 13 of them being in the immediate vicinity, within the first 300 m of the reservoir shore called the "*absolute protection zone.*" Oil spills at these wells and along pipelines connecting wells and other facilities are considered as potential pollution

sources effecting the reservoir water quality. Spill records revealed that, during the peak oil production years, 95 tons of annual average spill occurred, resulting in an average total petroleum hydrocarbons (TPHs) concentration of 20,300 ppm in contaminated soils. As a consequence, contaminant mass leaching to the reservoir from soils contaminated by oil spills is viewed as a primary concern for reservoir water quality. In addition to soil and possible reservoir water pollution problems, another primary concern at this site is pollution of the Midyat aquifer due to injection of nearly 20 million m^3 of formation water between the years of 1971 and 1996. Injected formation water contains high amounts of brine (with a chloride concentration of 3,000 mg/L) and some emulsified oil (with a concentration of 500 mg/L). The Midyat aquifer overlies the Beykan Oil Field and a primary source of drinking water supply for the nearby community. For this site, studies concerning the assessment of the extent of contamination and appropriate remedial measures are currently underway (NATO/CCMS Pilot Study, 1998).

Incirlik PCB Contaminated Soils Site: At this site, soil contamination by polychlorinated biphenyls (PCB), oil leaking from storage drums at a military reutilization yard occurred during the operation of the yard between the years of 1970 and 1988. An excavation of 0,5 meters deep was made in October 1991, leaving the excavated soil stored in approximately 300 drums and in a pile. Estimated PCB-contaminated soil volume is 1,600 m^3. Site characterization investigations revealed that site soils are high in clay content (65 percent) and potential for groundwater contamination is low. PCB concentrations measured in composite contaminated soil samples range up to 750 ppm. For remediation of contaminated soils, various alternatives are being evaluated including incineration and *in situ*/*ex situ* solidification/stabilization (S/S) (NATO/CCMS Pilot Study, 1998).

Chromium Ore Processing Residue Dump Site: At this site, soil and groundwater contamination by Cr(VI) leaching from chromium ore processing residue (COPR) is of concern. COPR is produced by a chromate production factory providing mostly the needs of leather tanning industry. During the early production years, COPR is dumped at a temporary dump site near factory. The unprocessed row

chromites ore ($FeCr_2O_4$) contains nearly 45 percent of chromium oxide (Cr_2O_3). After a roasting process of chromites ore by adding Na_2CO_3 and $CaCO_3$ constituents, COPR contains nearly 25,000 ppm of total chromium. Due to high chromium content, COPR is partly recycled by mixing with chromium ore at a ratio of roughly 1:20. The current chromate production technology used yields approximately three tons of COPR to produce one ton of chromate. Currently, some research work is underway to evaluate soil and groundwater pollution potential of land-disposed COPR and to develop technical guidelines for appropriate management of COPR related wastes and remediation of COPR contaminated soils (NATO/CCMS Pilot Study, 1998).

In Turkey legal framework for contaminated soil rehabilitation studies have been started recently in 2004. Ministry of Environment has promulgated standards for soil. Table 1.1. presents maximum allowable concentration for soil media.

Table 1.1 Maximum allowable contaminant concentrations in soil (Official Gazette, 2001)

Heavy metal contents		**Frontier values (mg/kg oven dry soil)**
Lead		1200
Cadmium		40
Chromium		1200
Copper		1750
Nickel		400
Zinc		4000
Mercury		25
Polluting materials		**Frontier values**
Chloride Ion(mg CI/I)	(Total)	25
Sodium (mg Na/I)	(Total)	125
Cobalt (Co)	(mg/kg Oven Dry Soil)	20
Arsenic (As)	"	20
Molibden (Mo)	"	10
Tin (Sn)	"	20
Barrium (Ba)	"	200
Florur (F)	"	200
Free siyanid (CN)	"	1
Kompleks Cyanide CN)	"	5
Sulphide (S^{-1})	"	2
Bromid (Br)	"	20
Benzene	"	0,05
Butil benzen	"	0,05
Toliol	"	0,05
Xylol	"	0,05
Phenol	"	0,05
Selenium (Se)	"	5
Talium (Tl)	"	1
Uranium (U)	"	5
Polycylic Aromatic Hydrocarbons	"	5
Organochlorides	"	0,5
Pesticides-Individual Pesticides-Total	" "	0,5 2
PCB Polychlorinated Bipheniyls	"	0,5
Hexachlor Benzol	"	0,1
Pentachlor Benzol	"	0,1
HCH (Lindane)	"	0,1

1.3 Contaminated Site Rehabilitation

1.3.1 Land Treatment and Composting

Soil may be viewed as a natural filter for wastes. Soil has physical, chemical, and biological characteristics that can enable waste detoxification, biodegradation, chemical decomposition, and physical and chemical fixation. Therefore, land treatment of wastes may be accomplished by mixing the wastes with soil under appropriate conditions.

Soil is a natural medium for a number of living organisms that may effect biodegradation of hazardous wastes. Of these, the most important are bacteria, including those from the general *Agrobacterium, Arthrobacteri, Bacillus, Flavobacterium and Pseudomonas* (EPA, 1991).

Land treatment is mostly used for petroleum wastes and is applicable to the treatment of fuels and wastes leaking from underground storage tanks. It can also be applied to biodegradable organic chemical wastes, including some organohalide compounds. Land treatment is not suitable for the treatment of wastes containing acids, bases, toxic inorganic compounds, salts, heavy metals, and organic compounds that are excessively soluble, volatile, or flammable.

Composting of hazardous wastes is biodegradation of solid or solidified materials in a medium other than soil. Bulking material, such as plant residue, paper, municipal refuse, or sawdust may be added to retain water and enable air to penetrate to the waste material. Successful composting of hazardous waste depends upon a number of factors. The first of these is the selection of the appropriate microorganism or inoculums. Other parameters that must be controlled include oxygen supply, moisture content (which should be maintained at a minimum of about 40%) pit (usually around neutral), and temperature (EPA, 1991).

1.3.2 Immobilization, Stabilization and Solidification

Immobilization includes physical and chemical processes that reduce surface areas of wastes to minimize leaching.

Stabilization means the conversion of a waste from its original form to a physically and chemically more stable material. Stabilization is required for land disposal of wastes.

Fixation is a process that binds a hazardous wastes in a less mobile and less toxic form; it is similar to stabilization.

Solidification may involve chemical reaction of the waste with the solidification agent, mechanical isolation in a protective binding matrix, or a combination of chemical and physical processes. It can be accomplished by evaporation of water from aqueous wastes or sludge, sorption onto solid material, reaction with cement, reaction with silicates, encapsulation, or imbedding in polymers or thermoplastic materials. Vitrification or glassification consists of imbedding wastes in glass material. In this application, glass may be regarded as a high-melting-temperature inorganic thermoplastic (EPA, 1991).

Encapsulation is used to coat wastes with an impervious material so that they do not contact their surroundings (EPA, 1991).

Chemical fixation is a process that binds a hazardous waste in a less mobile, less toxic form by a chemical reaction that alters the waste chemically (EPA, 1991).

1.3.3 Thermal Treatment

Hazardous waste thermal technologies have been implemented for remediation at many contaminated sites. This technology can be used for the treatment of contaminated liquids, sludge, soils, and air streams. The most widely applied means of thermal treatment of hazardous wastes is incineration. Hazardous waste incineration is defined as a process that involves exposure of the waste materials to oxidizing conditions at a high temperature, usually in excess of 900˚ C. Normally,

the heat required for incineration comes from the oxidation of organically bound carbon and hydrogen contained in the waste material or in supplemental fuel:

$$C \text{ (organic)} + O_2 \longrightarrow CO_2 + \text{heat}$$

$$4H \text{ (organic)} + O_2 \longrightarrow 2H_2O + \text{heat}$$

1.3.4 Physical / Chemical Extraction

The basic processes in extraction installation consist of ;

a) Pre-screening of the soil
b) Mixing with an extraction agent
c) Fluid-solids separation
d) Treatment of the cleaned soil and
e) Water purification and sludge treatment

Physical / chemical extraction process is also known as soil-washing system. Extraction installation may incorporate a variety of other techniques besides the actual extraction. In general, particle separation techniques (separation on size and / or density) chemical treatment (mainly oxidation), and flotation techniques are integral components of extraction installations (EPA, 1991).

1.3.5 Soil Vapor Extraction

Soil Vapor Extraction (SVE), also know as in situ venting (ISV), can be a cost-effective remediation process for soils to remove volatile organic compounds (VOC's) and some semi volatile organics compounds (SVOC's) from the vadoze zone. The vadoze zone is the subsurface soil zone located between the surface soil and the top of the water table. SVE is used for contaminants from soil and groundwater to air and to condensed wastewater streams.

1.3.6 Pump and Treat

This treatment technology is intended to destroy dissolved organic contaminants, including chlorinated hydrocarbons and aromatic compounds, that are present in wastewater or groundwater with low level of suspended solids, oils and grease (EPA, 1991).

Pump and treat technology consist of the extraction of contaminated groundwater from the subsurface, followed by treatment of groundwater at the surface to remove the contaminants.

The application of pump and treat technology can be considered for essentially two cases: Containment of the contaminants in the groundwater to within a confined zone thereby preventing the spread of the contamination, and decontamination of the aquifer (EPA, 1991).

1.3.7 Biodegradation of Wastes

Biodegradation of wastes is their conversion by biological processes to simple inorganic molecules" (mineralization) and, to a certain extent, to biological materials. Usually the products of biodegradation are molecular forms that tend to occur in nature and that are in greater thermodynamic equilibrium with their surroundings than are the starting materials. Detoxification refers to the biological conversion of a toxic substance to a less toxic species. Microbial bacteria and fungi possessing enzyme systems required for biodegradation of wastes are usually best obtained from populations of indigenous microorganisms at a hazardous waste site where they have developed the ability to degrade particular kinds of molecules. Although it has some short-comings in the degradation of complex chemical mixtures, biological treatment offers a number of significant advantages and has considerable potential for the degradation of hazardous wastes. The biodegradability of a compound is influenced by its physical characteristics, such as solubility in water and vapor pressure, and by its chemical properties, including molecular mass, molecular structure, and presence of various kinds of functional groups (EPA, 1991).

Recalcitrant or biorefractory substances are those that resist biodegradation and tend to persist and accumulate in the environment. Aerobic waste treatment processes utilize aerobic bacteria and fungi that require molecular oxygen. These processes are often favored by microorganisms, in part because of the high energy yield obtained when molecular oxygen reacts organic matter. Anaerobic waste treatment in which microorganisms degrade wastes in the absence of oxygen can be practiced on a variety of organic hazardous wastes. The overall process for anaerobic digestion is a fermentation process in which organic matter is both oxidized and reduced. The simplified reaction for the anaerobic fermentation of a hypothetical organic substance "(CH_2O)" is the following:

$$2\,(CH_2O) \longrightarrow CO_2 + CH_4$$

Of all the environments inhabited by microorganisms, the soil is by far the most complex. It contains representatives of every major type of microorganisms; frequently in prodigious numbers.

The principal function of soil microorganisms is decomposition. The major producers in a soil ecosystem are the rooted green plants (as well as the bodies and wastes of their consumers) become grist for the decomposition mill. Thus, as in aquatic ecosystems, there is an ecological cycle in soil. Since the growth of the green plants depends on a continual supply of a biotic substances in the soil, soil fertility is very is largely a function of soil micro flora . Decomposition is not the only function of microorganisms in soil. Algae are widespread in soil, so they serve as producers to some extent.

A number of bacteria posses a rare capability: They can capture gaseous nitrogen from the atmosphere and convert it into nitrogenous compounds that are usable by green plants. In addition, many microorganisms influence the growth of plants though their complex biochemical activities. For example, certain fungi called *mycorrhizae* are associated with plant roots in what is apparently a mutualistic relationship.

The microorganisms in soil are mainly bacteria and fungi, although many algae and protozoa are also present . The bacteria are predominantly saprophytic forms, since the principal role of soil bacteria is decomposition. Fungi largely in the from of molds, make up a significant portion of the soil micro flora. These organisms are especially active in the decomposition of gross organic matter.

Although most algal species are aquatic, a large number is terrestrial. Algae serve principally as photosynthetic producers. Virtually all soil algae are green algae.

A great many protozoan species are found in soil, where they serve as first-level consumers or as scavengers. They break down participate organic matter into simpler compounds, which then become available to bacteria and decomposers.

It is desirable to conduct a biological remediation under aerobic conditions, with the supply of oxygen, in order to maximize the biodegradation rates.

The following stoichiometric equation allows us to determine the amount of oxygen needed for the biological process:

$$C_x H_y O_z + O_2 \longrightarrow xCO_2 + (y/2)H_2O$$

Current biological in-situ remedial action techniques solve this task by injecting water into the flushed contaminated subsoil. The water runs in a circulation system with infiltration and extracting wells and is saturated with air oxygen or is enriched with pure oxygen or hydrogen peroxide or ozone sometimes nitrates are also added (EPA, 1991).

CHAPTER TWO
FACTORS LIMITING BIOREMEDIATION

2.1 Bioremediation Methods

2.1.1 In Situ and Ex Situ Methods

Bioremediation technologies can be broadly classified as ex situ and in situ. Ex situ technologies are those treatments which involve the physical removal of the contaminated material for treatment process. In contrast, in situ techniques involve treatment of the contaminated material in place. Some of the examples of in situ and ex situ bioremediation are given below:

a) Land farming: Solid-phase treatment system for contaminated soils: may be done in situ or ex situ.
b) Composting: Aerobic, thermophilic treatment process in which contaminated material is mixed with a bulking agent; can be done using static piles or aerated piles.
c) Bioreactors: Biodegradation in a container or reactor; may be used to treat liquids or slurries.
d) Bioventing: Method of treating contaminated soils by drawing oxygen through the soil to stimulate microbial activity.
e) Biofilters: Use of microbial stripping columns to treat air emissions.
f) Bioaugmentation: Addition of bacterial cultures to a contaminated medium; frequently used in both in situ and ex situ systems.
g) Biostimulation: Stimulation of indigenous microbial populations in soils or ground water by providing necessary nutrients.
h) Intrinsic bioremediation: Unassisted bioremediation of contaminant; only regular monitoring is done.
i) Pump and treat: Pumping groundwater to the surface, treating, and re-injecting.

2.1.2 Advantages and Disadvantages of Bioremediation

For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microbes are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants. Bioremediation offers several advantages over conventional techniques such as land-filling or incineration. Bioremediation can be done on site, is often less expensive and site disruption is minimal, it eliminates waste permanently, eliminates long-term liability, and has greater public acceptance, with regulatory encouragement, and it can be coupled with other physical or chemical treatment methods.

Bioremediation has also its limitations. Some chemicals are not amenable to biodegradation, for instance, heavy metals, radio-nuclides and some chlorinated compounds. In some cases, microbial metabolism of contaminants may produce toxic metabolites. Bioremediation is a scientifically intensive procedure which use to be tailored to the site-specific conditions, which means one has to do treatability studies on a small scale before the actual clean-up of the sites. Some of the questions one has to answer before using bioremediation techniques are: is the contaminant biodegradable? is biodegradation occurring in the site naturally? are environmental conditions appropriate for biodegradation? if the waste does not completely biodegrade, where will it go? These questions can be answered by doing site characterization and also by treatability studies (Boopathy and Manning, 1998).

2.1.3 Physiology of Biodegradative Microbes

A bioremediation process is based on the activities of aerobic or anaerobic heterotrophic microorganisms. Microbial activity is affected by a number of physicochemical environmental parameters. The factors that directly impact on bioremediation are energy sources (electron donors), electron acceptors, nutrients, pH, temperature, and inhibitory substrates or metabolites. One of the primary distinctions between surface soils, vadose zone soils and groundwater sediments is the content of organic material. Surface soils, which typically receive regular inputs

of organic material from plants, will have higher organic matter content. The high organic matter content is typically associated with high microbial numbers and a great diversity of microbial populations. The organic matter serves as a storehouse of carbon and energy as well as a source of other macronutrients such as nitrogen, phosphorous, and sulfur. Subsurface soils, and groundwater sediments have lower levels of organic matter and thus lower microbial numbers and population diversity than surface soils (Adriaens and Hickey, 1993). Bacteria become more dominant in the microbial community with increasing depth in the soil profile as the numbers of other organisms such as fungi or actinomycetes decrease. This is attributed to the ability of bacteria to use alternative electron acceptors to oxygen. Other factors that control microbial populations are moisture content, dissolved oxygen, and temperature.

2.1.4 Metabolic Processes

Primary metabolism of an organic compound has been defined as the use of the substrate as a source of carbon and energy. This substrate serves as an electron donor resulting in microbial growth. Application of co metabolism to site-remediation of xenobiotics is required when the compound cannot serve as a source of carbon and energy by nature of the molecular structure, which does not induce the required catabolic enzymes. The term co-metabolism has been defined as the metabolism of a compound that does not serve as a source of carbon and energy or as an essential nutrient which can be achieved only in the presence of a primary (enzyme inducing) substrate.

Aerobic processes are characterized by metabolic activities involving oxygen as a reactant. Dioxygenases and monooxygenases are two of the primary enzymes employed by aerobic organisms during transformation and mineralization of xenobiotics. Anaerobic microbes take advantage of a range of electron acceptors, which, depending on their availability and the prevailing redox conditions, include nitrate, iron, manganese, sulfate, and carbon dioxide.

2.2 Scientific Factors Affecting Bioremediation

2.2.1 Energy Sources

One of the primary variables affecting the activity of bacteria is the ability and availability of reduced organic materials to serve as energy sources.

Whether a contaminant will serve as an effective energy source for an aerobic heterotrophic organism is a function of the average oxidation state of the carbon in the material. In general, higher oxidation states correspond to lower energy yields which thus provide less energetic incentive for microorganism degradation.

The outcome of each degradation process depends on microbial (biomass concentration, population diversity, enzyme activities), substrate (physico-chemical characteristics, molecular structure, and concentration), and a range of environmental factors (pH, temperature, moisture content, Eh, availability of electron acceptors and carbon and energy sources). These parameters affect the acclimation period of the microbes to the substrate. The molecular structure and contaminant concentration have been shown to strongly affect the feasibility of bioremediation and the type of microbial transformation occurring, and whether the compound will serve as a primary, secondary or co-metabolic substrate.

Major factors affecting bioremediation are given below:

Microbial

- Growth until critical biomass is reached
- Mutation and horizontal gene transfer
- Enzyme induction
- Enrichment of the capable microbial populations
- Production of toxic metabolites

Environmental

- Depletion of preferential substrates
- Lack of nutrients
- Inhibitory environmental conditions

Substrate

- Too low concentration of contaminants
- Chemical structure of contaminants
- Toxicity of contaminants
- Solubility of contaminants

Biological aerobic vs. anaerobic process

- Oxidation/reduction potential
- Availability of electron acceptors
- Microbial population present in the site

Growth substrate vs. co-metabolism

- Type of contaminants
- Concentration
- Alternate carbon source present
- Microbial interaction (competition, succession, and predation)

Physico-chemical bioavailability of pollutants

- Equilibrium sorption
- Irreversible sorption
- Incorporation into humic matters

Mass transfer limitations

- Oxygen diffusion and solubility
- Diffusion of nutrients
- Solubility/miscibility in/with water

2.2.2 Bioavailability

The rate at which microbial cells can convert contaminants during bioremediation depends on the rate of contaminant uptake and metabolism and the rate of transfer to the cell (mass transfer). Increased microbial conversion capacities do not lead to higher biotransformation rates when mass transfer is a limiting factor (Boopathy and Manning, 1998). This appears to be the case in most contaminated soils and sediments. For example, the contaminating explosives in soil did not undergo biodegradation process even after 50 years. Treatments involving rigorous mixing of

the soil and breaking up of the larger soil particles stimulated biodegradation drastically (Manning et al., 1995). The bioavailability of a contaminant is controlled by a number of physico-chemical processes such as sorption and desorption, diffusion, and dissolution. A reduced bioavailability of contaminants in soil is caused by the slow mass transfer to the degrading microbes. Contaminants become unavailable when the rate of mass transfer is 0. The decrease of the bioavailability in the course of time is often referred to as aging or weathering. It may result from:

a) chemical oxidation reactions incorporating contaminants into natural organic matter,
b) slow diffusion into very small pores and absorption into organic matter, and
c) the formation of semi-rigid films around non-aqueous phase liquids (NAPL) with a high resistance toward NAPL-water mass transfer.

These bioavailability problems can be overcome by the use of food-grade surfactants (Boopathy and Manning, 1999), which increase the availability of contaminants for microbial degradation. In recent years, application of biosurfactants to increase bioavailability is studied by many researchers.

2.2.3 Bioactivity and Biochemistry

The term bioactivity is used to indicate the operating state of microbiological processes. Improving bioactivity implies that system conditions are adjusted to optimize biodegradation (Blackburn and Hafker, 1993). For example, if the use of bioremediation requires meeting a certain minimum rate, adjustment of conditions to improve biodegradation activity becomes important and a bioremediation configuration that makes this control possible has an advantage over one that does not.

In nature, the ability of organisms to transfer contaminants to both simpler and more complex molecules is very diverse. In light of our current limited ability to measure and control biochemical pathways in complex environments, favorable or

unfavorable biochemical conversions are evaluated in terms of whether individual or groups of parent compounds are removed, whether increased toxicity is a result of the bioremediation process, and sometimes whether the elements in the parent compound are converted to measurable metabolites. These biochemical activities can be controlled in an in situ operation when one can control and optimize the conditions to achieve a desirable result.

CHAPTER THREE

IMPROVEMENT OF IN SITU BIOREMEDIATION OF CONTAMINATED SOIL

3.1 Factors Limiting Efficiency of In Situ Bioremediation

Soil contaminated with various organic recalcitrant compounds is a very widespread problem throughout the world, particularly in industrialized areas. There are many reasons for organic compounds being degraded very slowly or not at all in the soil environment, even though they are per se biodegradable. Among those are:

a) Low temperature. In soil, particularly in northern industrialized countries in Europe and North America, the soil temperature during a large part of the year is too low for efficient microbial degradation of soil contaminants. The same may be true also for deeper soil layers in other parts of the world.

b) Anaerobic conditions. Degradation in anaerobic conditions is slow; some compounds are not degraded anaerobically and some are degraded only partly and may give rise to toxic compounds.

c) Low levels of nutrients and co-substrates. A contaminated site usually has a suboptimal nutrient balance. If the contaminant is a hydrocarbon, e.g. oil contamination, there is likely to be a shortage of at least nitrogen, but each site has to be evaluated case by case, taking into account also matters such as solubility of the contaminant in order not to over-fertilize.

d) Bioavailability. Spatial distribution of contaminants in relation to degrading organisms and solubility of the contaminant; these variables are in part interrelated and are both separately and in combination major factors affecting degradation velocity.

e) Absence of degradation potential. A biological degradation pathway for synthetic, xenobiotic compounds may not exist precluding biodegradation, or genes encoding enzymes that may be active on the compound are not induced by the contaminant. Suitable pathways are, however, likely to evolve, either naturally or accelerated in laboratory conditions.

3.2 Increase of Bioavailability by Using Surfactants

One of the reasons for recalcitrance of many organic soil and groundwater contaminants is their hydrophobicity, low solubility in water and thereby poor bioavailability (Van Loosdrecht et al., 1990; Weissenfelset al., 1992). Some bacteria produce biosurfactants that help them to access hydrophobic compounds as carbon and energy sources (Francy et al., 1991; Volkering et al., 1998). Bacteria with this capacity may in themselves be good candidates for bioremediation purposes (Falatko and Novak, 1992; Banat, 1995), but both synthetic and biosurfactants have, in many studies, been added to contaminated sites or in bench-scale test as a means of accelerate biodegradation (Volkering et al., 1998). As with addition of nutrients, one difficulty is then the dissemination of the surfactant during in situ bioremediation.

3.3 Natural Attenuation/Intrinsic Bioremediation

Natural attenuation is increasing in use as a low cost means of remediating contaminated soil and groundwater. According to the USEPA (1999), natural attenuation is the ‘‘use of natural processes to contain the spread of the contamination from chemical spills and reduce the concentration and amount of pollutants at contaminated sites’’. It can also be termed as intrinsic remediation, bioattenuation and intrinsic bioremediation. In this case, the contaminants are left on site and the naturally occurring processes are left to clean up the site. The natural processes include biological degradation, volatilization, dispersion, dilution, radioactive decay, and sorption of the contaminant onto the organic matter and clay minerals in the soil.

Natural attenuation is mainly used for benzene, toluene, ethyl benzene and xylene (BTEX) and more recently for chlorinated hydrocarbons. Other contaminants that could potentially be remediated by natural attenuation include pesticides and inorganic compounds. The success of natural attenuation depends on the subsurface geology, hydrology and microbiology.

There are various advantages to natural attenuation including:

a) Possibility of destroying the contaminant completely. Remediation method that is potentially more acceptable to the public than other remediation technologies.
b) Can be used with other methods as a pre- or post treatment at a site.
c) Can potentially reduce remediation costs significantly.

Potential disadvantages are:

a) Longer remediation times compared to other technologies.
b) Lack of knowledge concerning mechanisms for remediation particularly with regard to inorganic contaminants.
c) Substantial requirements for monitoring.
d) By-products can be more environmentally hazardous than the parent compounds.
e) Desorption or resolubilization of contaminants can occur.
f) Modeling data has to be evaluated with caution and may be subject to significant uncertainty.

Natural attenuation could cynically be looked upon as doing nothing, but what it does involve is monitoring the natural attenuation process and the self-cleaning potential. The process is monitored and if non-functional abandoned in favour of more direct and drastic measures. The minimum is to monitor the site so that further spread of contaminating compounds is limited.

Among the approaches in this context is to ensure that the genetic potential (Stapleton et al., 1998) or actual capacity of the intrinsic micro flora (Solano-Serena etal., 1998) for degradation is present in the soil. The presence of a particular gene or degradation capacity in a soil can be traced by molecular genetics methods, once homologous genes have been isolated or suitable primers designed. This potential gives a possibility to test a contaminated site for the potential of intrinsic remediation (self cleaning) once the conditions support the growth of the carrier microbe. If this is the case, no addition of degraders or of degradation potential in the form of, for example, conjugative plasmids may be needed. Also, an evaluation of the site and the conditions is needed: Are the ambient natural attenuation mechanisms; biodegradation, dilution and sorption efficiency enough to meet regulatory requirements? Is intrinsic bioremediation, the deliberate use of natural attenuation mechanisms, sufficient, or are more drastic measures required (Chapelle, 1999)? Further, is the localization of the contaminants of a kind that spreading of the contaminant plume to and in, for example, groundwater does not pose an increased environmental threat? If there is little or no risk for the situation to worsen, and natural events more or less efficiently improve the situation, then possibly all that is needed is monitoring (Chapelle, 1999).

3.4 Utilizing Plants to Improve Soil Contaminant Biodegradation

One way to achieve truly in situ bioremediation is by utilizing plants to perform rhizosphere bioremediation. The roots of plants penetrate into the soil, changing locally the conditions in the rhizosphere and mycorrhizosphere. The roots themselves are colonized by bacteria (Suominen et al., 2000) or mycorrhiza-forming fungi, in which the fungal hyphae are more of less densely colonized by a bacterial biofilm (Sarand et al., 1998). Plants growing at a contaminated site themselves improve the appearance of the site, their roots prevent erosion and they stabilize and increase the microbial population density and diversity of the soil. Plant root exudates may also provide co-substrates for the bacteria which can improve degradation on certain contaminating compounds by inducing the right set of genes. The roots also change the porosity of the soil, improving the aeration.

The presence of a large diversity of bacteria in close proximity may also aid the sequential degradation of compounds through the concerted activities of different bacterial species rather than individual strains. On the other hand, a key question is whether the bacteria, in the presence of more easily degradable substrates, utilize the contaminating compound. Microcosm studies in the laboratory indicate that upon availability of higher levels of substrate, the bacterial cell density increases. In the conditions used, availability of carbon and energy source remains the growth-limiting factor for the bacteria, thus ensuring efficient contaminant-degradation.

When the carbon and energy source is the growth-limiting factor, also the sub-optimal substrates will be used, as long as the concentration is high enough to induce the promoters of the degradation pathway genes.

In all, the perceived advantages of rhizosphere bioremediation approach include:

a) Maintenance of high numbers of target bacteria in the nutrient-rich root zone;
b) High energy conditions allowing the expression and functioning of the degradative enzymes;
c) Likely association of degradative bacteria with other beneficial microorganisms such as root symbiotic mycorrhizal fungi that may be able to co-degrade the organic pollutants or their intermediates;
d) The plant roots may in some cases produce compounds that function as natural co-substrates, inducing the degradative genes of the bacteria also when the contaminant level is low;
e) Perennial plants promote survival of the associated bacteria over the years;
f) Plant roots reach different layers of the soil thereby distributing microbes without need for soil mixing;
g) Plants bind the surface soil and prevent erosion and flooding, while mixing may actually lead to worsened leaching of toxins into the ground water;

h) Plants take up water, counteracting the downward flow of possibly contaminated soil water towards the groundwater level, or into the waterways;
i) Plants may take up and enrich heavy metals from the soil; and
j) Plants also have an aesthetic function by improving the appearance of the site.

3.5 Soil Fauna

The role of soil fauna in decontaminating soil is in most cases indirect, but still important in that they redistribute microbes or help reintroduce them from less contaminated soil layers. Particularly, worms of various sizes also mix the soil and make it more porous, and thereby improve aeration. Different aspects of soil fauna in relation to bioremediation is reviewed in depth elsewhere in this issue (Haimi, 2000).

CHAPTER FOUR
BIOSURFACTANTS

4.1 Biosurfactants

Some surfactants, known as biosurfactants, are biologically produced by yeast or bacteria from various substrates including sugars, oils, alkanes and wastes (Lin, 1996).

Biosurfactants are grouped as glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids, polymeric and particulate compounds (Biermann et al., 1987). Most of these compounds are either anionic or neutral. Only a few are cationic such as those containing amine groups. The hydrophobic part of the molecule is based on long-chain fatty acids, hydroxy fatty acids or α-alkyl-β-hydroxy fatty acids. The hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. A wide variety of microorganisms can produce these compounds. The critical micelle concentration (CMC) of the biosurfactants generally range from 1 to 200 mg/L (Lang and Wagner, 1987).

4.2 Characteristics of Biosurfactants

Microbial surface-active agents or biological surfactants (biosurfactants) are a structurally diverse group of surfactants mainly produced by hydrocarbon-utilizing microorganisms during growth on insoluble substrates or produced on water-soluble compounds such as glucose, sucrose, glycerol, or ethanol.

The hydrophilic component of biosurfactants generally consists of carbohydrate, a hydrophilic amino acid such as glutamate, aspartate, lysine or arginine, or a hydrophilic peptide. The hydrophobic or water-insoluble portion frequently consists of a lipid structure, an isoprenold structure such as cholesterol, or a hydrophobic amino acid or peptides including amino acids such as phenylalanine, leucine, isoleucine, valine, or alanine.

Biosurfactant molecules are cell wall associated and are secreted into the surrounding media. Such extracellular and cell wall associated molecules have the potential to promote cellular attachment to hydrophobic surfaces, to affect the distribution of cell between oil and water phases, to emulsify water-insoluble substances into the cell. Biosurfactants, which are high molecular weight lipid complexes, share the same properties with synthetic surfactants and have both hydrophilic and hydrophobic functional groups (Kosaric, 1992).

In some cases, microbial surfactants are more effective in creating microemulsions in which micelle formation occurs and hydrocarbons can solubilize in water. Biosurfactants mainly reduce surface tension, interfacial tension, and CMC (the critical micelle concentration) in both aqueous solutions and hydrocarbon mixtures; therefore they increase the aqueous concentration of poorly soluble compounds, which leads to improving the accessibility of these substrates to microorganisms (Banat, 1995).

Surface tension, interfacial tension and solubilization as a function of surfactant concentration is presented in Figure 4.1.

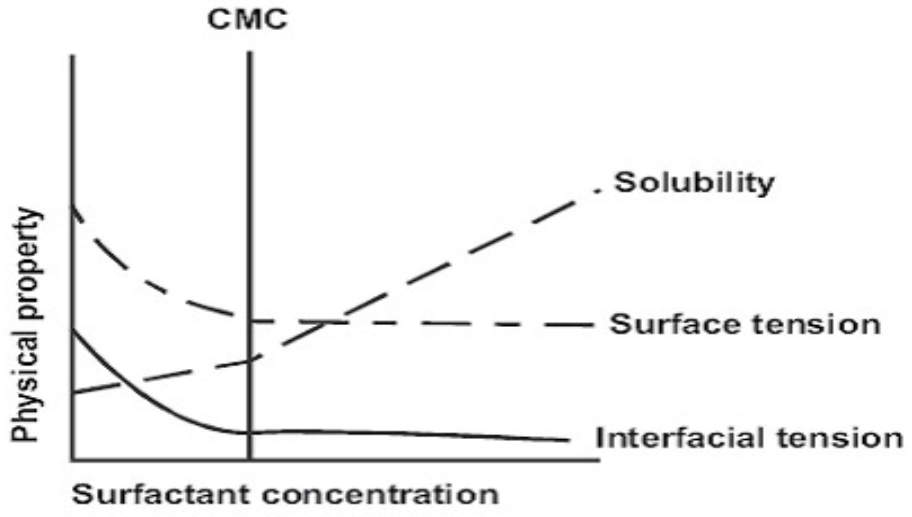


Figure 4.1 Surface tension, interfacial tension and solubilization as a function of surfactant concentration (CMC represents critical micelle concentration) Becher, P., (1965).

4.3 Classification of Biosurfactants

The major types of biosurfactants with their general properties and biosurfactant-producing microbial species are listed in Table 4.1.

Table 4.1 Biosurfactant producing microorganisms (Banat, B. Tech., 1995)

Microorganism	Biosurfactant
Athrobacter RAG-1	Hetropolysaccharides
Athrobacter MISS 8	Lipopeptide
Athrobacter sp.	Trehalose, sucrose, and fructose lipids
Bacillus licheniformis JF-2	Lipopeptides
Bacillus licheniformis 86	Lipopeptides
Bacillus subtilus	Sufactin
Bacillus pumilus A 1	Sufactin
Bacillus sp. AB-2	Rhamnolipids
Bacillus sp. C-14	Hydrocarbon-lipid-protein
Candida Antarctica	Mannosylerthritol
Candida bombicola	Sophorolipids
Candida tropicalis	Mannan fatty acid
Candida lipolytica Y-91 7	Sophorolipids
Closlridium pasteurianum	Neutral lipids
Cornyebacteriiim hydrocarbolastus	Protein-lipid-carbohy
Cornyebacterium insidiosum	Phospholipids
Cornyebacterium lepus	Fatty acids
Strain MM1	Glucose, lipid and hydroxydecanoic acid
Nocardia erythropolis	Neutral lipids
Ochrobactrum anthropii	Protein
Penicilliiim spiculisporum	Spiculosporic acid
Pseudomonas aeruginosa	Rhamnolipid
Pseudomonasfluorescem	Lipopeptide
Phaffia rhodozyma	Carbohydrate-lipid
Phodococcus erythropolis	Trehalose-dicorynomycolate
Phodococcus sp. ST-5	Glycolipid
Phodococcus sp. H13-A	Glycolipid
Phodococcus sp. 33	Polysaccharide
Tondopsis bombicola	Sophorolipids

Unlike chemical synthesized surfactants, biosurfactants are differentiated mainly on their biochemical compositions or nature, structures and microbial origins.

The major classes of biosurfactants include:

a) glycolipids
b) lipopeptides and lipoproteins
c) phospholipids and fatty acids
d) polymeric surfactants and
e) participate surfactants

4.4 Effect of Rhamnolipids on Petroleum Hydrocarbons Biodegradation

The various components of petroleum hydrocarbons are alkanes, cycloalkanes, aromatics, polycyclic aromatic hydrocarbons, asphaltenes, and resins. Although alkanes, represented by the formula C_2H_{2n+2} can have many isomers as the number of carbons increase, relatively few exist in petroleum. They are sometimes referred to as aliphatic compounds. Low molecular weight alkanes are the most easily degraded by microorganisms. Various studies have examined the effect of rhamnolipids on biodegradation of organic contaminants with mixed results. There has been particular focus on various hydrocarbons of low solubility. A recent review by Maier and Soberon-Chavez (2000) indicated that rhamnolipid addition can enhance biodegradation of hexadecane, octadecane, n-paraffin, and phenanthrene in liquid systems, in addition to hexadecane, tetradecane, pristine, creosote and hydrocarbon mixtures in soils. Two mechanisms for enhanced biodegradation are possible, enhanced solubility of the substrate for the microbial cells, and interaction with the cell surface, which increases the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily (Shreve et al., 1995; Zhang and Miller, 1992). Zhang and Miller (1992) demonstrated that a concentration of 300 mg/L of rhamnolipids increased the mineralization of octadecane to 20% from 5% for the controls. Beal and Betts (2000) showed that the cell surface hydrophobicity increased by the biosurfactant strain more than a non-biosurfactant producing strain during growth on hexadecane. The rhamnolipids also increased the solubility of the

hexadecane from 1,8 to 22,8 mg/L. There have been indications that inhibition can also occur. Other studies by Churchill et al. (1995) showed that rhamnolipids with a fertilizer (Inipol EAp-22) enhanced biodegradation of aromatic and aliphatic compounds in aqueous phase and soil reactors. Noordman et al. (2002) studied the effect of the biosurfactant from *P. aeruginosa* on hexadecane degradation.They determined that the biosurfactant could enhance biodegradation if the process is rate limited. For example in the case of small soil pore sizes (6 nm), the hexadecane is entrapped and of limited availability. The rhamnolipid stimulates release of entrapped substrates (if mixing conditions are low such as in a column) and enhances uptake by cells (if the substrate is available). This could then become important in the stimulation of bacterial degradation under in situ conditions. Some evidence of this was shown by Al-Awadhi et al. (1994) during in situ studies on oil-contaminated desert sands who determined that up to 82,5% reduction of total petroleum hydrocarbons and 90,5% reduction of total alkanes could be achieved in 12 months. Rahman et al. (2003) examined the bioremediation of n-alkanes in petroleum sludge (2003). The sludge contained an oil and grease content of 87,4%. C8–C11 alkanes in 10% sludge were degraded 100%, while C12–C21, 83–98%, C22–C31, 80–85% and C32–C40, 57–73% after 56 days with addition of a bacterial consortium, nutrients, and rhamnolipids. Lower rates of biodegradation occurred as the chain length increased.

However, the rates were still significant even for C32–C40 compounds, indicating the benefit of rhamnolipid addition for assisting the biodegradation of these low solubility compounds.

CHAPTER FIVE
MATERIALS AND METHODS

The sampling procedures for the soils, materials used for the study, the methods of the analysis, and the properties of the experimental setup are described in this section.

5.1 Soil Sampling

Three different types of soils are used for the study, namely; sand, agricultural soil, and field sample. Sand samples are taken from Altınkum Beach, Didim. The top 10 cm of the sand is collected into a zip lock plastic bag and stored at 5°C until it is used. Agricultural soil is obtained from the Menemen Research Centre of Turkish Ministry of Agriculture and stored in zip lock plastic bags at 5°C. Field sample is collected from Raman Petroleum Drilling Area, Batman. In this area petroleum spill first occurred on 1940s. Since then the area is polluted by many spills. The area is the belonging of Türkiye Petrolleri A.O. (Turkish National Petroleum Company) and the sample is taken from the bottom of the drilling well of West Raman# 132. Sampling depth is 10 cm. The samples are stored in zip-lock plastic bags at 5°C until the experiments.

5.2 Materials Used

- Engine oil is used to spike the soil samples in this study. The oil is the product of PETROFER Industrial Oils and Chemicals Company which is located in Çiğli, İzmir. The type of the oil is PETROFER Petrolube Lubrimax 20W/50 Four Seasons Engine Oil, API: SF-US MIL: 46152-B.
- Biosurfactant is used in this study to observe its effect on both increasing remediation efficiency and accelerated biodegradation of petroleum hydrocarbons in soil. Rhamnolipid ($C_{26}H_{48}O_9$) is used as biosurfactant. It is the

- product of JBR Company and its commercial name is “Natural Biosurfactant JBR-425”. The properties of Rhamnolipid used is given as follows:

MW (Molecular Weight) ($C_{26}H_{48}O_9$):	504 g
ℓ (Density):	1,05 g/cm^3
CMC (Critical Micelle Concentration):	30 mg/L

The cost of the biosurfactant application during different enhancing trials are calculated and the results are summarized in Table 5.1.

Table 5.1 The cost of the biosurfactant application

1 L Rhamnolipid : 5 $ = 500 cent	Amount of Remediated Soil for 1L Rhamlolipid	Costs for 1 Ton Soil
Compost	71 ton soil	7 cent
Activated Sludge	33,6 ton soil	15 cent
Natural	35 ton soil	14 cent

The results in Table 5.1 are show that the most economical application of Rhamnolipid is observed in the enhancement with compost addition (7 cents/tons of soil).

The unit costs for Rhamnolipids application are 15 and 14 cents for activated sludge enhancement and natural remediation respectively.

Rhamnolipid application cost in compost enhancement is low since CMC (Critical Micelle Concentration) is applied 30 mg/L while the soil is saturated with water and most of the soil water content is obtained by compost in that case.

- Compost is used as an enhancing material for bioremediation of petroleum hydrocarbons in soil. Compost is obtained from Uzundere Domestic Solid Waste Composting Plant which is operated by Izmir Municipality.

- Activated Sludge is also used as another enhancing material for bioremediation of petroleum hydrocarbons in soil. It is obtained from the activated sludge reactors located in Wastewater Treatment Plant of Izmir Municipality.

The properties of compost and activated sludge are determined before the experimental study and the findings are given in the Chapter 6 - Results and Discussion.

5.3 Analytical Methods

5.3.1 Preparation and Screening of Soil Samples

Three different types of soils used for the experiments are going to be called as SAND, MENEMEN and RAMAN in the following parts of the thesis. The soils are dried and screened through 2 mm sieve to remove the gravels and rocks before they are used for the experiments. All the samples are homogenized before the experiments. 100 grams of dry soil sample is weighted and particle size distribution is determined. The screen meshes used are: 600 μm, 300 μm, 90 μm, and 45 μm. The results are given in the next chapter.

5.3.2 Analytical Methods

Water content (WC), organic matter content (OM), pH, chemical oxygen demand (COD), total petroleum hydrocarbons (TPHCs), and total organic carbon (TOC) and heavy metal (Ni, Cu, Pb, Fe, Cd) analysis are completed in soil and procedures given in Table 5.2.

Table 5.2 Analytical methods and procedures

Parameter	Method	Procedure
Water Content (WC)	Standard Methods, AWWA 1981	The sample dried at 105°C overnight and differences between the initial and final weights are used to determine the water content. MEMMERT Loading Model 100-800 drying oven is used for the experiments.
Organic Matter Content (OM)	Standard Methods, AWWA 1981	The dried sample combusted at 550°C for 2 hours and differences between the initial and final weights are used to determine the organic matter content of dry portion. MEMMERT LM 100-800 furnace is used for the experiments.
pH	EPA Method 9045C	20 grams of sample is mixed with 20 ml of distilled water for 5 minutes and centrifuged at 4000 rpm for 10 minutes. The pH value of the supernatant is measured. NUVE NF 815 centrifuge and HANNAH HI 8314 pH meter is used for the experiments.
Chemical Oxygen Demand (COD-Dissolved)	EPA Method 1310 and Standard Methods, AWWA 1981	Dried sample is extracted according to the EPA1310 and the supernatant is tested for COD with Closed Reflux Method given in Standard Methods.
Total Petroleum Hydrocarbons (TPHCs)	EPA Method 3550B	Sample is extracted with hexane in an ultrasonic extractor according to EPA 3550B, 5 ml extract is transferred into a crucible and evaporated under fume hood. The TPHCs is determined gravimetrically by using initial and final weights of the crucible. The recovery rate of the method is 96%. EIA CP102 ultrasonic extractor is used for the experiments.
Total Organic Carbon (TOC- Dissolved)	EPA Method 1310	Dried sample is extracted according to the EPA1310 and the supernatant is tested for TOC with a Dohrmann-Tekmar DC-190 High Temperature TOC Analyzer.
Heavy Metals (Pb, Cd, Cu, Ni, Fe)	EPA Method 3050B	Dried samples are digested according to EPA3050B and the heavy metals are determined in the extracts by using MERCK NOVA 60 Photometer Kits.

In addition to these analyses, Mixed Liquor Suspended Solids (MLSS) of activated sludge is determined according to Standard Methods (Standard Methods, AWWA 1981)

5.4 Experimental Setup

Three different types of soil used for the experiments are SAND, MENEMEN, and RAMAN. Since the first two soils are not contaminated by TPHCs, they are spiked by using engine oil. RAMAN is a field of contaminated soil. In Raman, soil contamination occurred due to accidental spill and two different enhancing materials -compost and activated sludge- are used to determine their effects on biodegradation of TPHCs in soil. Another set of experiments is conducted without any enhancing material added into the soil to compare the results. These three set of trials are going to be called as COMPOST, ACTIVATED SLUDGE, and NATURAL sets in the next parts of the thesis. The efficiency of biodegradation of TPHCs in soil is also tested with the presence of biosurfactant Rhamnolipid. These set of trials are going to be indicated with a BS addition next to the soil name; such as SAND-BS. Total numbers of 18 sets of trials are conducted for the study. Half of these trials are completed with the presence of biosurfactant. The summary of experiment program is given in Table 5.3.

5.4.1 Description of the System

One and a half liter glass jars with sealed lids are used for the experiments. The dimensions of each jar are 19,5 cm*9,5 cm*9,5 cm as height, width and length and the volume is 1,5 L. A 100 ml cylindrical glass cup is installed in each jar to hold the experimental matrix.

SAND and MENEMEN are spiked with engine oil by using a syringe to obtain approximately 15000 ppm of TPHCs concentration in the dry matter at the beginning of the experiments. The samples are mixed slowly to obtain homogeneous distribution of oil in the matrix. The moisture contents of the matrices are arranged as 50% at the beginning of the experiments by addition of distilled water or activated sludge, considering the initial water content of the enhancing materials. The critical micelle concentration of Rhamnolipid is 30 mg/L therefore this concentration is prepared in either distilled water or activated sludge and added to the system for the

trials done with the biosurfactant. The amount of Rhamnolipid is arranged according to the total amount of water in the system which consists of water content of the compost or activated sludge and the distilled water added to the system.

Approximately 300 grams of soil mixtures are prepared and 50 grams of experimental matrices are placed into the glass cups and the cups are placed into the jars at the beginning of the experiments. The jars are closed and sealed. The initial properties of the matrices are determined by using the remaining soil mixtures.

The jars are kept in the incubator (MEMMERT Beschickung LM 100-800) at 30°C. Each set of trials are prepared as three jars. The first jar is opened at the 7^{th} day of the experiment, the second jar opened at the 14^{th} day of the experiment and the last jar is opened at the 28^{th} day and the properties of the matrices and the changes in the amounts of contaminants with time are determined.

5.4.2 Definition of the Set of Trials

Mainly three different trials are conducted during the study: The trials with compost amendment, the trials with activated sludge amendment, and the trials with natural soil (no amendment). The sets of trials are indicated in Table 5.3 with the amounts of materials used for each one.

Table 5.3 The sets of trials

TRIALS	SETS	MATERIALS USED
COMPOST ENHANCED TRIALS	SAND-BS	120 g Compost + 120 g Sand +58.8 ml Ionized Water with Rhamnolipid + 2,52ml Engine Oil
	SAND	120 g Compost + 120 g Sand +58.8 ml Ionized Water + 2,52 ml Engine Oil
	MENEMEN-BS	120 g Compost + 120 g Menemen Soil +58.8 ml Ionized Water with Rhamnolipid + 2,52ml Engine Oil
	MENEMEN	120 g Compost + 120 g Menemen Soil +58.8 ml Ionized Water + 2,52 ml Engine Oil
	RAMAN-BS	120 g Compost + 120 g Raman Soil + 60 ml Ionized Water with Rhamnolipid
	RAMAN	120 g Compost + 120 g Raman Soil + 60 ml Ionized Water
ACTIVATED SLUDGE ENHANCED TRIALS	SAND-BS	147 g Sand +153 ml Activated Sludge with Rhamnolipid + 2,52 ml Engine Oil
	SAND	147 g Sand +153 ml Activated Sludge + 2,52ml Engine Oil
	MENEMEN-BS	147 g Menemen Soil +153 ml Activated Sludge with Rhamnolipid + 2,52 ml Engine Oil
	MENEMEN	147 g Menemen Soil +153 ml Activated Sludge + 2,52ml Engine Oil
	RAMAN-BS	147 g Raman Soil +153 ml Activated Sludge with Rhamnolipid
	RAMAN	147 g Raman Soil +153 ml Activated Sludge
NATURAL SOIL TRIALS	SAND-BS	150 g Sand +150 ml Ionized Water with Rhamnolipid + 2,52 ml Engine Oil
	SAND	150 g Sand +150 ml Ionized Water + 2,52ml Engine Oil
	MENEMEN-BS	150 g Menemen Soil +150 ml Ionized Water with Rhamnolipid + 2,52 ml Engine Oil
	MENEMEN	150 g Menemen Soil +150 ml Ionized Water + 2,52ml Engine Oil
	RAMAN-BS	150 g Raman Soil +150 ml Ionized Water with Rhamnolipid
	RAMAN	150 g Raman Soil +150 ml Ionized Water

CHAPTER SIX
RESULTS AND DISCUSSION

The results of the experimental study and the discussion of these results are given in this section of the thesis.

6.1 Results

6.1.1 Properties of Soil Samples

The properties of the soil samples used for the experiments are given in Table 6.1. All samples are dried at 60°C overnight prior to experiments to remove water content.

Table 6.1 Initial properties of soil samples

Soil Sample	**Cu, ppm dw**	**Cd, ppm dw**	**Pb, ppm dw**	**Fe, ppm dw**	**Ni, ppm dw**	**pH**	**Water Content, %**	**Organic Matter Content, % dw**	**Total Petroleum Hydrocarbons (TPHCs), ppm dw**
Sand	158	100	500	400	712	7,57	0,0	0,63	nd*
Menemen	33	5,7	143	400	818	8,05	11,77	5,58	nd*
Raman	291	45,8	55	440	600	8,23	1,89	27,21	23160

nd*: not detected, dw: dry weight

Also, the particle size distribution of soil samples is determined. The results can be seen in Table 6.2.

Table 6.2 Particle size distribution of soil samples

Screens	SAND, % dw	MENEMEN, % dw	RAMAN, % dw
2mm>A>600µm	43,12	21,36	57,02
600µm >B>300µm	52,79	10,42	19,35
300µm >C>90µm	4,05	37,20	16,31
90µm >D>45µm	0,03	22,52	4,22
45µm >E	0,00	8,50	3,10

dw: dry weight

As can be seen in Table 6.2, while almost 100% of the sand particles and 93% of Raman soil particles are larger than 90 µm, 31% of Menemen soil particles are smaller than 90 µm.

6.1.2 Properties of Compost and Activated Sludge

The properties of compost and activated sludge are determined and the results are given in Table 6.3.

Table 6.3 Properties of compost and activated sludge

Material	Cu, ppm dw	Cd, ppm dw	Pb, ppm dw	Fe, ppm dw	Ni, ppm dw	pH	Water Content, %	Organic Matter Content, % dw	Mixed Liquor Volatile Suspended Solids, mg/L
Compost	5	2,5	12	400	64	8,94	76,1	39,26	nd*
Activated Sludge	nd*					7,84	98,31	63,65	11000

nd*: not detected, dw: dry weight

6.1.3. Results of the Trials with Compost Enhancement

The parameters of water content, organic matter content, pH, dissolved chemical oxygen demand (COD_d), total petroleum hydrocarbons (TPHCs), and dissolved total organic carbon (TOC_d) are determined in the experimental matrices enhanced with compost at the days of 0, 7, 14, and 28 and the results are given in Table 6.4.

Table 6.4 Results of compost enhanced TPHCs remediation in soil

SETS	Time, day	pH	WC, %	OM, % dw	COD_d, ppm dw	TPHC, ppm	TOC_d, ppm dw
SAND-BS	0	8,14	30,4	25,2	39040	21000	5616
	7	8,21	31,1	19,8	86080	16320	7517
	14	9,00	30,9	15,5	64000	16720	5363
	28	9,05	29	13,3	28800	15320	1592
SAND	0	8,07	30,98	19,2	37600	14800	6973
	7	8,40	32,54	15,8	53344	16600	8288
	14	9,28	32,16	15,8	52160	4280	6390
	28	9,18	32,08	10,7	38400	9320	3571
MENEMEN-BS	0	8,67	38,6	18,6	37800	14280	27277
	7	8,61	38,41	18,6	31680	11520	32864
	14	8,58	38,1	16,3	28080	9560	22406
	28	8,83	38,34	15,7	24480	7420	12480
MENEMEN	0	8,44	32,37	18,72	36000	13440	25053
	7	8,37	28,27	18,55	35838	10960	25309
	14	8,31	33,43	18,14	32400	8440	24259
	28	8,66	35,36	16,8	29880	6851	22963
RAMAN-BS	0	8,61	44,68	21,4	40960	10480	5366
	7	8,67	45,76	20,9	83840	7240	15165
	14	8,77	45,4	18,8	45440	4480	11885
	28	8,64	44,62	17,5	29760	1800	9312
RAMAN	0	8,65	24,54	18,95	39680	7640	6742
	7	8,61	30,64	18,24	63808	6920	5760
	14	8,61	37,12	16,03	57600	5200	5718
	28	8,60	40,77	15,12	55360	3840	5677

6.1.4 Results of the Trials with Activated Sludge Enhancement

The parameters of water content, organic matter content, pH, dissolved chemical oxygen demand (COD_d), total petroleum hydrocarbons (TPHCs), and dissolved total organic carbon (TOC_d) are determined in the experimental matrices enhanced with activated sludge at the days of 0, 7, 14, and 28 and the results are given in Table 6.5.

Table 6.5 Results of activated sludge enhanced TPHCs remediation in soil

SETS	Time, day	pH	WC, %	OM, % dw	COD_d, ppm dw	TPHC, ppm	TOC_d, ppm dw
SAND-BS	0	7,17	53,3	5,07	35200	6360	18083
	7	6,36	52,7	2,39	46080	5640	15898
	14	7,26	34,4	2,16	30400	3240	6214
	28	7,62	46,9	2,11	20800	1240	2429
SAND	0	7,41	53,73	4,54	29760	6040	19360
	7	6,61	34,97	4,5	42240	5360	15430
	14	8,48	35,3	4,41	40320	4210	6992
	28	8,3	42,54	4,12	37760	4000	3139
MENEMEN-BS	0	7,12	52,51	9,48	15797	9920	21024
	7	6,27	50,41	9,45	12960	7440	19882
	14	7,48	47,93	7,87	6480	6520	14320
	28	7,61	43,77	6,28	720	5422	4678
MENEMEN	0	7,95	45,76	7,16	15077	9720	19651
	7	7,11	44,01	7,05	14350	7240	18262
	14	8,4	47,18	6,86	13680	6240	16230
	28	8,34	43,03	6,45	13320	5124	2269
RAMAN-BS	0	7,86	43,85	34,93	72960	22000	3558
	7	8,63	53,82	29,91	131520	18920	11648
	14	9,09	53,6	12,92	112640	16800	11280
	28	8,68	46,36	12,59	76800	14120	2644
RAMAN	0	8,06	52,93	14,26	68480	15240	20490
	7	8,6	46,38	11,8	104320	13080	9974
	14	7,97	49,46	10,14	78400	10440	9414
	28	8,32	46,99	9,99	62720	9560	8883

6.1.5 Results of the Trials without Enhancement

The parameters of water content, organic matter content, pH, dissolved chemical oxygen demand (COD_d), total petroleum hydrocarbons (TPHCs), and dissolved total organic carbon (TOC_d) are determined in the experimental matrices without enhancement at the days of 0, 7, 14, and 28 and the results are given in Table 6.6.

Table 6.6 Results of TPHCs remediation in natural soil

SETS	Time, day	pH	WC, %	OM, % dw	CODd, ppm dw	TPHC, ppm	TOCd, ppm,dw
SAND-BS	0	7,58	34,1	1,46	9720	8640	2256
	7	7,12	35,2	1,38	7560	6840	1226
	14	8,38	31,9	1,35	5760	6360	1024
	28	7,24	33,6	1,11	3600	5862	1264
SAND	0	7,47	59,86	1,84	9360	5120	2246
	7	6,69	59,88	1,83	7920	5160	2397
	14	7,88	32,16	1,8	7560	5000	2461
	28	7,85	32,32	1,77	7492	4960	2555
MENEMEN-BS	0	8,21	44,88	13,4	31040	12640	3555
	7	9,43	48,36	10,7	78080	22640	5232
	14	9,29	46,74	5,97	65920	15000	5034
	28	9,15	44,37	5,13	65280	16550	4730
MENEMEN	0	8,5	44,9	13,5	24320	18800	6128
	7	8,84	51,49	12,64	25920	15760	15174
	14	9,27	52,52	5,92	24960	15800	9674
	28	8,7	48,48	5,44	20480	14840	1441
RAMAN-BS	0	8,47	49,45	14,08	32400	12280	23696
	7	8,44	48,98	11,33	29160	10240	26442
	14	9,02	38,12	9,58	13320	7440	13027
	28	7,96	40,24	7,86	8640	7261	4726
RAMAN	0	8,44	29,19	14,26	31680	11020	22979
	7	8,43	28,45	11,48	29998	9640	25533
	14	8,54	29,2	10,38	23040	7040	14320
	28	8,36	29,94	10,17	18720	6658	13434

6.2 Discussion of Results

6.2.1 Variations and Reductions in Soil Parameters during Remediation

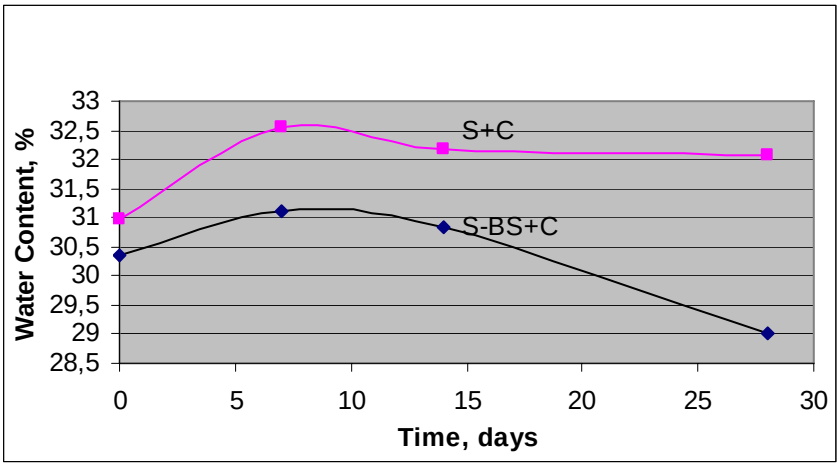
In this section the variation of concentration of pollutant parameters and water content and pH values in soil media are shown against time. In the graphs the explanation of medium is indicated as; M: Menemen Soil, R: Raman Soil, S: Sand, and the enhancement material, AS: Activated Sludge, C: Compost and BS: Biosurfactant.

6.2.1.1 Variations in pH, Water Content and Contaminant Concentrations in Soil

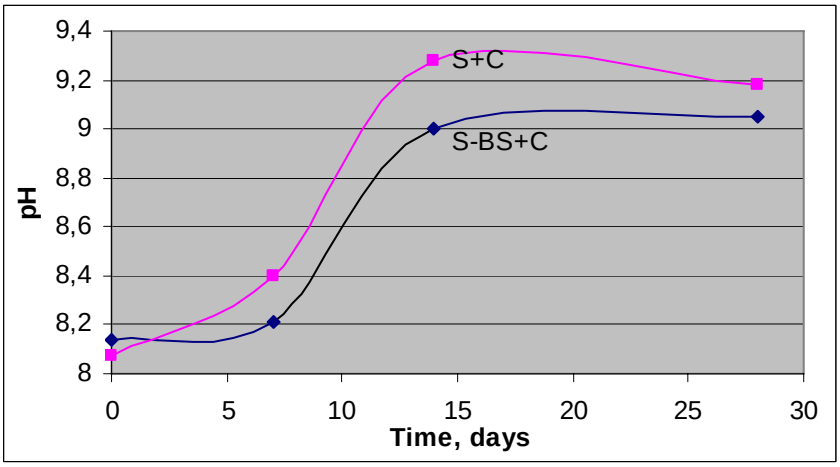
6.2.1.1.1 Compost Enhanced Trials

- **Trials with Sand**

a)



b)



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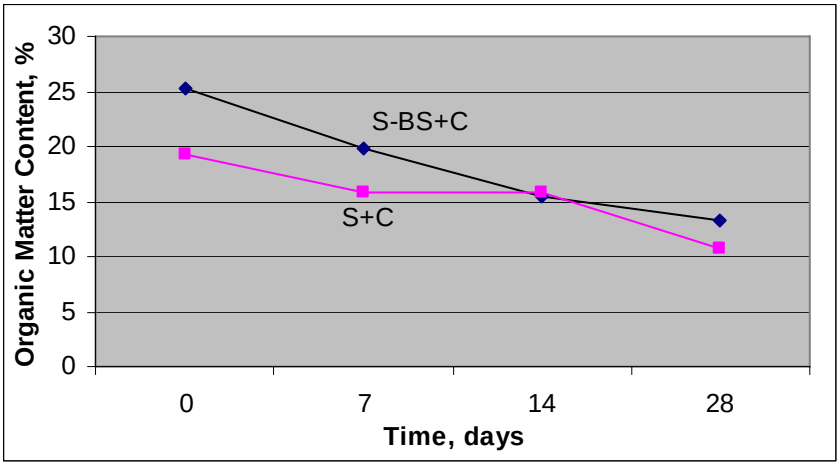
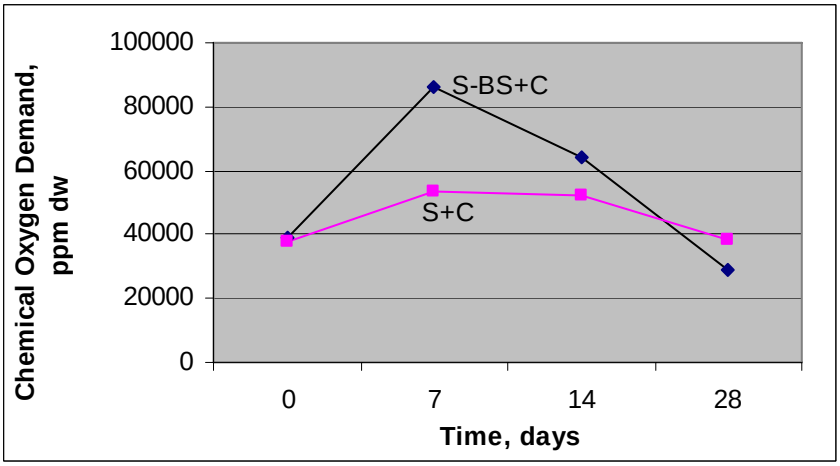
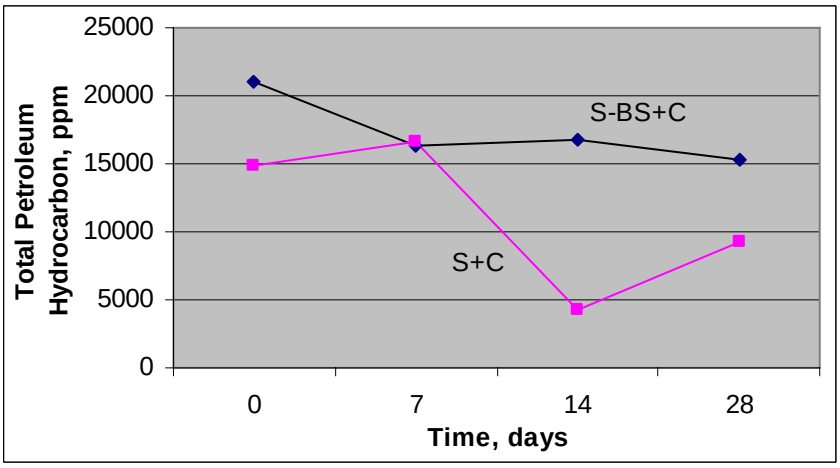

Organic Matter Content, %
30
25
20
15
10
5
0
S-BS+C
S+C
0
7
14
28
Time, days

d)


Chemical Oxygen Demand, ppm dw
100000
80000
60000
40000
20000
0
S-BS+C
S+C
0
7
14
28
Time, days

e)


Total Petroleum Hydrocarbon, ppm
25000
20000
15000
10000
5000
0
S-BS+C
S+C
0
7
14
28
Time, days

f)

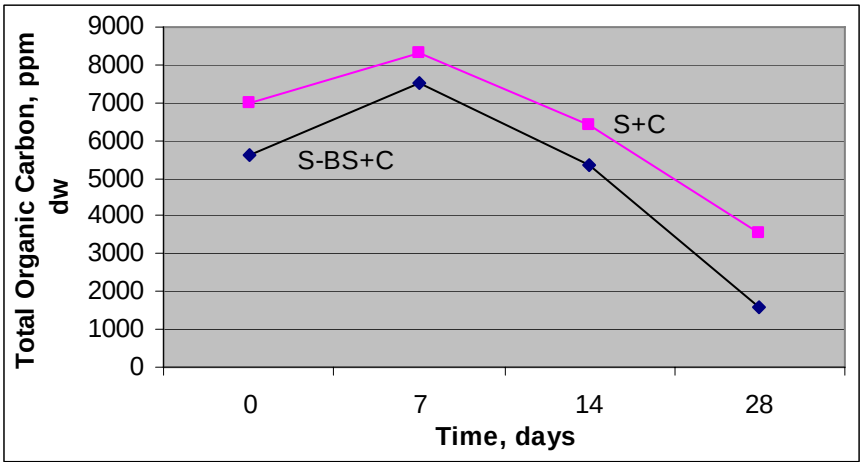


Figure 6.1 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with compost enhanced soil remediation (Sand-BS, Sand).

Water content of the soil samples varied between 29% and 32,5%. Therefore the water content was not the limiting factor for the decomposition. pH values have shown a steady increase starting from 8,1 up to 9-9,3.

After a slight rise in COD contents of the soil in the first week of the experiments, a significant decline has been observed with biosurfactant. Since the added biosurfactant contain organic matter, initial concentration is expected to be higher.

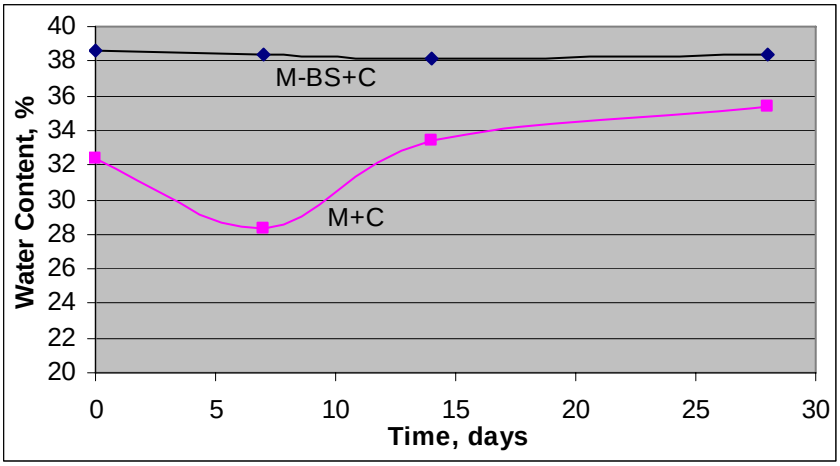
pH of the soil media has increased to alkaline levels as the organic matter content has decreased in both cases of biosurfactant added soil and without biosurfactant.

As can be seen in Figure 6d and 6f water soluble chemical oxygen demand and total organic carbon values show an increase in the first week of the trial. This can be caused by the effect of microbial activities that expose enzymes which may help dissolving the carboniferous material. Decreases in water soluble chemical oxygen demand and total organic carbon can be seen in the following days of the experiment.

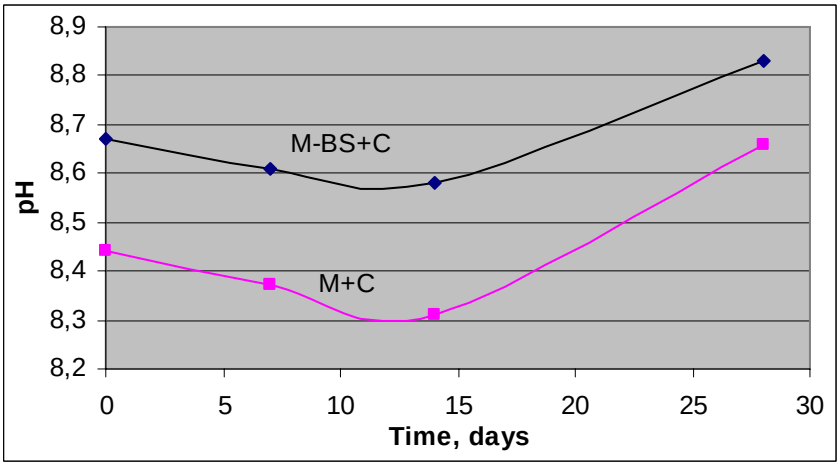
Total petroleum hydrocarbons have decreased with the presence of biosurfactant, but a stable regime can not be seen in the set without biosurfactant.

- **Trials with Menemen Soil**

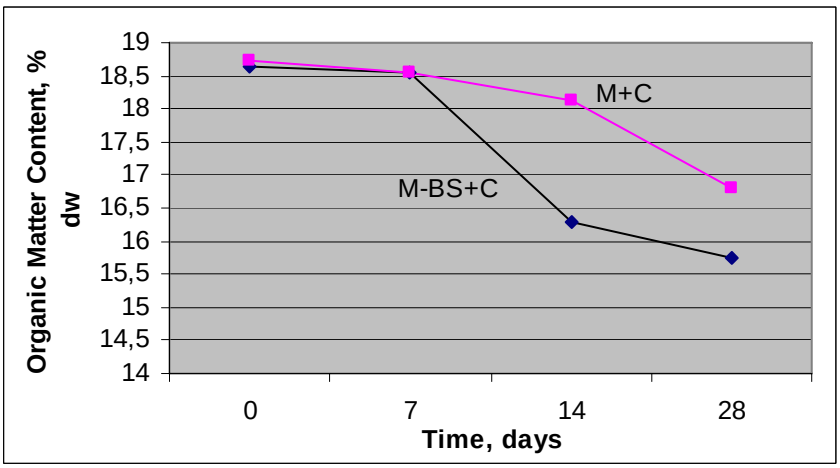
a)



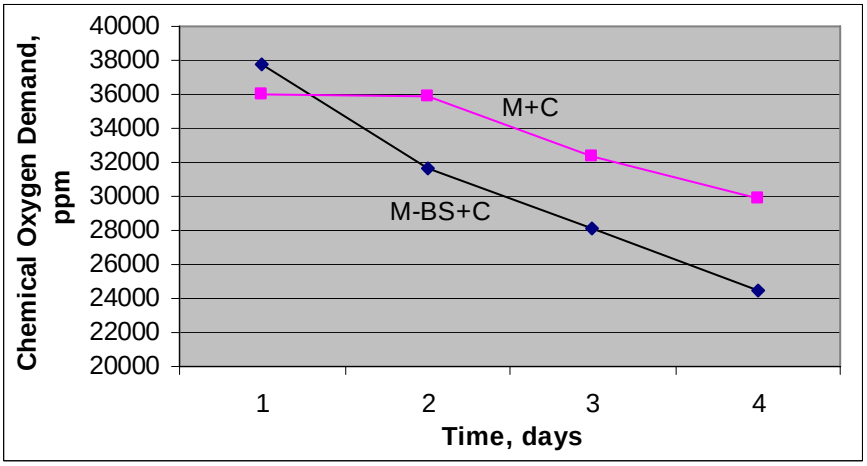
b)



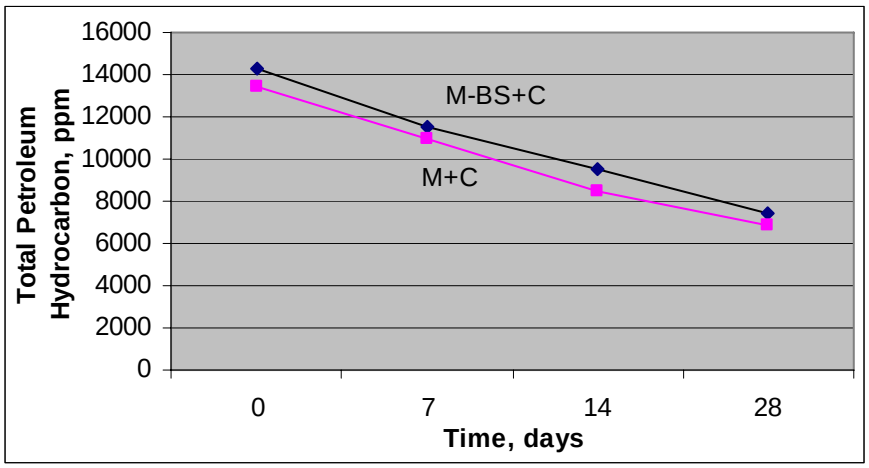
c)



d)



e)



f)

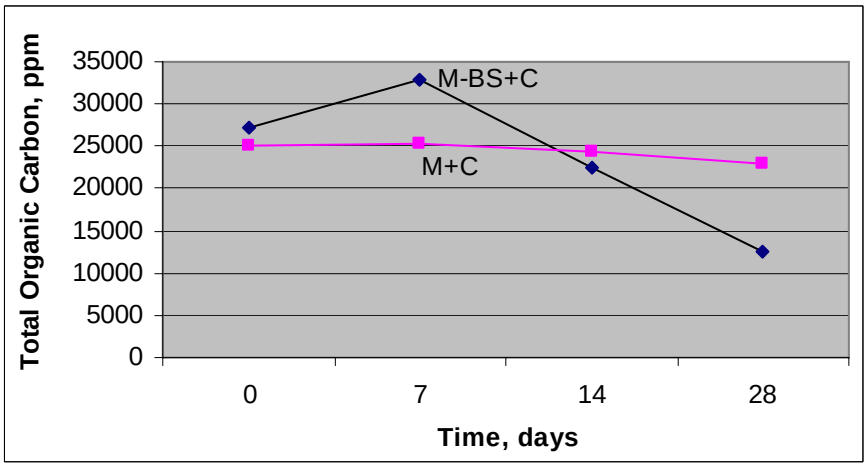


Figure 6.2 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with compost enhanced soil remediation (◆ Menemen-BS, ■ Menemen).

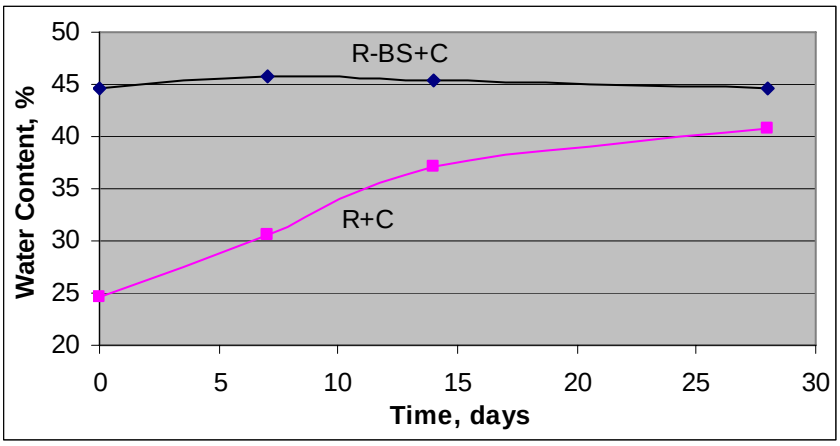
Water content of the soil samples from Menemen remained almost constant with biosurfactant and some variation has been observed without biosurfactant.

COD values showed a steady and considerable decrease in both soil samples with and without biosurfactant the positive effect of biosurfactant on organic matter decay was obvious.

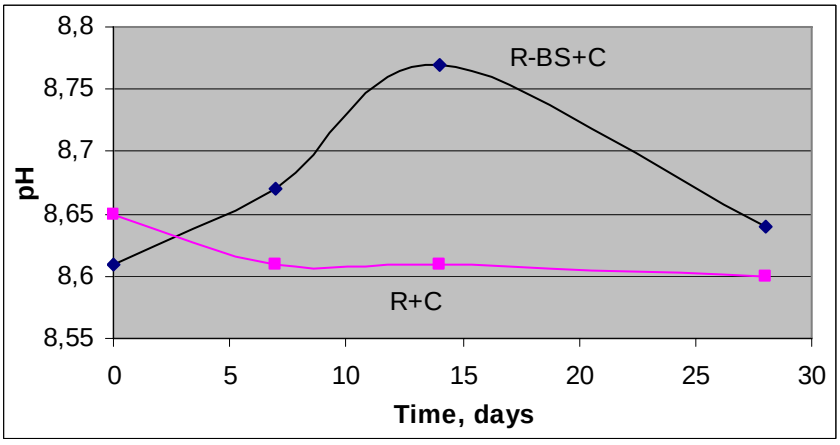
After a decline in the first 14 day of the experiment pH values climbs to alkaline levels. Water content shows as light decline in the presence of biosurfactant while the set without biosurfactant rises. Organic matter content, water soluble chemical oxygen demand and total petroleum hydrocarbons decline steadily in these set of experiments with Menemen soil. Water soluble total organic carbon shows a rise in the first week with the presence of biosurfactant, and then declines. Decrease in concentration of water soluble total organic carbon without biosurfactant is not sufficient.

- **Trials with Raman Soil**

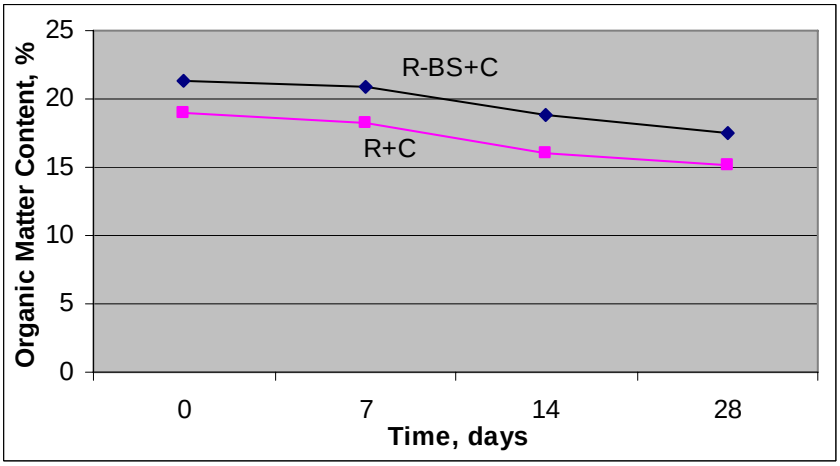
a)



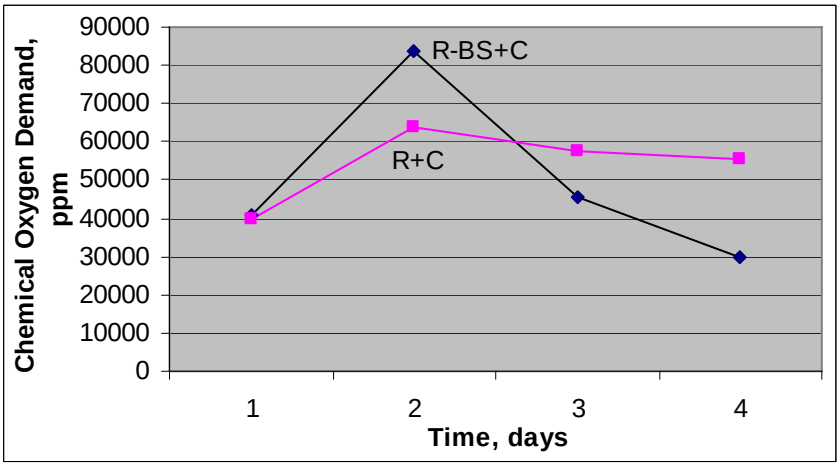
b)



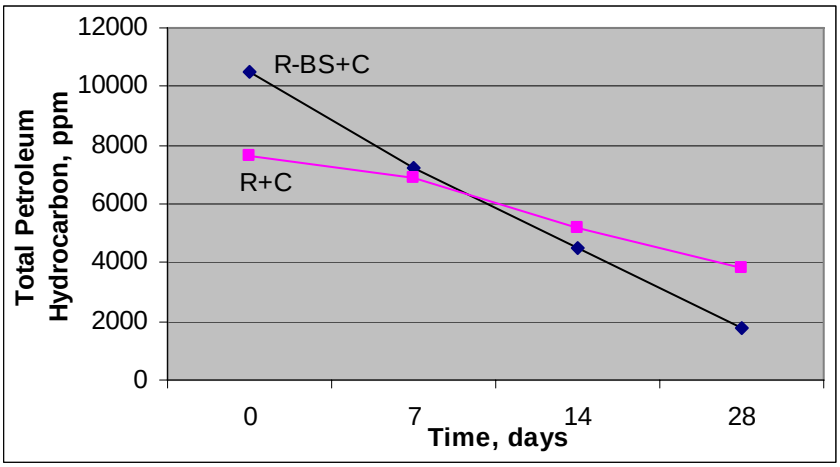
c)


Organic Matter Content, %
25
20
15
10
5
0
R-BS+C
R+C
0
7
14
28
Time, days

d)


Chemical Oxygen Demand, ppm
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
R-BS+C
R+C
1
2
3
4
Time, days

e)


Total Petroleum Hydrocarbon, ppm
12000
10000
8000
6000
4000
2000
0
R-BS+C
R+C
0
7
14
28
Time, days

f)

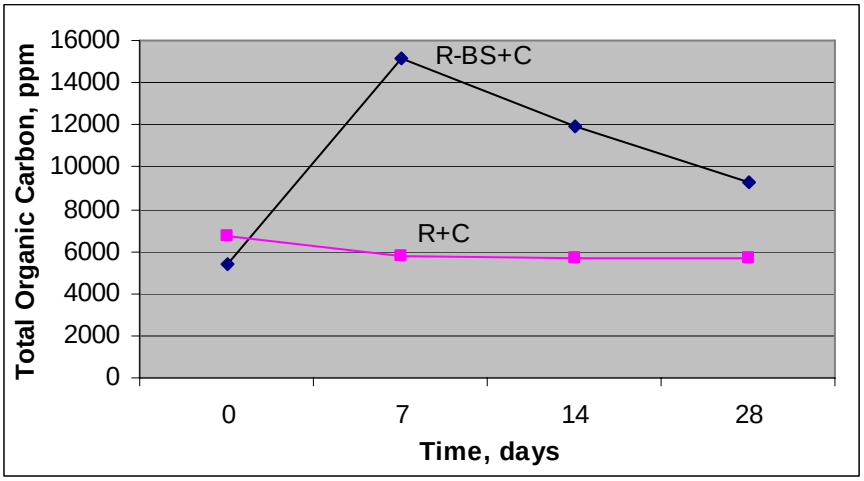


Figure 6.3 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with compost enhanced soil remediation (Raman-BS, Raman).

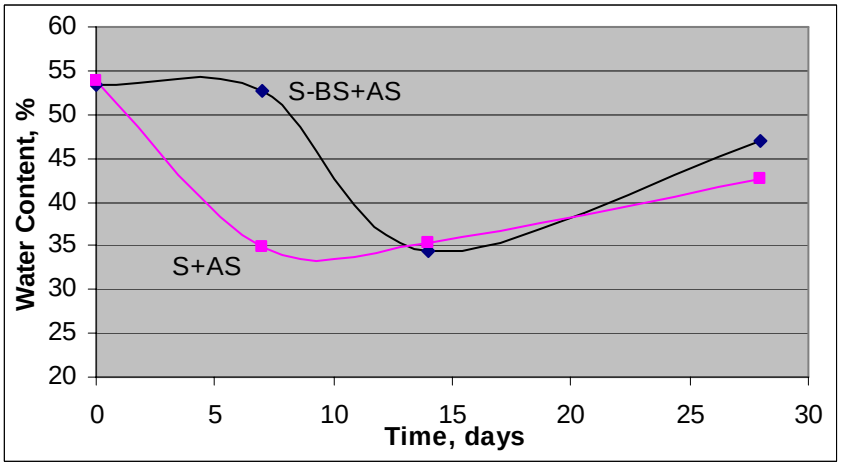
As can be seen from figures c and e; both organic matter content and total petroleum hydrocarbons versus time graphs indicate the positive effect of biosurfactants on the enhancement of biodegradation. Especially the difference between the removal efficiency of petroleum hydrocarbons was 33,08%.

In the trials with Raman soil; water content has been almost constant during the experiment with the presence of biosurfactant, but a significant raise has seen without biosurfactant.

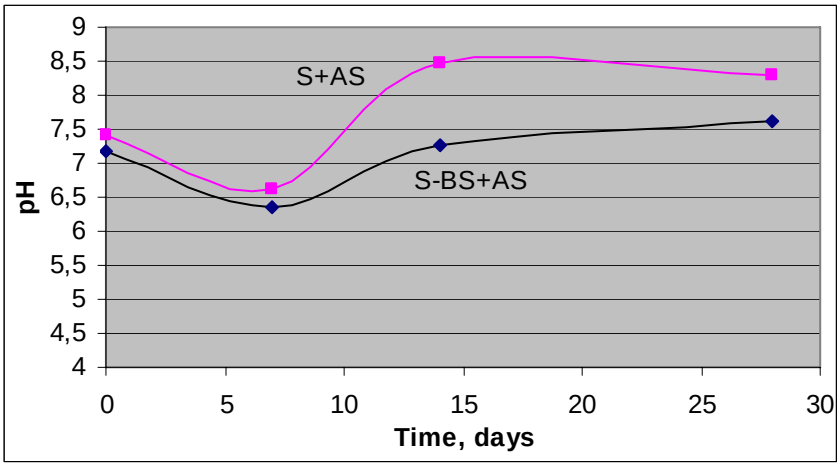
6.2.1.1.2 Activated Sludge Enhanced Trials

- **Trials with Sand**

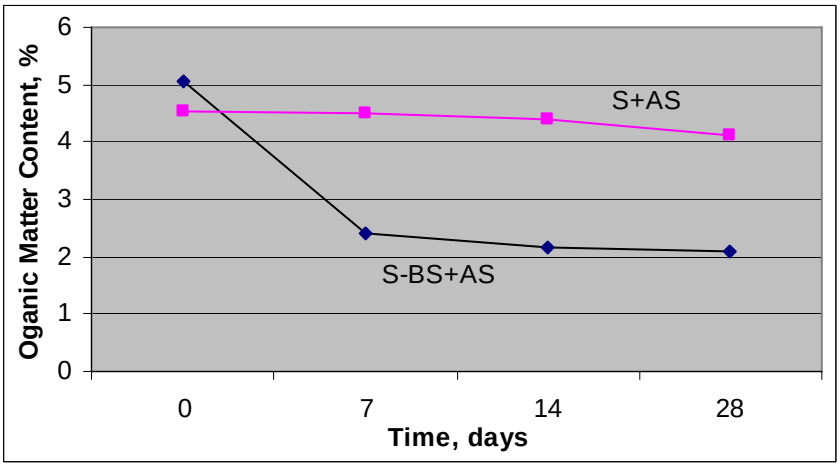
a)



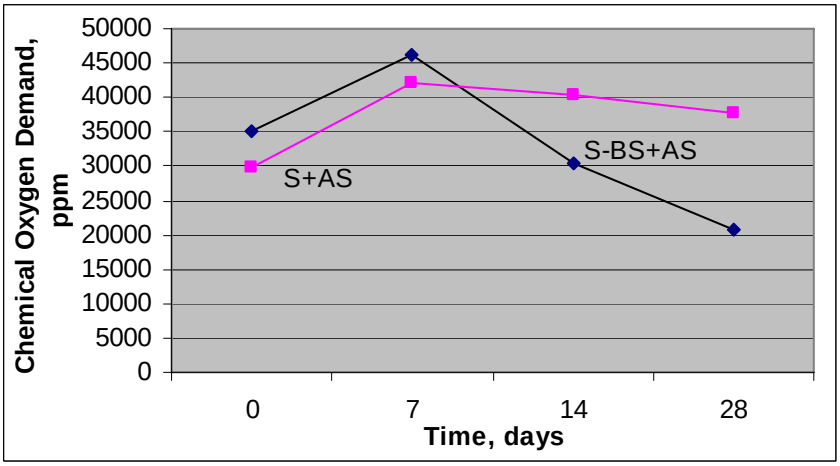
b)


9
8,5
8
7,5
7
6,5
6
5,5
5
4,5
4
pH
S+AS
S-BS+AS
0
5
10
15
20
25
30
Time, days

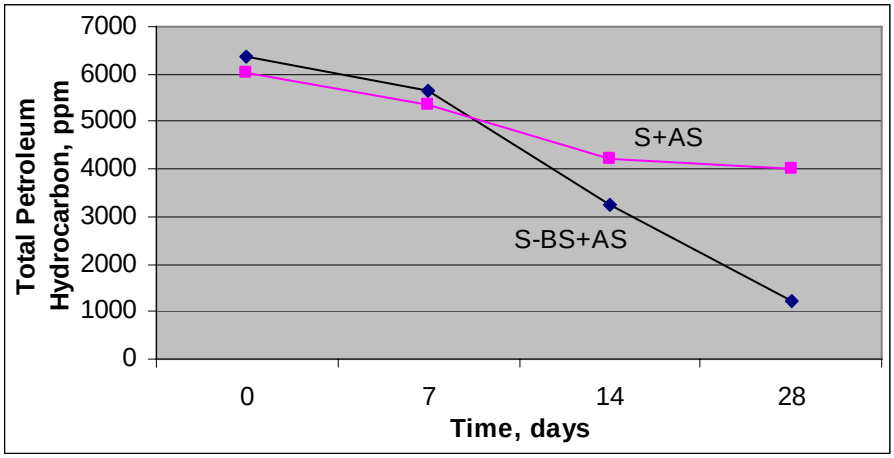
c)


6
5
4
3
2
1
0
Oganic Matter Content, %
S+AS
S-BS+AS
0
7
14
28
Time, days

d)


50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Chemical Oxygen Demand, ppm
S-BS+AS
S+AS
0
7
14
28
Time, days

e)



f)

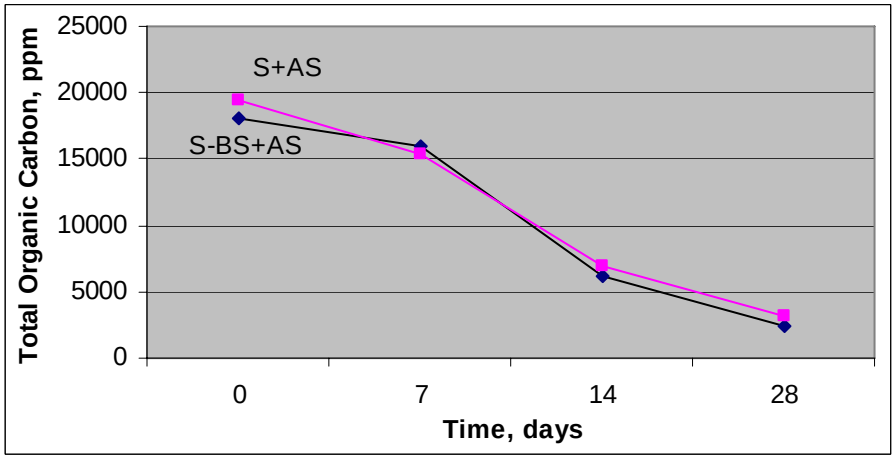


Figure 6.4 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with activated sludge enhanced soil remediation (◆ Sand-BS, ■ Sand).

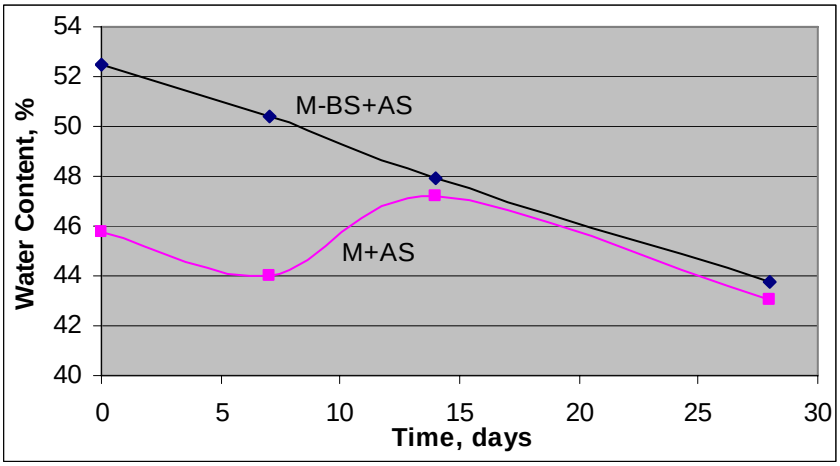
Water content of the media drop in the first two weeks of the experiments, and it slightly rises again until the end of the experiment. pH values of the both sets drop in the first week and then show a slight rise to alkaline conditions.

Organic matter content, total petroleum hydrocarbons and water soluble total organic carbon values gradually decline during these set of experiments, especially in the set with the presence of biosurfactant. Water soluble chemical oxygen demand values have also decreased after an increase seen in the first week. Drop in total petroleum hydrocarbons concentration was 5120 ppm for the biosurfactant added soil although without biosurfactant addition the removal was 2040 ppm This series

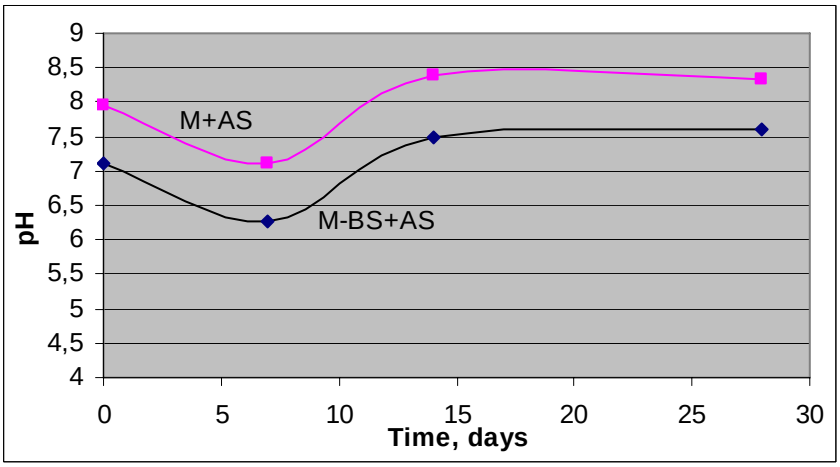
indicates the positive effect of biosurfactant addition under the same conditions natural decay.

- **Trials with Menemen Soil**

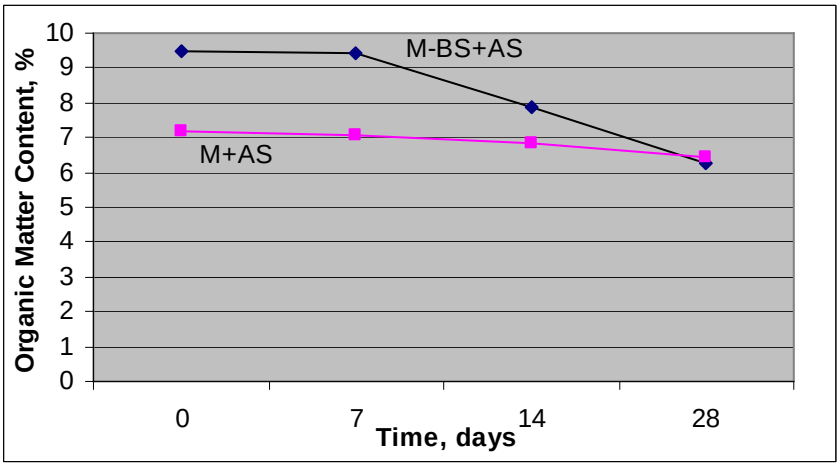
a)



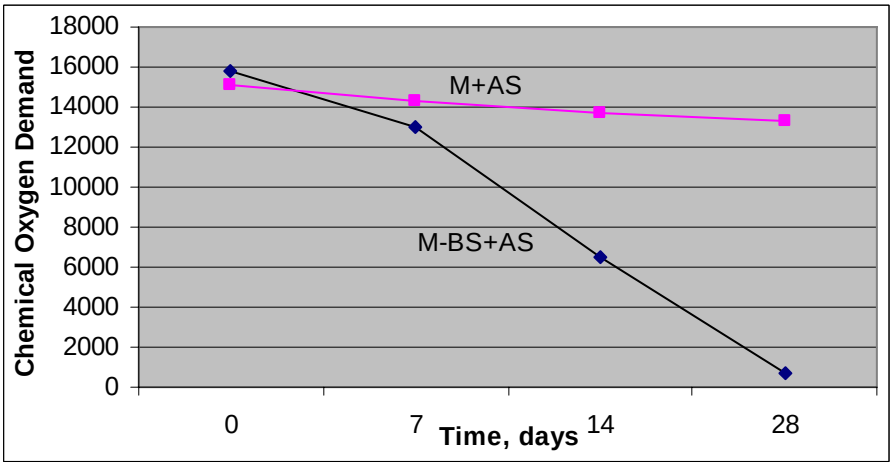
b)



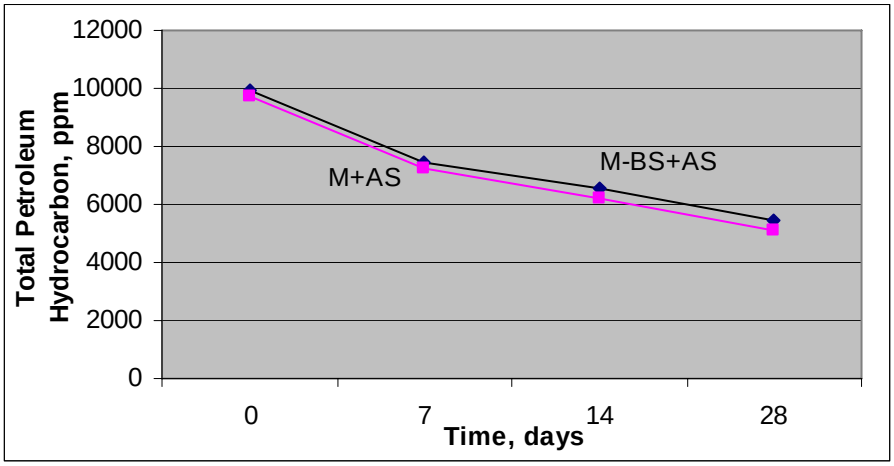
c)



d)



e)



f)

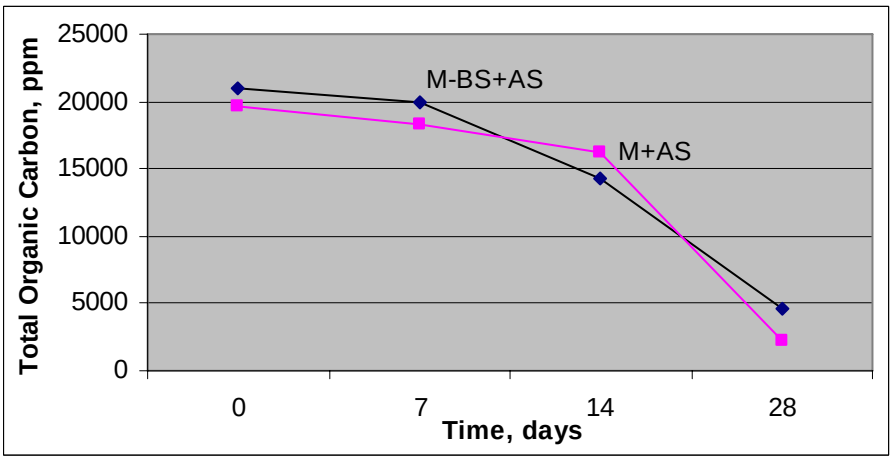


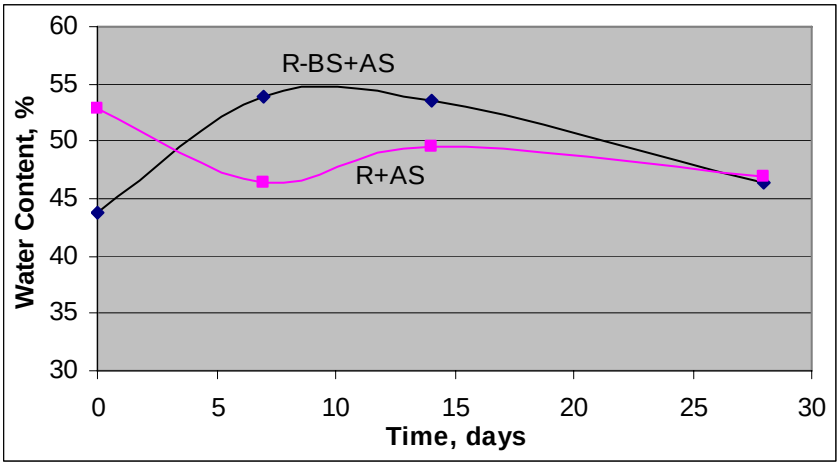
Figure 6.5 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with activated sludge enhanced soil remediation (◆ Menemen-BS, ■ Menemen).

In these set of experiments water contents in both cases, with and without presence of biosurfactant, decline significantly. pH values show a decrease in the first week then raise to more alkaline levels. The removal efficiency of TPHCs in natural was 21,06% but removal efficiency of TPHCs in activated sludge was 47,28%.

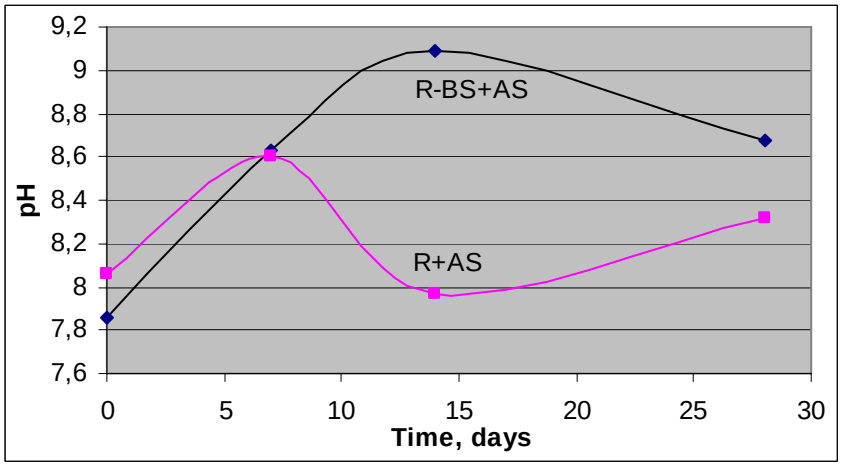
Organic matter content, total petroleum hydrocarbons, water soluble chemical oxygen demand, and total organic carbon values show rapid declines during these set of experiments and the positive effect in the presence of biosurfactant has been observed.

- **Trials with Raman Soil**

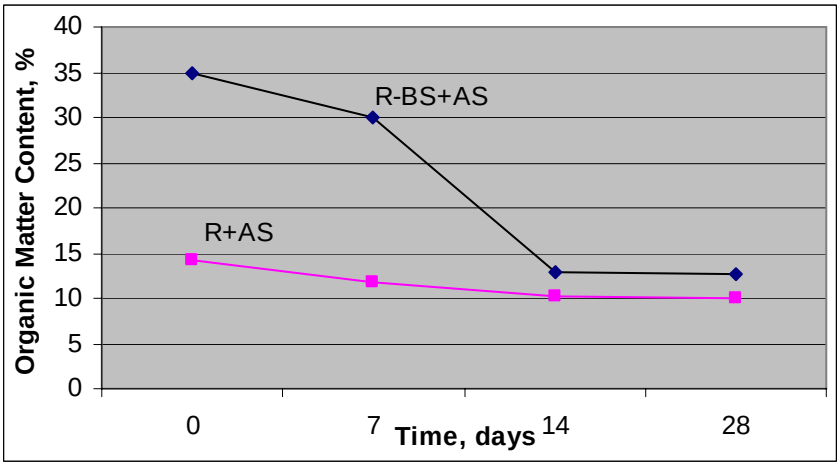
a)



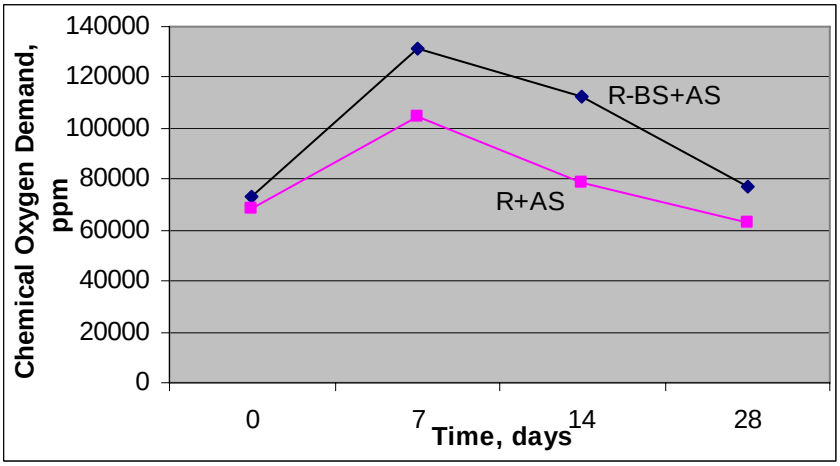
b)



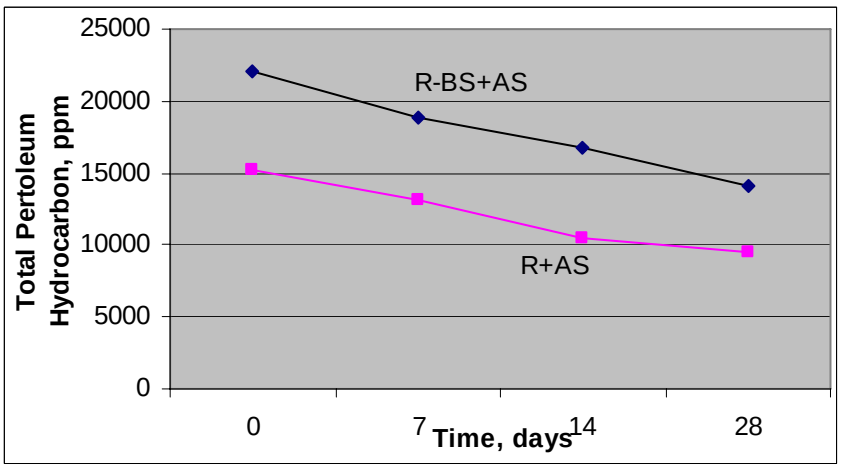
c)


Organic Matter Content, %
40
35
30
25
20
15
10
5
0
R-BS+AS
R+AS
0
7
14
28
Time, days

d)


Chemical Oxygen Demand, ppm
140000
120000
100000
80000
60000
40000
20000
0
R-BS+AS
R+AS
0
7
14
28
Time, days

e)


Total Pertoleum Hydrocarbon, ppm
25000
20000
15000
10000
5000
0
R-BS+AS
R+AS
0
7
14
28
Time, days

f)

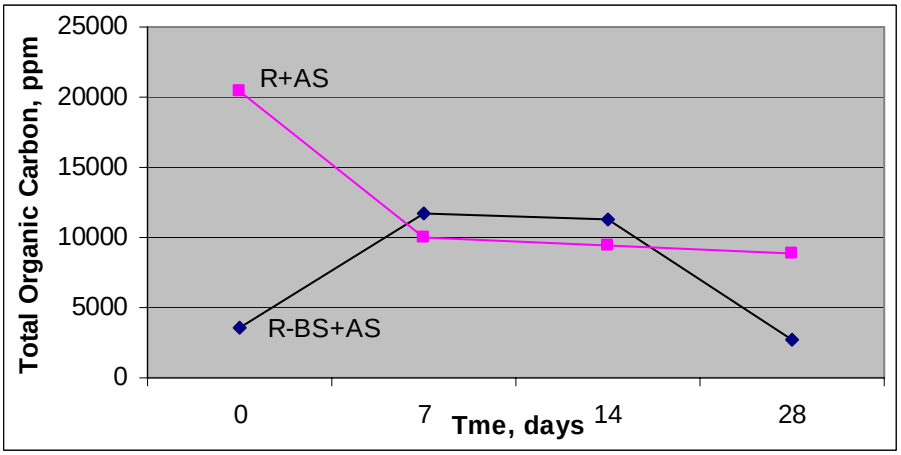


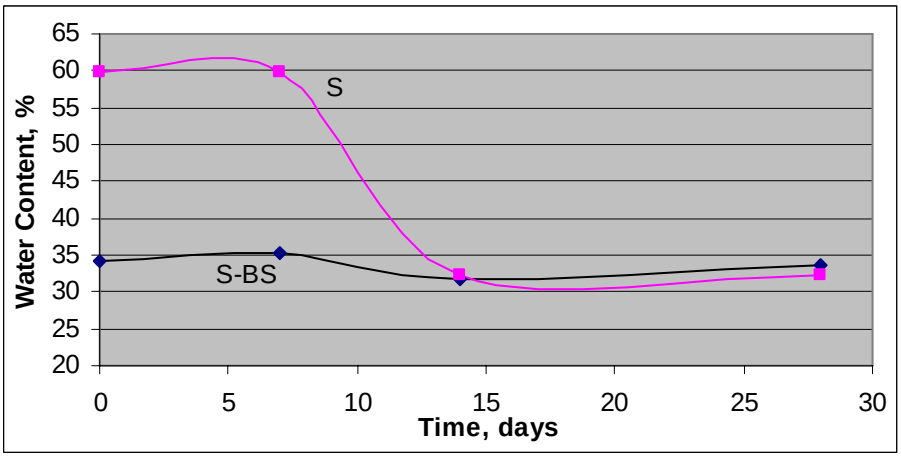
Figure 6.6 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with activated sludge enhanced soil remediation (Raman-BS, Raman).

In this series of experiments water content values of the soil are around 50 %, while pH raises in the presence of biosurfactant and fluctuates without any biosurfactant. Organic matter content and total petroleum hydrocarbons declines in the presence of biosurfactant considerably. Water soluble chemical oxygen demand shows an increase in the first week and declines in the next days of the experiment. Total organic carbon values fluctuates during the experimental period.

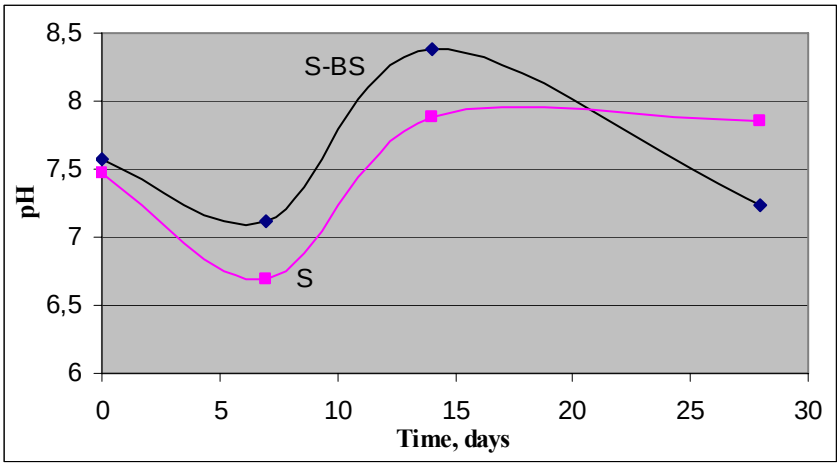
6.2.1.1.3 Trials with No Enhancement (Natural)

- **Trials with Sand**

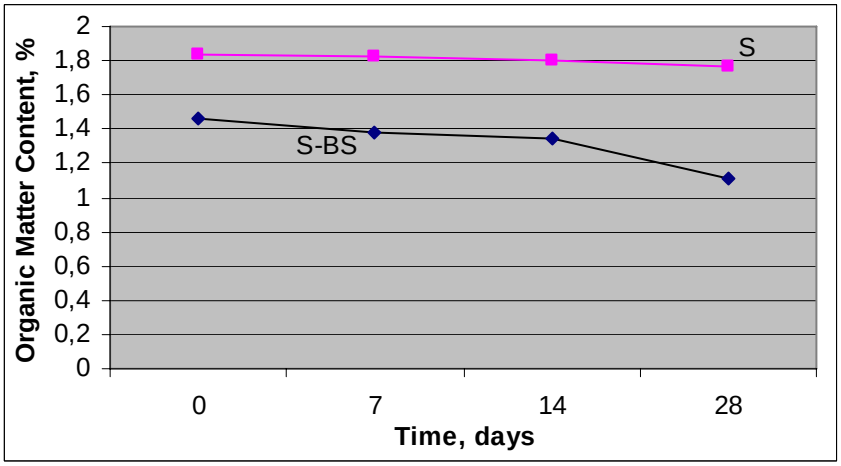
a)



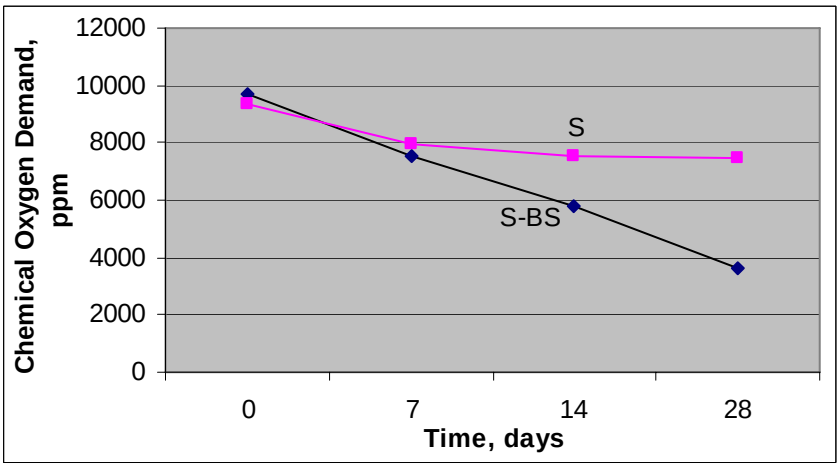
b)


8,5
8
7,5
7
6,5
6
pH
S-BS
S
0
5
10
15
20
25
30
Time, days

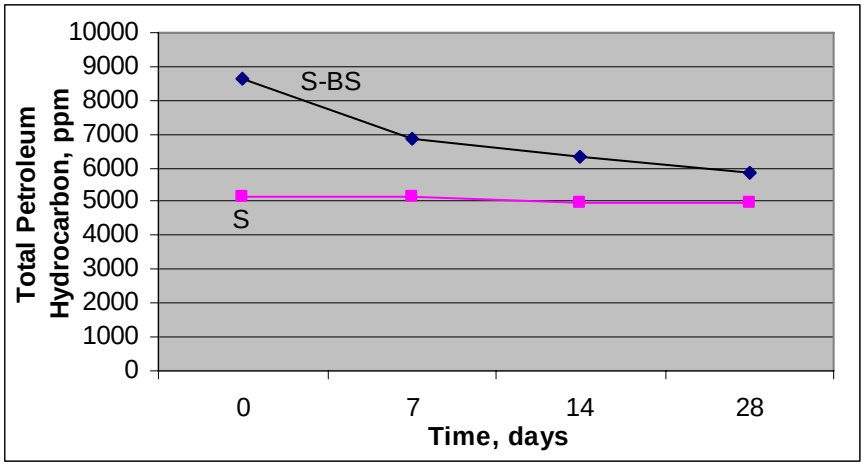
c)


2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
Organic Matter Content, %
S
S-BS
0
7
14
28
Time, days

d)


12000
10000
8000
6000
4000
2000
0
Chemical Oxygen Demand, ppm
S
S-BS
0
7
14
28
Time, days

e)



f)

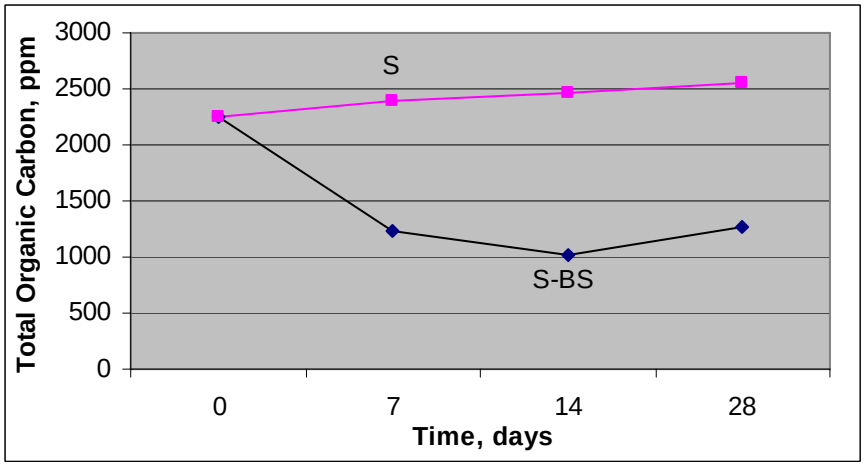


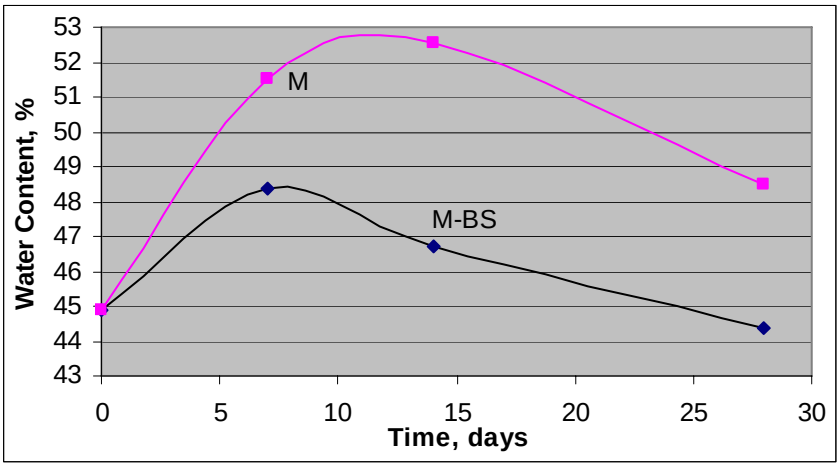
Figure 6.7 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with no enhancement enhanced soil remediation (—◆— Sand-BS, —■— Sand).

Water content in the set with biosurfactant doesn't change much, but a rapid decline has been observed in the set without any biosurfactant. pH values drop in the first week, then they increase to alkaline levels in the second week. At the end of the experimental time period neutral values has been reached in both sets.

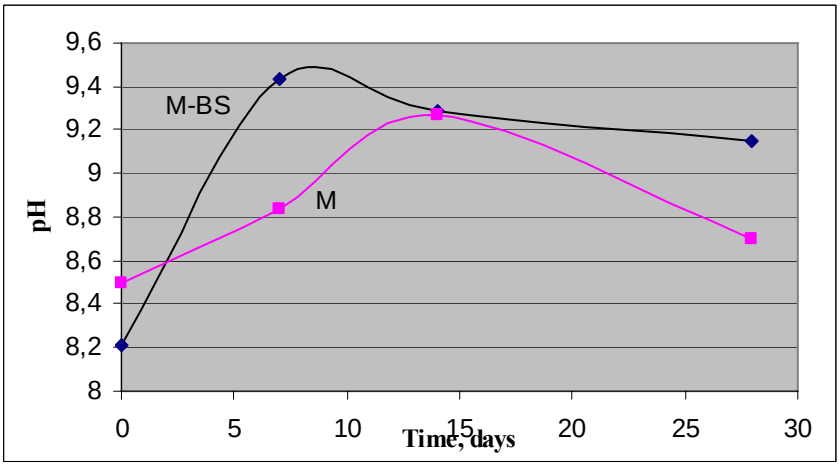
Organic matter content, total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand values in the set with biosurfactant are significantly decreases and the positive effect of the presence of biosurfactant is apparent.

- **Trials with Menemen Soil**

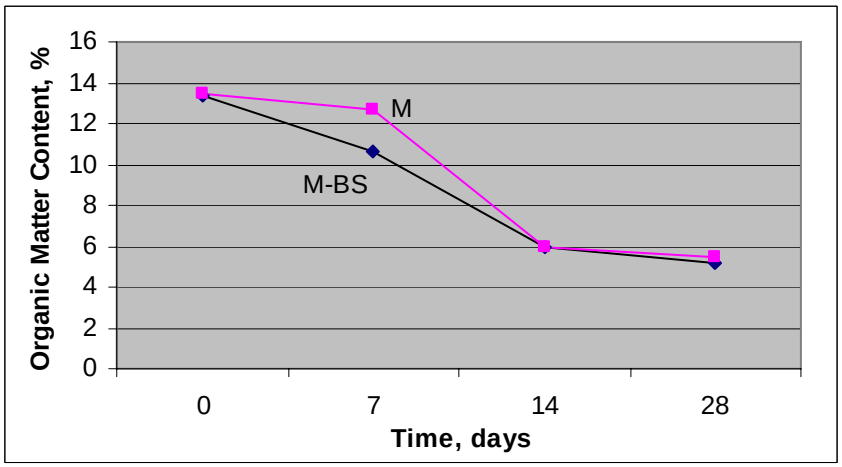
a)



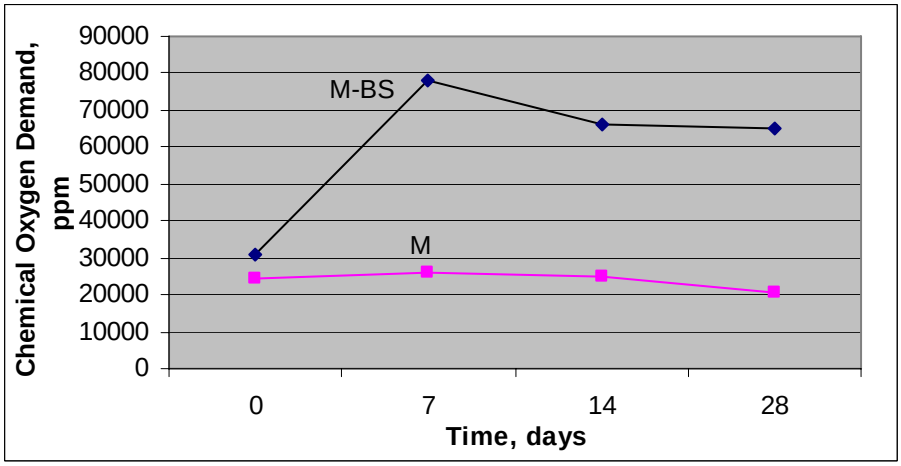
b)



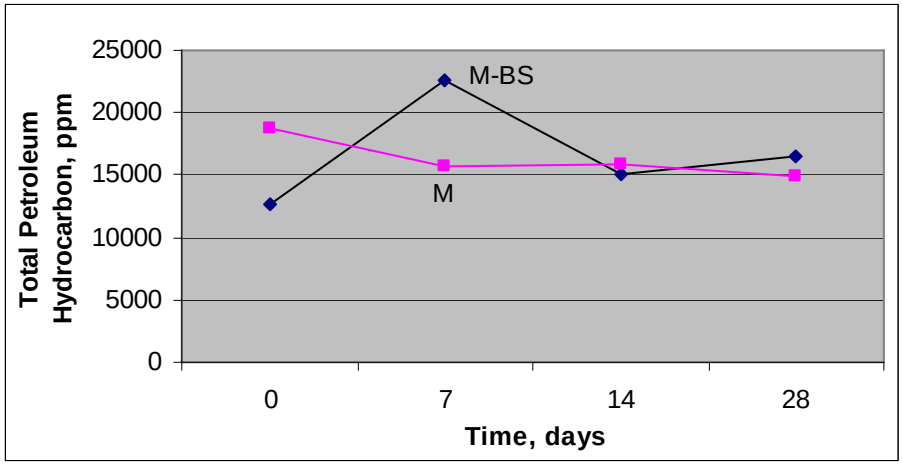
c)



d)



e)



f)

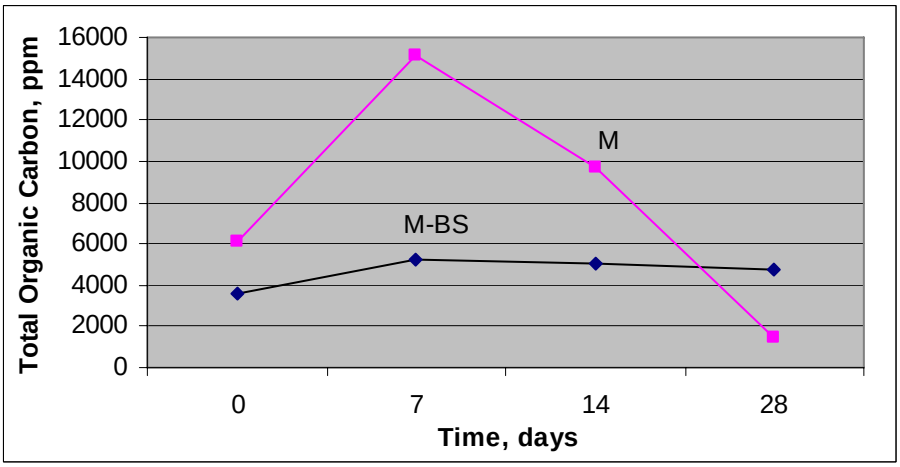


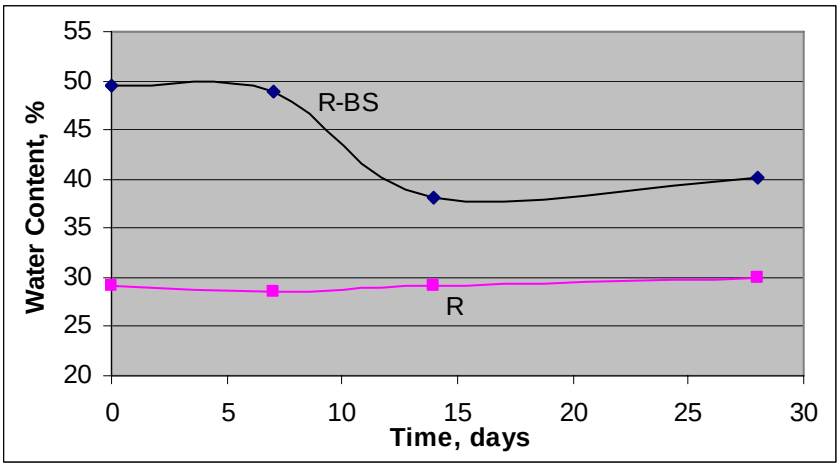
Figure 6.8 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with no enhancement enhanced soil remediation (—◆— Menemen-BS, —■— Menemen).

Water contents rise in the first week of the experiment, and then decrease slowly in the next days. pH values has increased to alkaline levels in the first steps of the experimental period and then show a slow decline at the end of the time period.

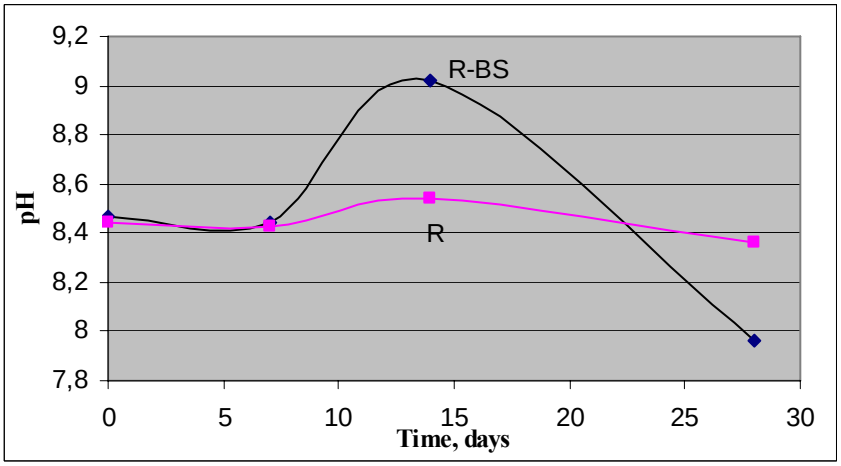
Organic matter content decreases during the experiment in both cases while total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand show peaks in first week. The decreases in total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand values have not shown a steady regime in these set of trial.

- **Trials with Raman Soil**

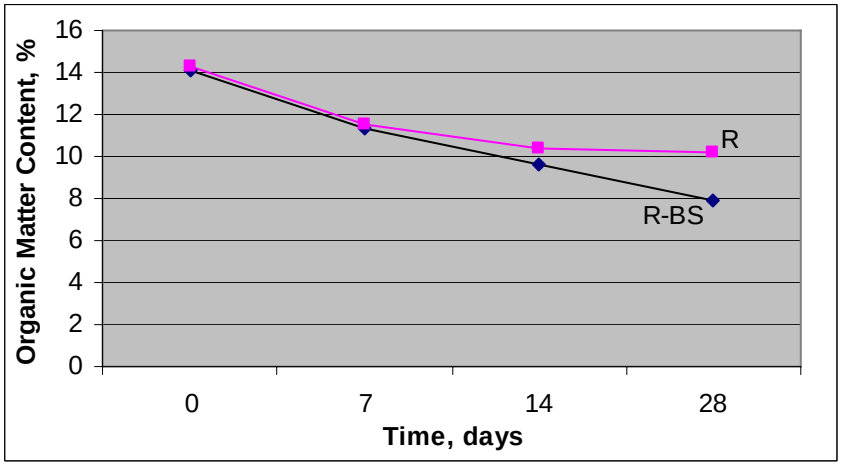
a)



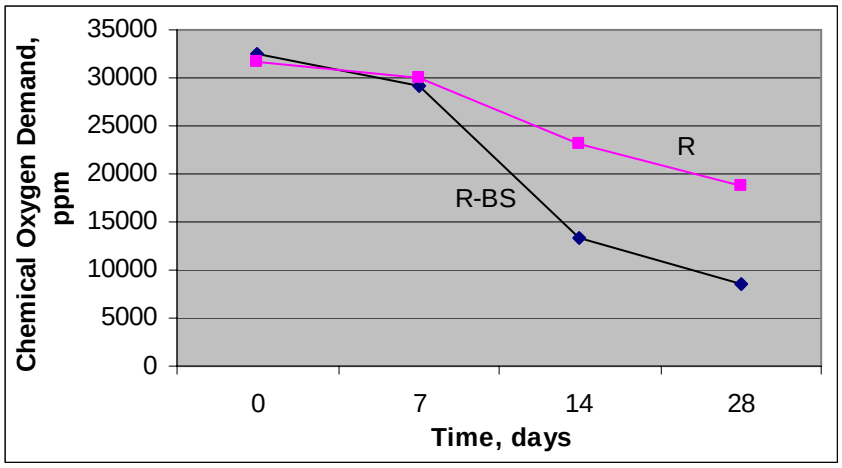
b)



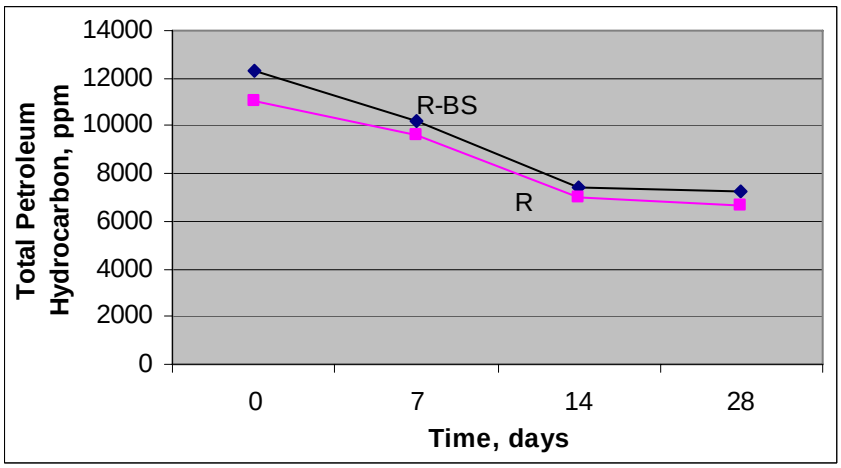
c)


Organic Matter Content, %
16
14
12
10
8
6
4
2
0
0
7
14
28
Time, days
R
R-BS

d)


Chemical Oxygen Demand, ppm
35000
30000
25000
20000
15000
10000
5000
0
0
7
14
28
Time, days
R
R-BS

e)


Total Petroleum Hydrocarbon, ppm
14000
12000
10000
8000
6000
4000
2000
0
0
7
14
28
Time, days
R-BS
R

f)

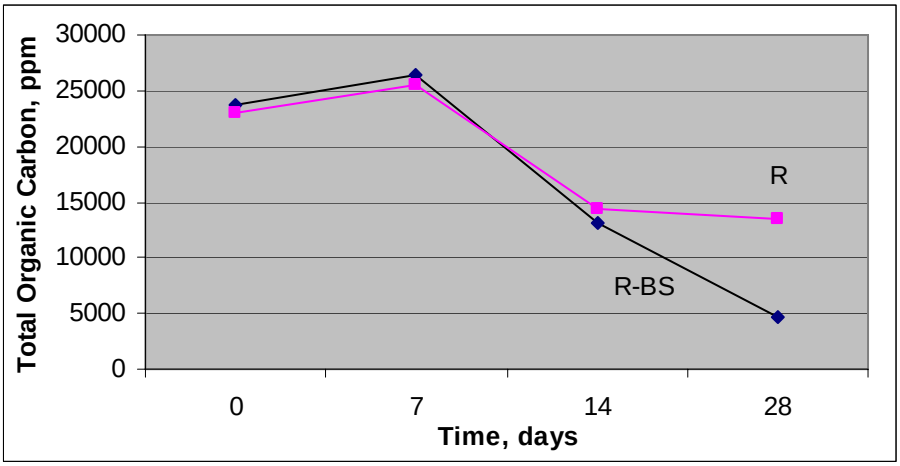


Figure 6.9 The variation of (a) water content, (b) pH, (c) organic matter content, (d) COD_d, (e) TPHCs, and (f) TOC_d in with no enhancement enhanced soil remediation (—◆— Raman-BS, —■— Raman).

Water content in the set with biosurfactant has dropped during the experiment and it hasn't changed without the presence of biosurfactant. pH raised to alkaline level until the second week of the experimental period, then it has dropped to neutral values at the end of the experiment.

Organic matter content, total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand values has showed satisfactory decreases in this trial, especially in the presence of biosurfactant.

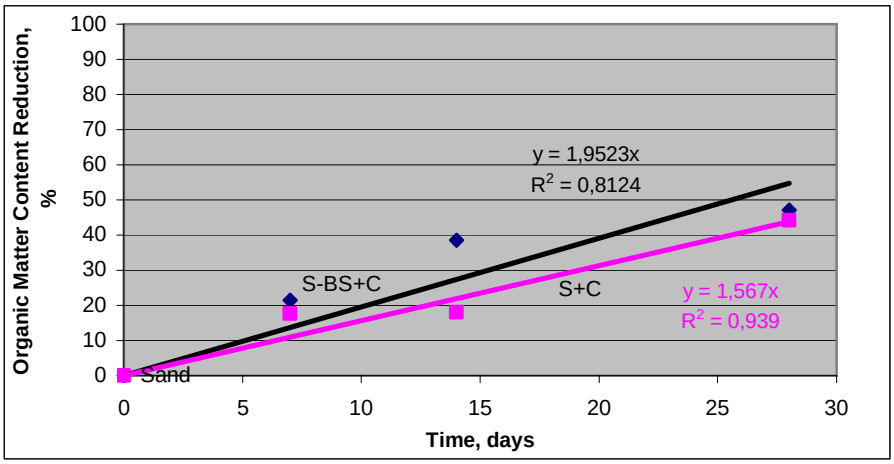
6.2.1.2 Reductions in Contaminant Concentration

Since the initial contaminant concentrations and the properties of soil mediums are not exactly the same, the concentration changes during the experiments are not reflecting the real treatment efficiencies in the different set of trials. In this part of the thesis, removals in organic matter content (OMC), total petroleum hydrocarbons (TPHCs), water soluble total organic carbon (TOC_d) and water soluble chemical oxygen demand (COD_d) for the sets of trials are shown according to the percent initial value with time. Here, the effect of biosurfactant on remediation of engine oil can be seen much clearly.

6.2.1.2.1 Compost Enhanced Trials

- **Trials with Sand**

a)



b)

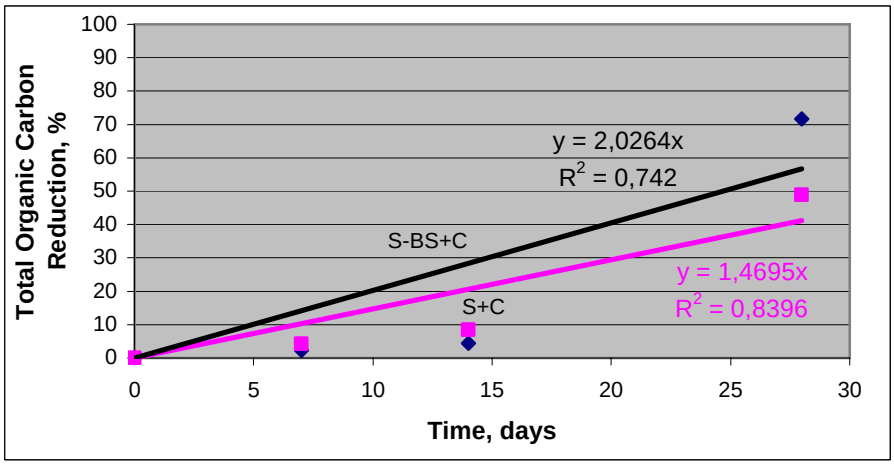
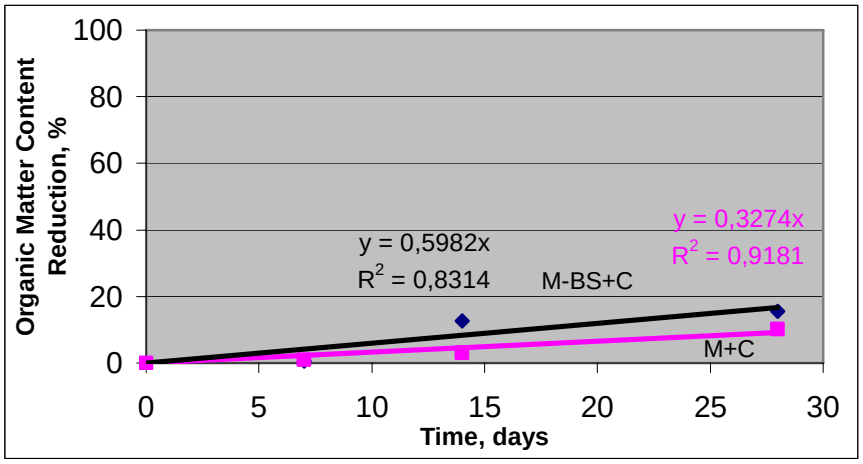


Figure 6.10 The reduction rate of (a) organic matter (b) TOC_d in engine oil spiked Sand (◆ Sand-BS, ■ Sand).

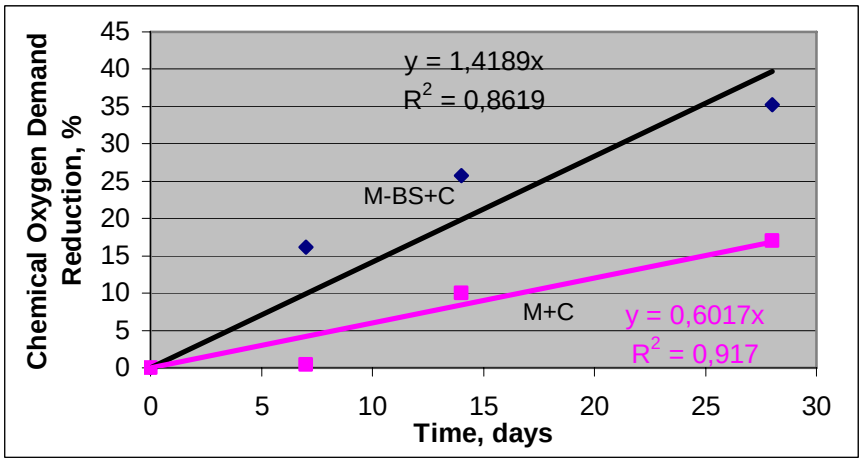
The organic matter reduction and water soluble total organic carbon reduction of the trial with compost in sand are almost the same. For the set with biosurfactant, the reductions of organic matter content and water soluble total organic carbon are around 50%, while they are around 40% in the set without biosurfactant.

- **Trials with Menemen Soil**

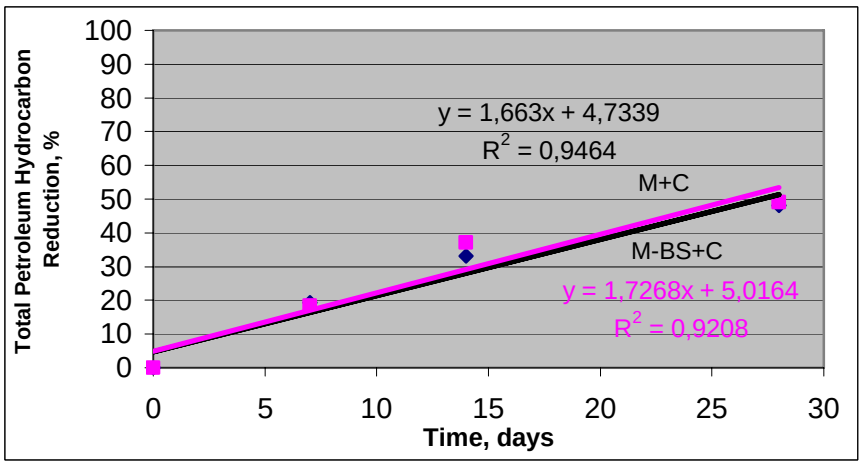
a)



b)



c)



d)

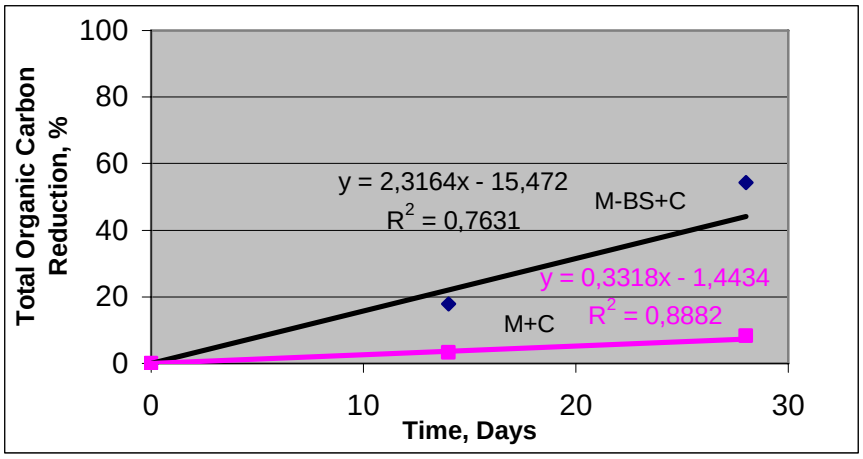
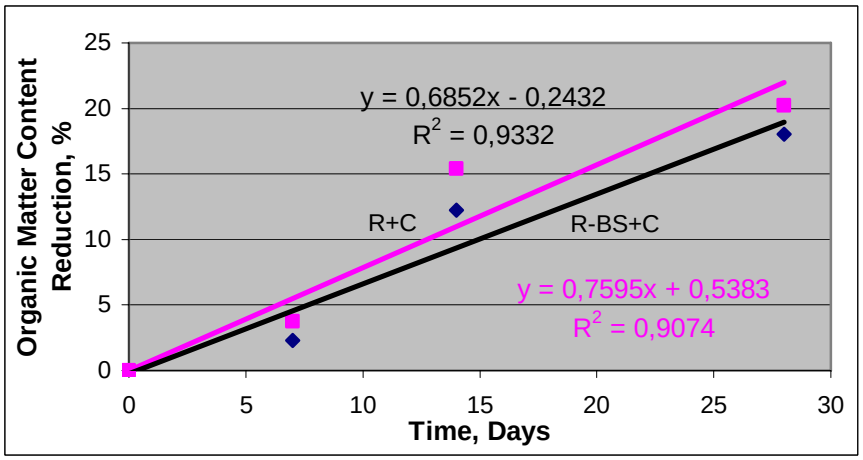


Figure 6.11 The reduction rate of (a) organic matter (b) COD_d (c) TPHCs (d) TOC_d in engine oil spiked Menemen soil (◆ Menemen-BS, ■ Menemen).

Almost all parameters reflects remediation effects in the trials with compost in Menemen soil. The reductions in concentration of total petroleum hydrocarbons are almost the same in the cases with and without biosurfactant (around 50%). The positive effect of presence of biosurfactant can be seen much clearly in the reductions in organic matter content, water soluble total organic carbon, and chemical oxygen demand. Reductions in the set with biosurfactant are 5%, 10% and 46% more for organic matter content, water soluble total organic carbon, and chemical oxygen demand respectively, then the set without biosurfactant.

- **Trials with Raman Soil**

a)



b)

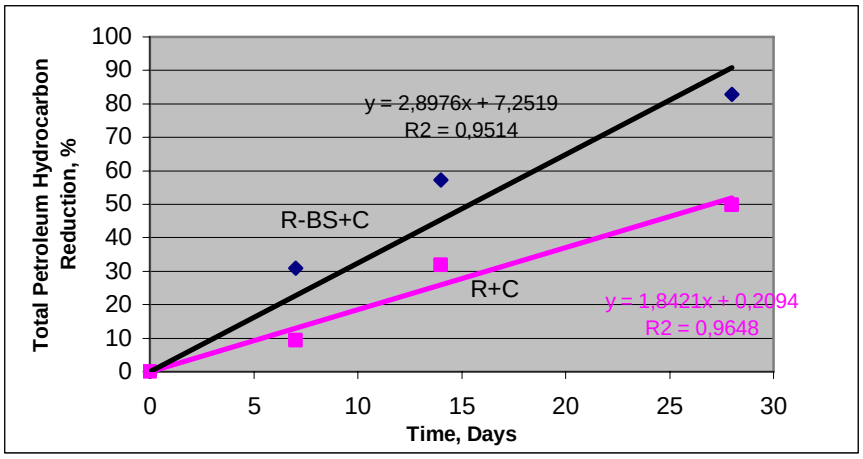


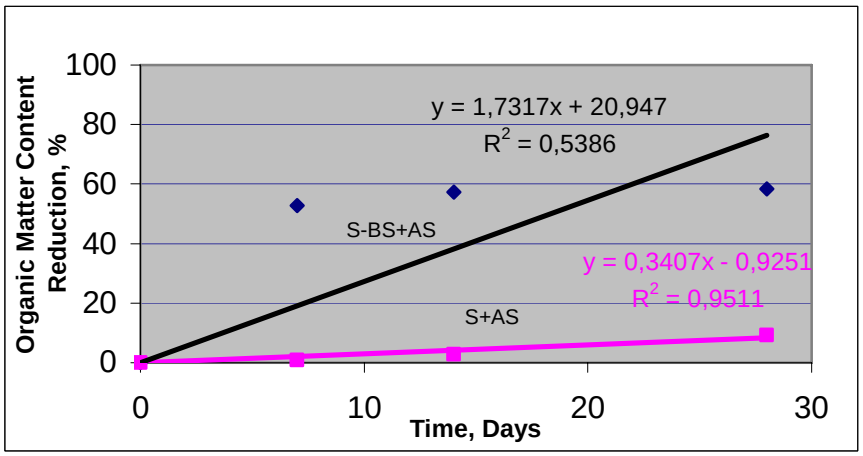
Figure 6.12 The reduction rate of (a) organic matter (b) TPHCs in Raman soil (◆ Raman-BS, ■ Raman).

In the trials with compost in Raman soil, the reduction in TPHCs concentration is significant in the set with biosurfactant (82%), while the reduction without biosurfactant is 49%. Organic matter reductions look like the same in both cases.

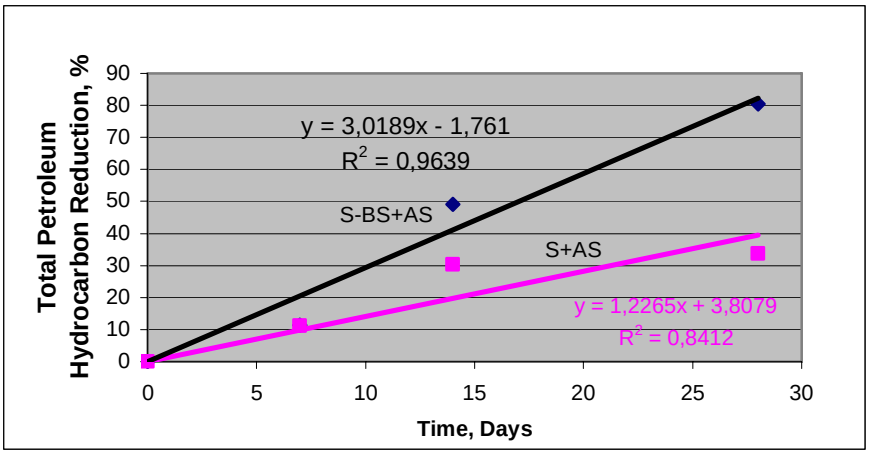
6.2.1.2.2 Activated Sludge Enhanced Trials

- **Trials with Sand**

a)



b)



c)

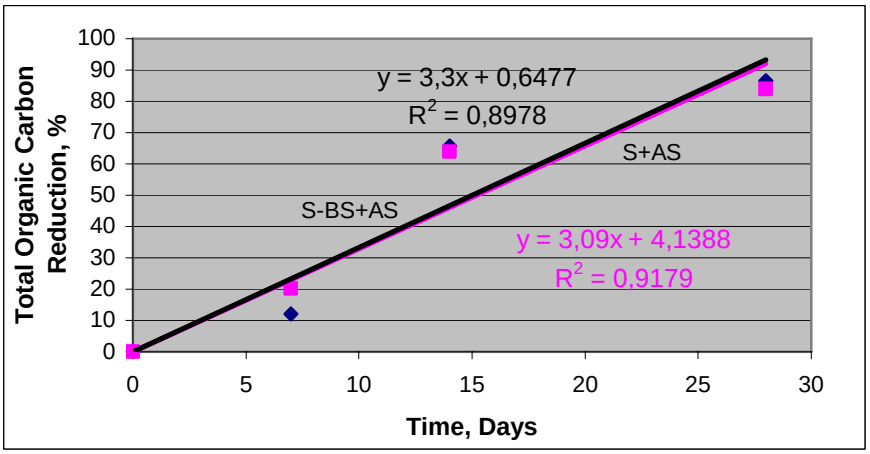
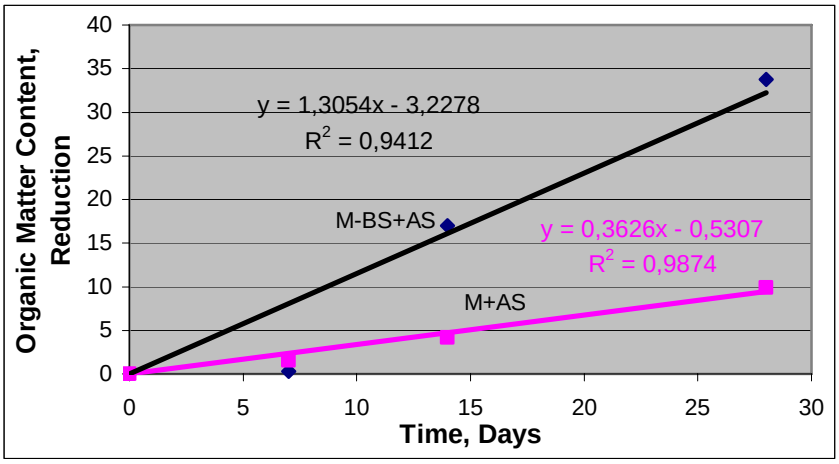


Figure 6.13 The reduction rate of (a) organic matter (b) TPHCs reduction (c) TOC in engine oil spiked Sand (—◆— Sand-BS, —■— Sand).

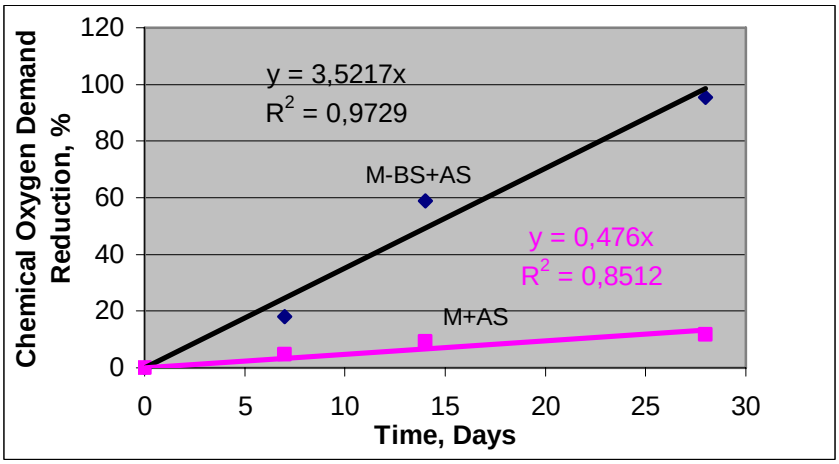
In the activated sludge enhanced sand trials the reductions in organic matter content, total petroleum hydrocarbons, and water soluble total organic carbon in the set with biosurfactant are higher then the set without biosurfactant. The reductions in total petroleum hydrocarbons is as high as 80% during the experiment.

- **Trials with Menemen Soil**

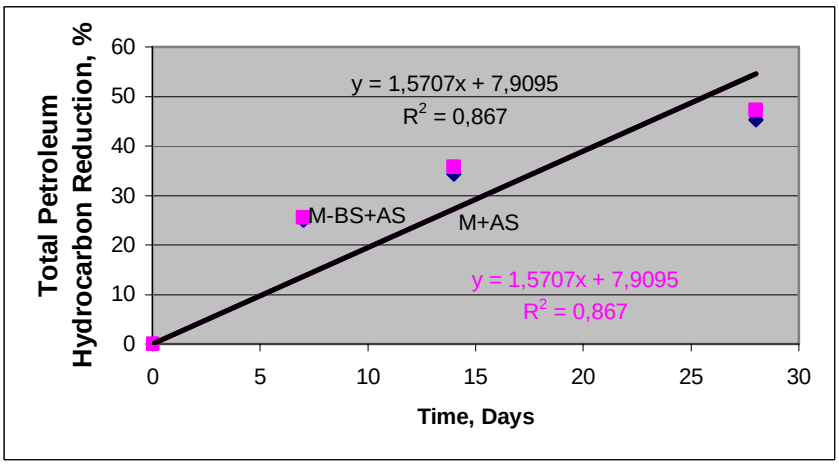
a)



b)



c)



d)

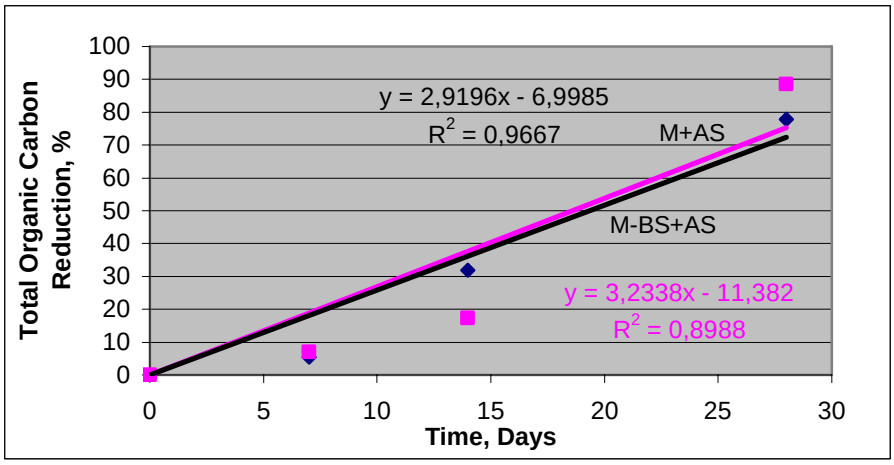
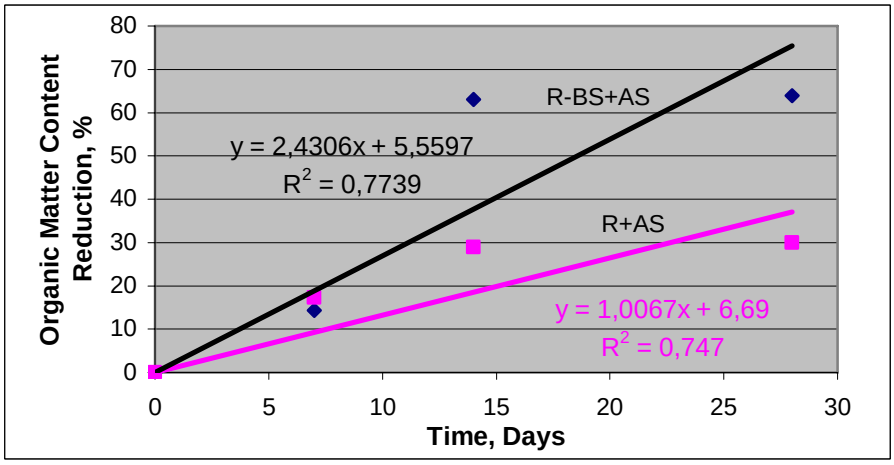


Figure 6.14 The reduction rate of (a) organic matter (b) COD_d (c) TPHCs (d) TOC in engine oil spiked Menemen soil (—◆— Menemen-BS, —■— Menemen).

In Menemen Soil remediation trials with activated sludge enhancements the reduction of total petroleum hydrocarbons and water soluble total organic carbon in the sets with and without biosurfactant are almost the same , while organic matter and water soluble chemical oxygen demand reduction with biosurfactant is much higher then the reduction without biosurfactant.

- **Trials with Raman Soil**

a)



b)

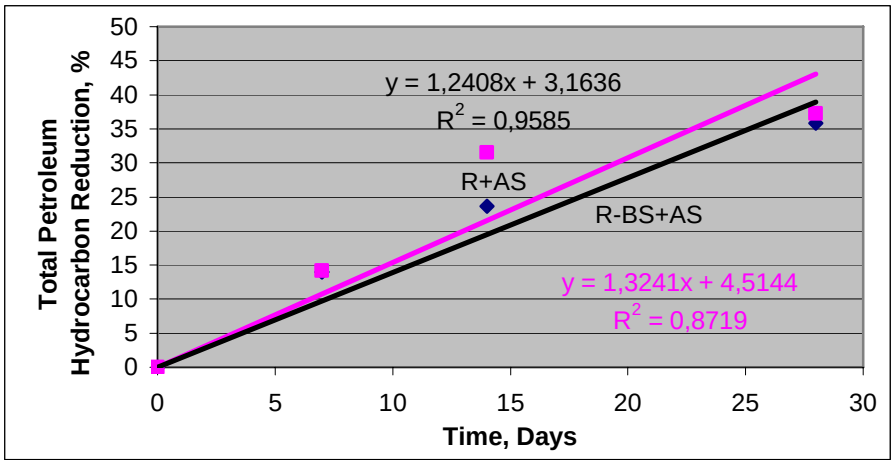


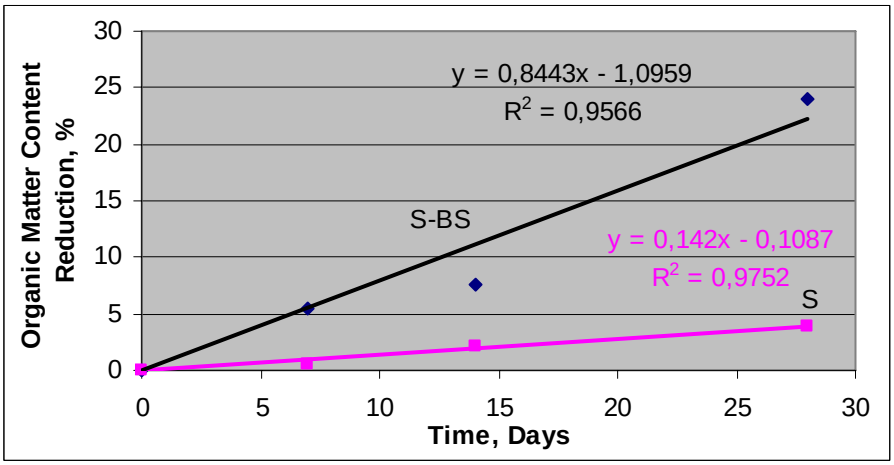
Figure 6.15 The reduction rate of (a) organic matter (b) TPHCs in Raman soil (Raman-BS, Raman).

Organic matter reduction rate is as high as 63% in the set with biosurfactant for the activated sludge enhanced trial in Raman soil, while it is only 29% at the end of the experiment for the set without biosurfactant. But this difference between the organic matter reduction rates in two cases cannot be seen for the parameter of total petroleum hydrocarbons.

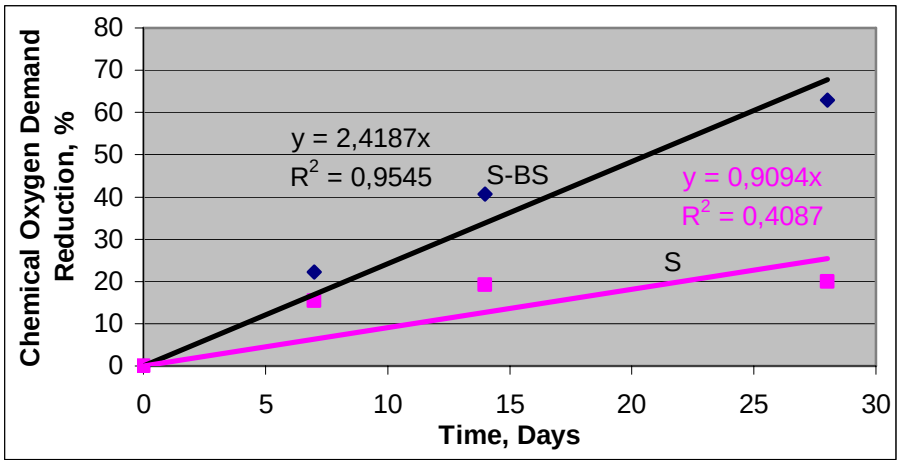
6.2.1.2.3 Natural (No Amendment) Soil Enhanced Trials

- **Trials with Sand**

a)



b)



c)

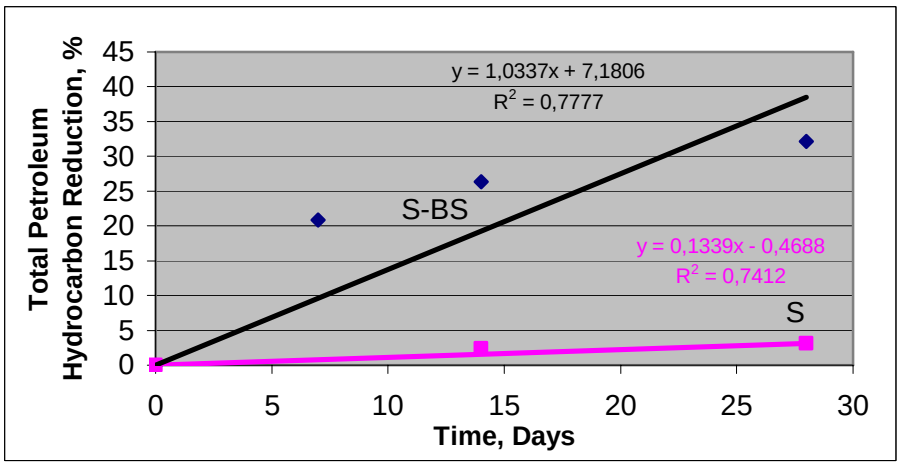


Figure 6.16 The reduction rate of (a) organic matter (b) COD_d (c) TPHCs in engine oil spiked Sand (—◆— Sand-BS, —■— Sand).

Reductions of organic matter content, water soluble chemical oxygen demand and total petroleum hydrocarbons are significantly higher in the set with biosurfactant in the trial in Raman soil with no amendment. Total petroleum hydrocarbons reduction rate with biosurfactant is observed 32% at the end of the experiment. Although without biosurfactant application it remained as low as 4%.

- **Trials with Menemen Soil**

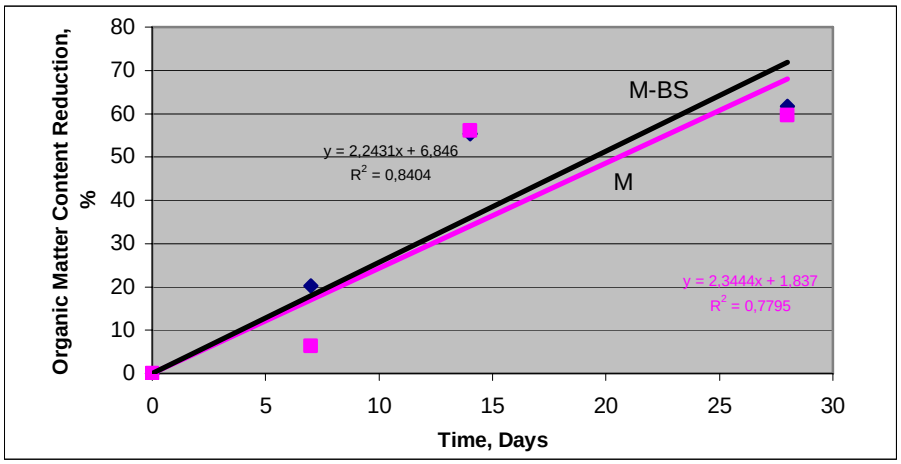
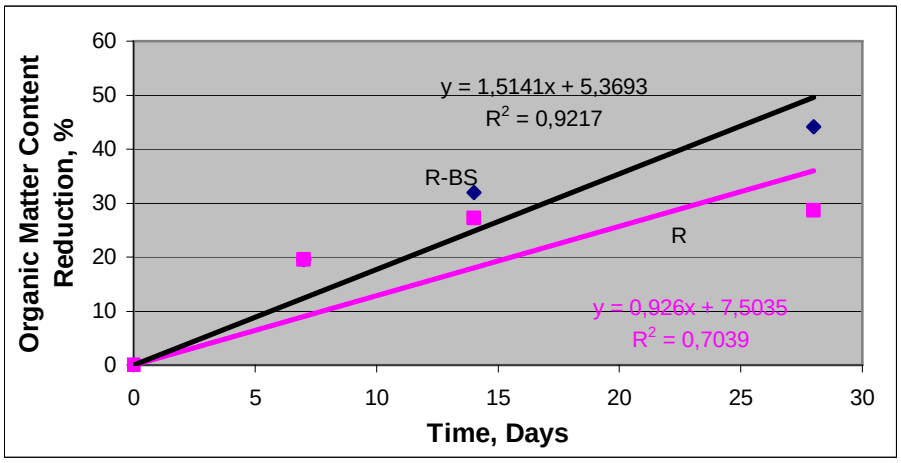


Figure 6.17 The reduction rate of organic matter in engine oil spiked Menemen soil (Menemen-BS, Menemen).

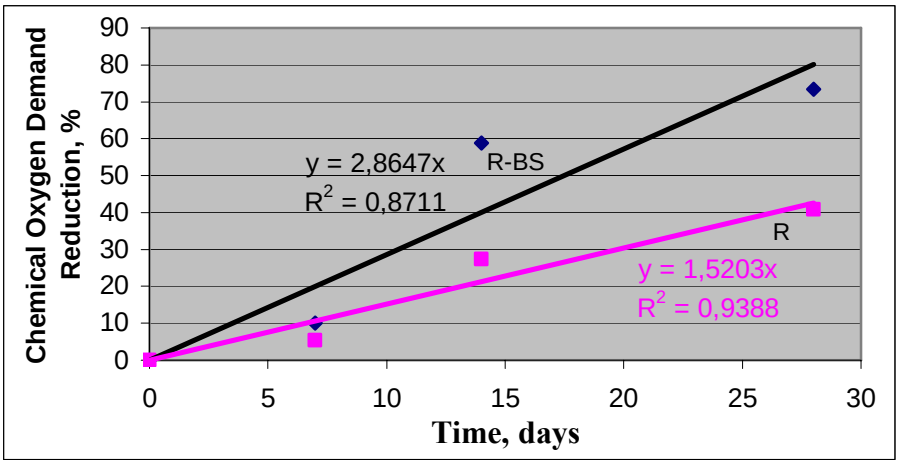
In Menemen soil, organic matter content reductions has been observed in both cases; with and without biosurfactant. The reductions in total petroleum hydrocarbons, water soluble total organic carbon, and chemical oxygen demand are not very meaningful at this trial set.

- **Trials with Raman Soil**

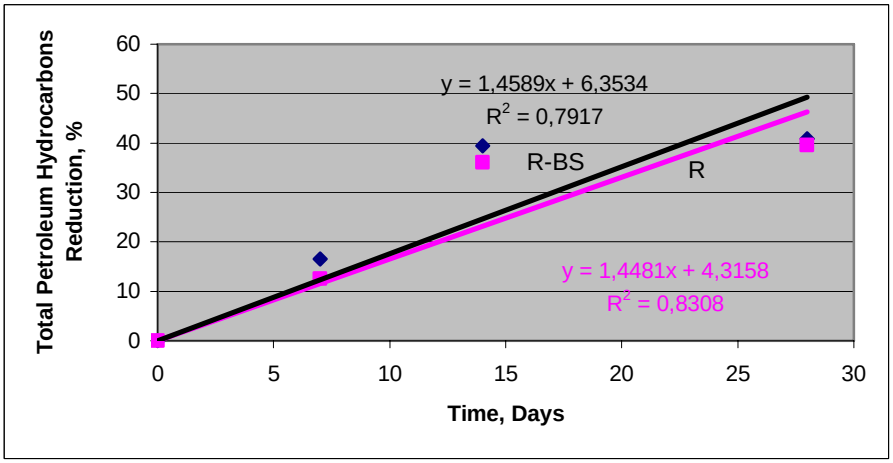
a)



b)



c)



d)

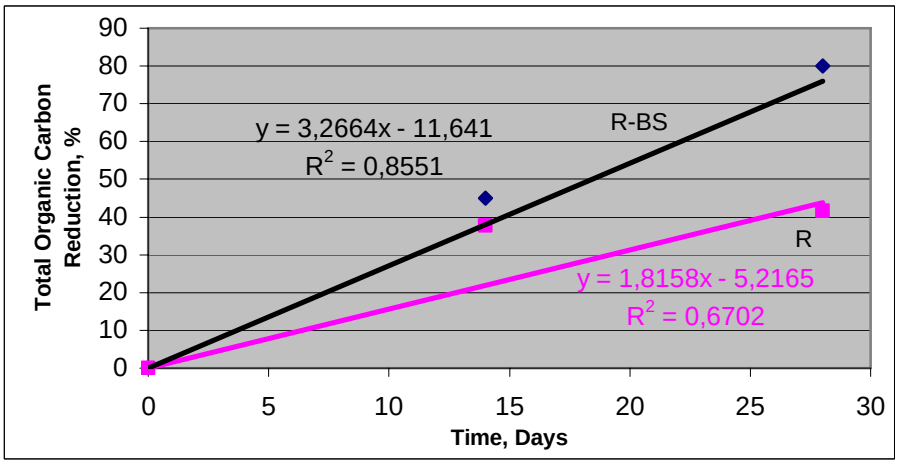


Figure 6.18 The reduction rate of (a) organic matter (b) COD_d c) TPHCs, (d) TOC in Raman soil (Raman-BS, Raman).

Reductions are higher in the set with biosurfactant for Raman soil with no amendment. Especially, water soluble total organic carbon, and chemical oxygen demand reduction rates are much higher in the presence of biosurfactant.

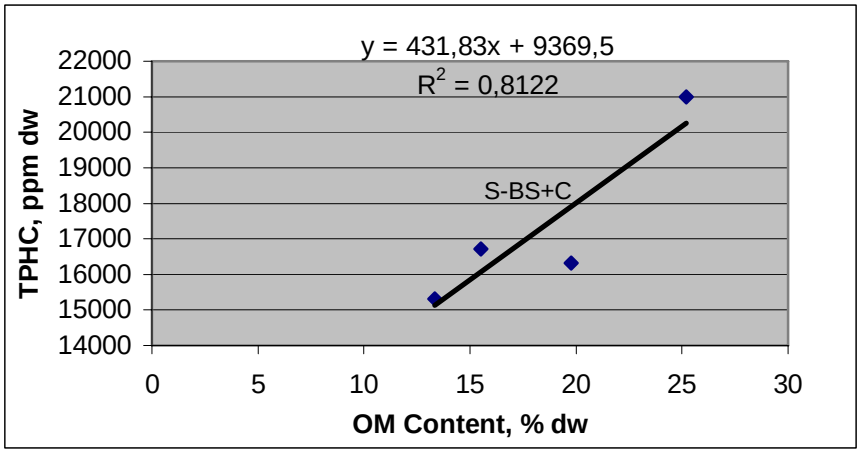
6.2.2 Correlations in Variation of Contaminant Concentration during Remediation

In this part of the study correlations between different parameters are tried to be determined. Of course COD, organic matter, TOC and TPHC parameters are correlated with each other. Although the biosurfactant is measured with COD, TOC and organic matter content, TPHC measurement excludes biosurfactant. Due to the measurement technique. The aim of determining the correlation between the parameters is to determine to most reliable parameter and to check the sensitivity of measurement. Although the analysis include the control of the parameters (parallel analysis) meaningful correlation coefficients were seeked in order to be sure about correctness of analysis and the data which should be omitted.

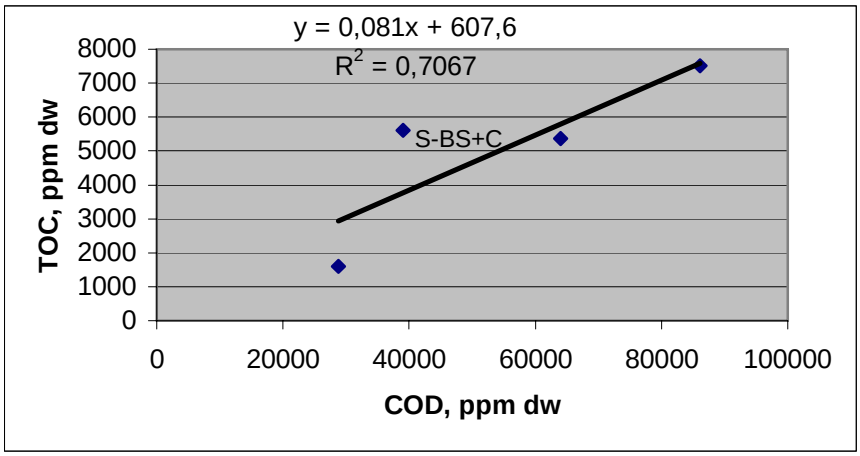
6.2.2.1 Compost Amended Soil Remediations

- **Trials with Sand**

a)



b)



c)

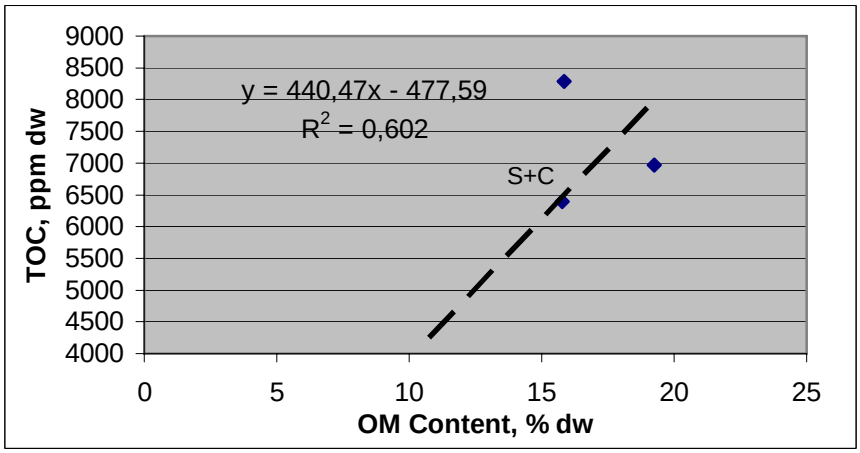
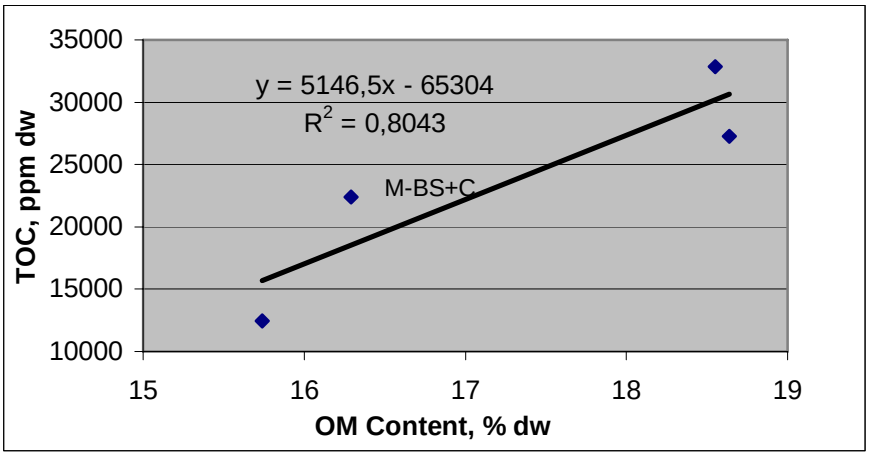


Figure 6.20 The correlation in variation of contaminant concentrations for Sand in compost enhancement (a) OM vs. TPHCs (b) COD_d vs. TOC (c) OM vs. TOC (——— Sand-BS, – – – – Sand).

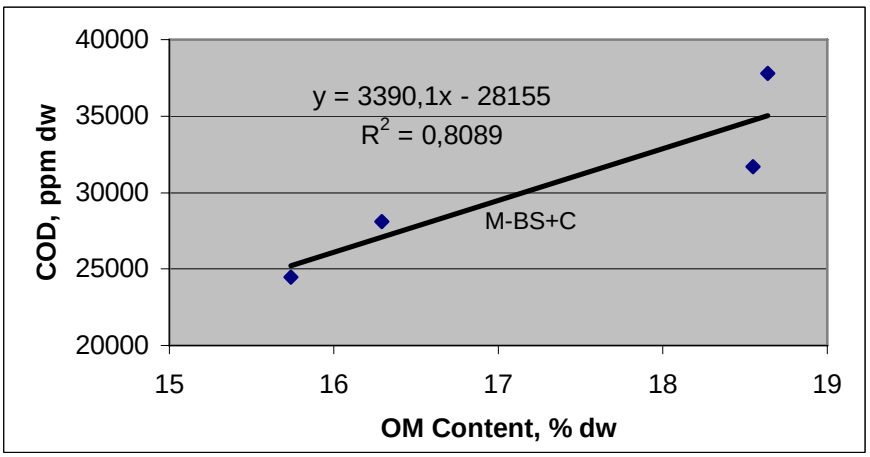
Strong correlations between organic matter content and total petroleum hydrocarbons concentrations and water soluble chemical oxygen demand and total organic carbon concentrations has seen in the remediation trials of compost enhanced sand remediation with the presence of biosurfactant. Also, the correlation between organic matter content and water soluble total organic carbon draws attention in the same case without biosurfactant.

- **Trials with Menemen Soil**

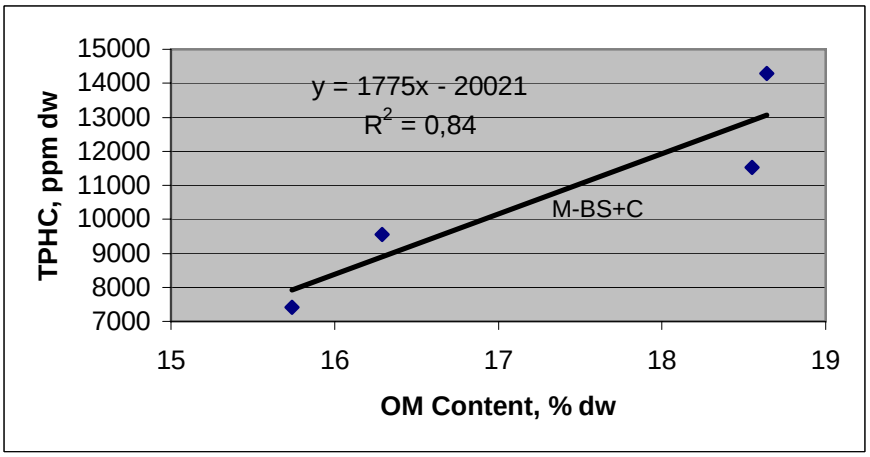
a)



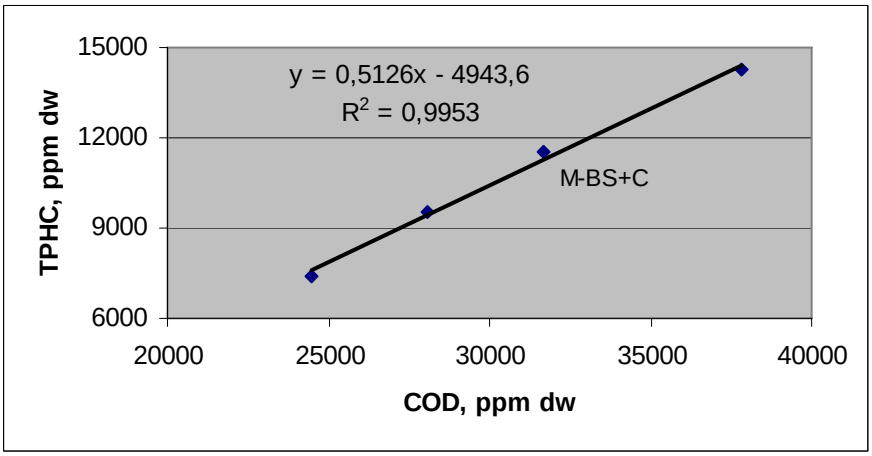
b)



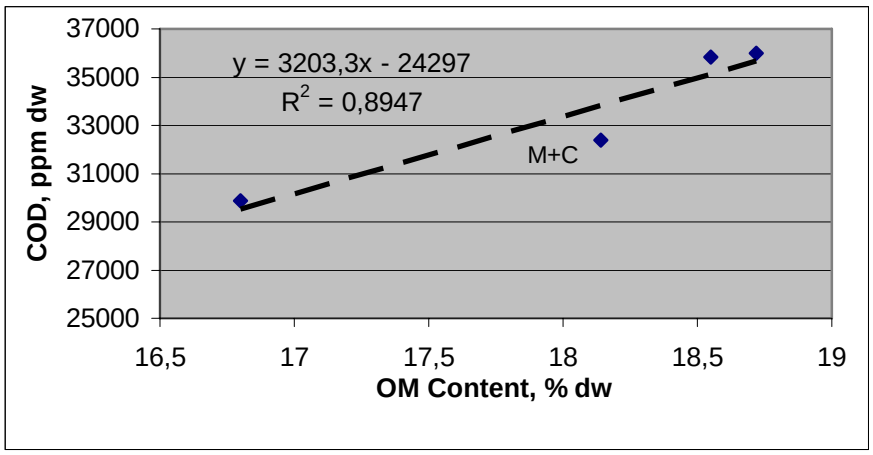
c)



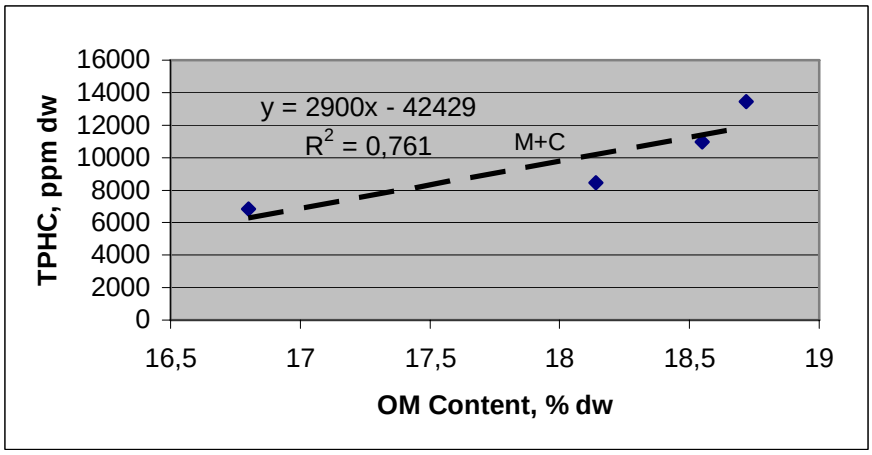
d)


y = 0,5126x - 4943,6
R2 = 0,9953
M-BS+C
TPHC, ppm dw
COD, ppm dw
15000
12000
9000
6000
20000
25000
30000
35000
40000

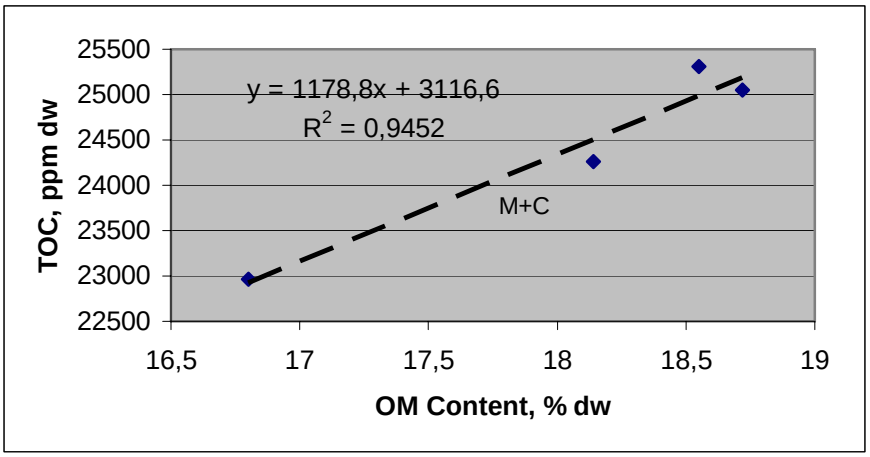
e)


y = 3203,3x - 24297
R2 = 0,8947
M+C
COD, ppm dw
OM Content, % dw
37000
35000
33000
31000
29000
27000
25000
16,5
17
17,5
18
18,5
19

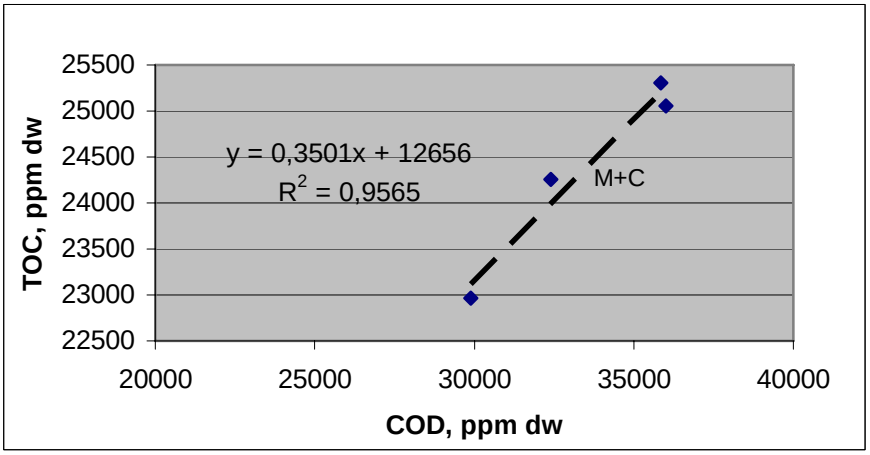
f)


y = 2900x - 42429
R2 = 0,761
M+C
TPHC, ppm dw
OM Content, % dw
16000
14000
12000
10000
8000
6000
4000
2000
0
16,5
17
17,5
18
18,5
19

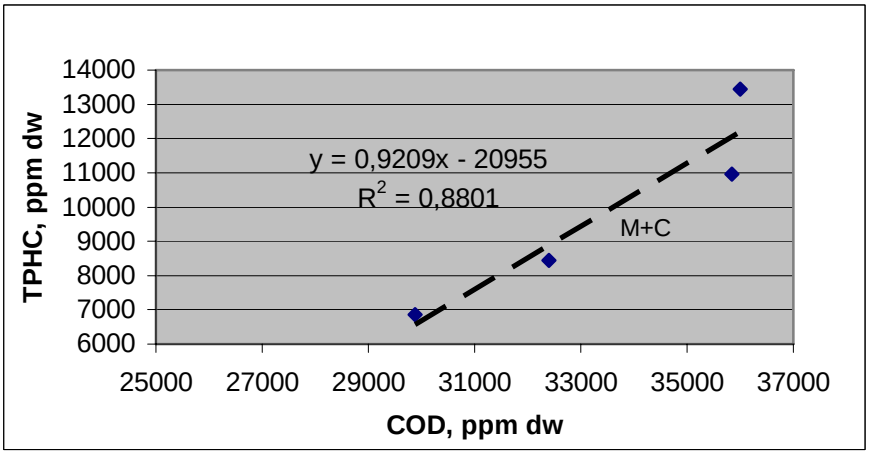
g)


y = 1178,8x + 3116,6
R2 = 0,9452
M+C
TOC, ppm dw
OM Content, % dw
25500
25000
24500
24000
23500
23000
22500
16,5
17
17,5
18
18,5
19

h)


y = 0,3501x + 12656
R2 = 0,9565
M+C
TOC, ppm dw
COD, ppm dw
25500
25000
24500
24000
23500
23000
22500
20000
25000
30000
35000
40000

i)


y = 0,9209x - 20955
R2 = 0,8801
M+C
TPHC, ppm dw
COD, ppm dw
14000
13000
12000
11000
10000
9000
8000
7000
6000
25000
27000
29000
31000
33000
35000
37000

j)

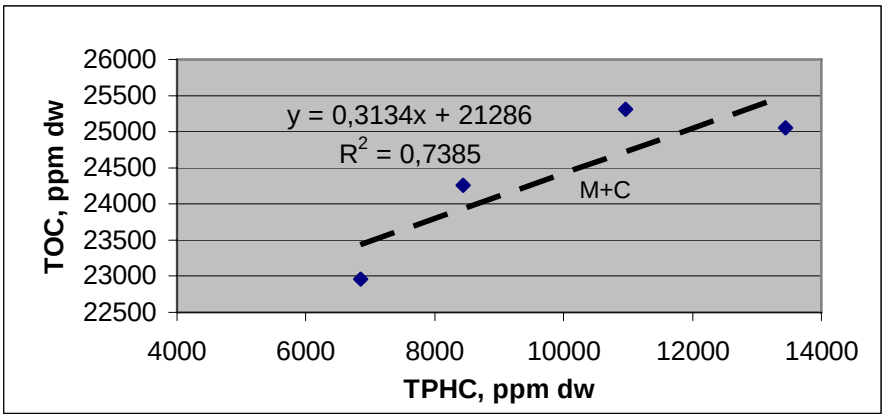
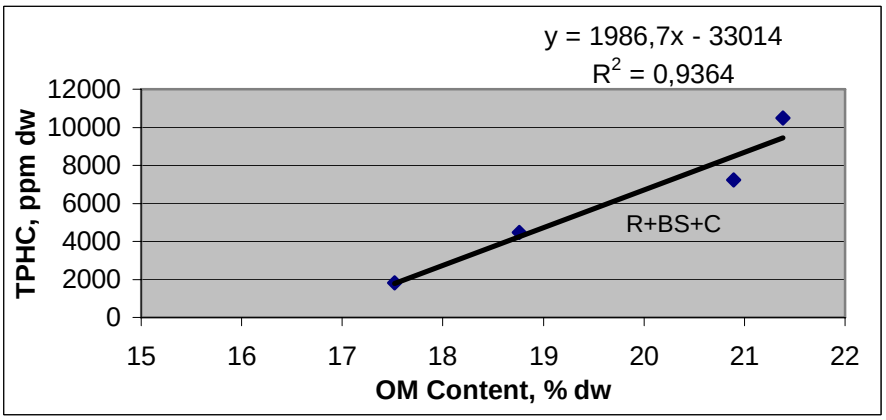


Figure 6.21 The correlation in variation of contaminant concentrations for Menemen soil in compost enhancement (a) OM vs. TOC (b) OM vs. COD_d (c) OM vs. TPHCs (d) COD_d vs. TPHCs (e) OM vs. COD_d (f) OM vs. TPHCs (g) OM vs. TOC (h) COD_d (i) COD vs. TPHC (j) TPHCs vs. TOC
(– – – Menemen, ——— Menemen-BS).

Organic matter content of the soil medium is correlated with total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand in Menemen soil with presence of biosurfactant. When the same set has conducted without biosurfactant, organic matter content looks correlated with total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand. Correlations between total petroleum hydrocarbons, and water soluble chemical oxygen demand are also present in both cases.

- **Trials with Raman Soil**

a)



b)

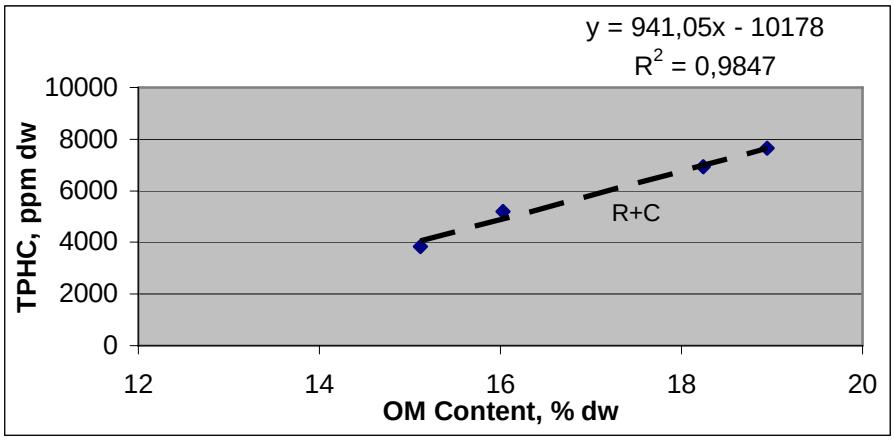


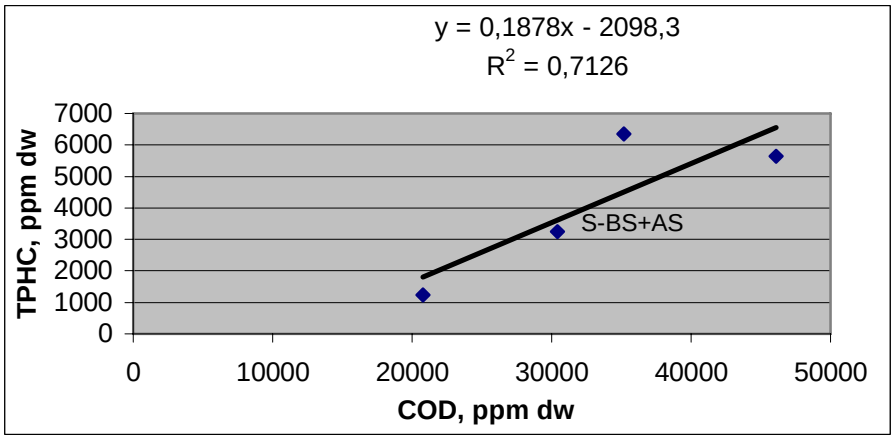
Figure 6.22 The correlation in variation of contaminant concentrations for Raman soil in compost enhancement (a) OM vs. TPHCs (b) OM vs. TPHCs
(– – – Raman, ——— Raman-BS).

In the trials with Raman soil enhanced with compost addition organic matter content vs. total petroleum hydrocarbons correlations are looking significantly strong.

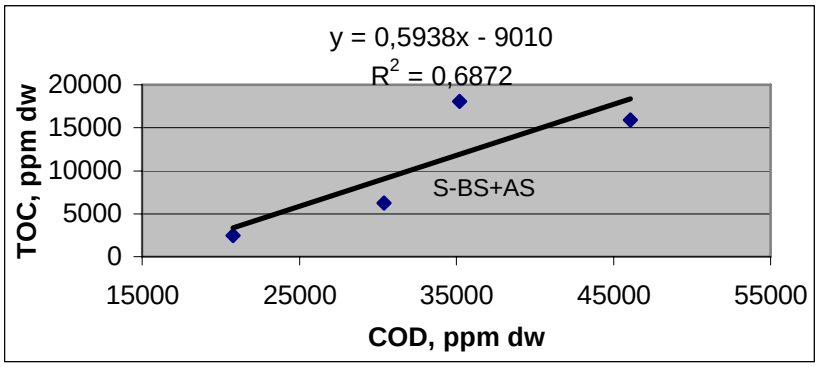
6.2.2.2 Activated Sludge Amended Soil Remediations

- **Trials with Sand**

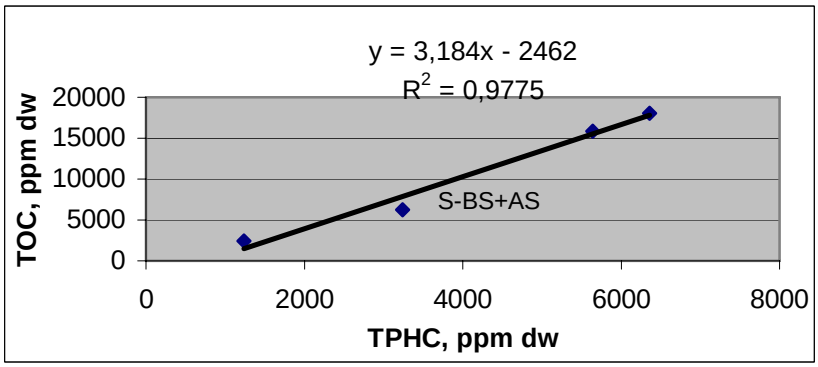
a)



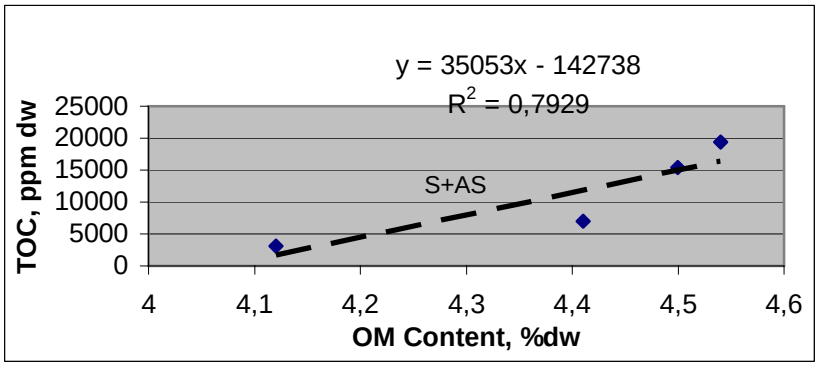
b)


y = 0,5938x - 9010
R2 = 0,6872
S-BS+AS
TOC, ppm dw
20000
15000
10000
5000
0
15000
25000
35000
45000
55000
COD, ppm dw

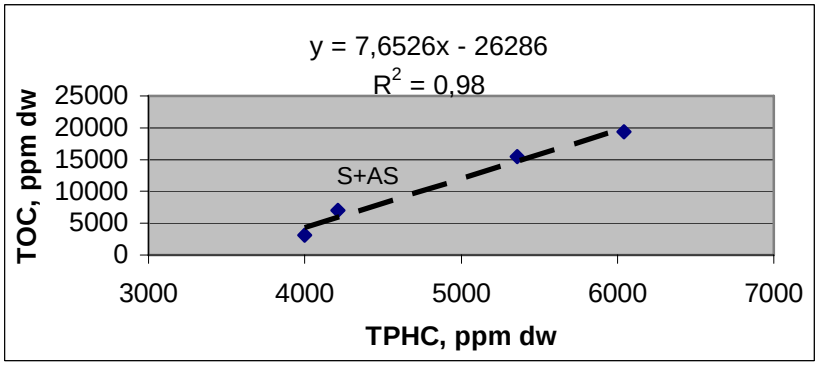
c)


y = 3,184x - 2462
R2 = 0,9775
S-BS+AS
TOC, ppm dw
20000
15000
10000
5000
0
0
2000
4000
6000
8000
TPHC, ppm dw

d)


y = 35053x - 142738
R2 = 0,7929
S+AS
TOC, ppm dw
25000
20000
15000
10000
5000
0
4
4,1
4,2
4,3
4,4
4,5
4,6
OM Content, %dw

e)


y = 7,6526x - 26286
R2 = 0,98
S+AS
TOC, ppm dw
25000
20000
15000
10000
5000
0
3000
4000
5000
6000
7000
TPHC, ppm dw

f)

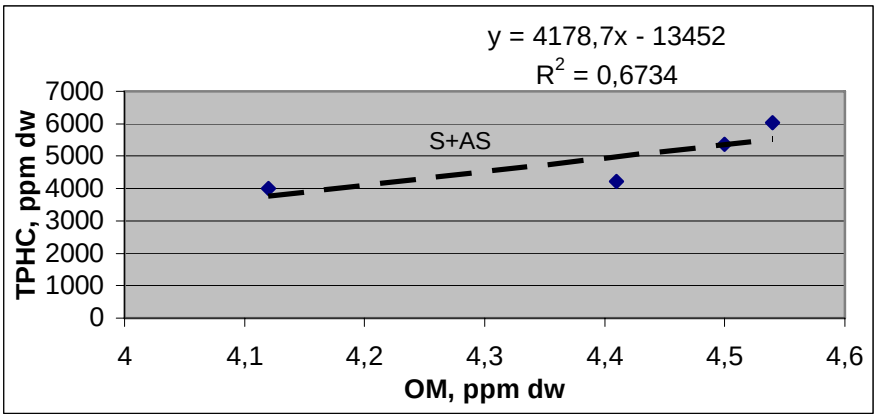
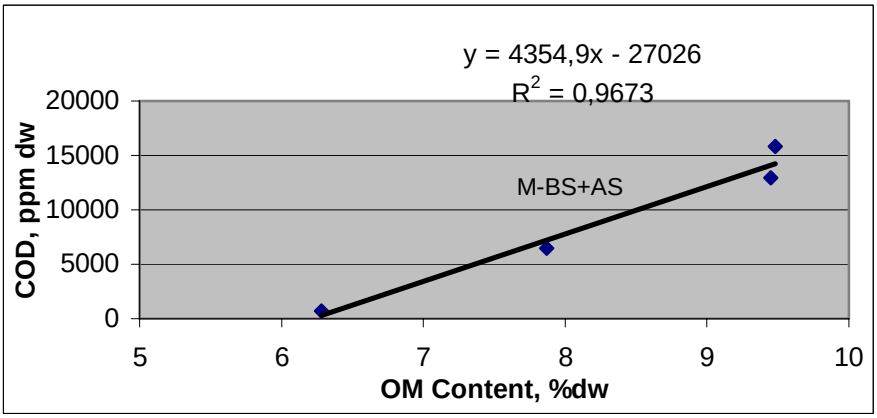


Figure 6.23 The correlation in variation of contaminant concentrations for Sand in activated sludge enhancement (a) COD vs. TPHCs (b) COD vs. TOC (c) TPHCs vs. TOC (d) OM vs. TOC (e) TPHCs vs. TOC (f) OM vs. TPHCs (– – – Sand, ——— Sand-BS).

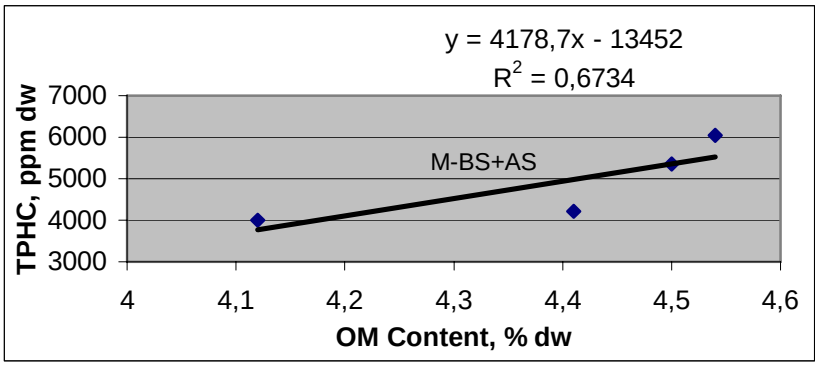
Correlations among total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand has seen in the case of engine oil remediation in sand with compost addition in the presence of biosurfactant. The same set of experiments reflects correlations among organic matter, water soluble total organic carbon and total petroleum hydrocarbons in the soil medium without any biosurfactant.

- **Trials with Menemen Soil**

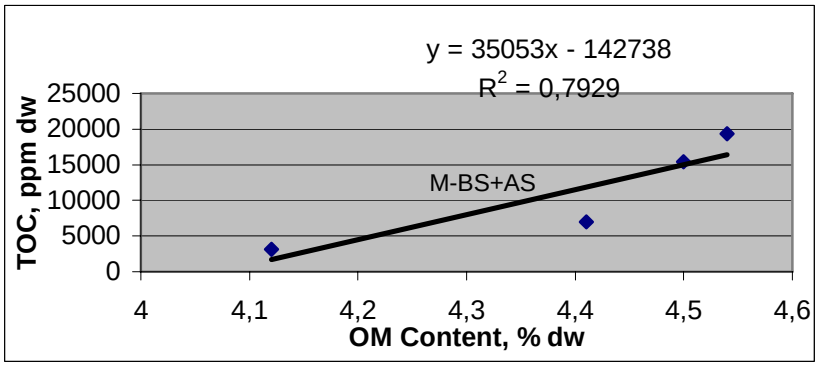
a)



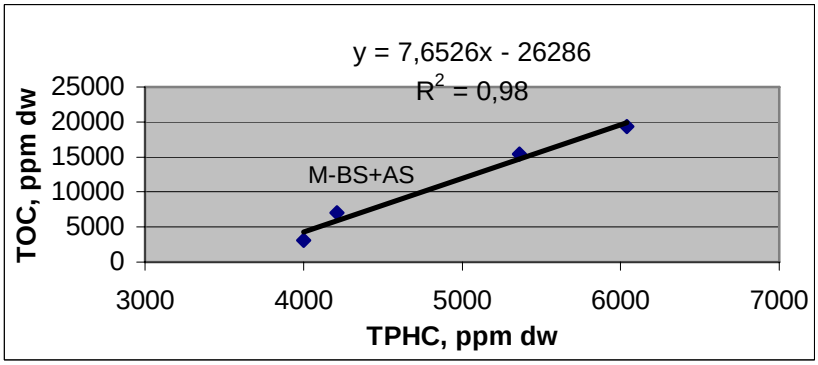
b)


y = 4178,7x - 13452
R2 = 0,6734
M-BS+AS
TPHC, ppm dw
7000
6000
5000
4000
3000
4
4,1
4,2
4,3
4,4
4,5
4,6
OM Content, % dw

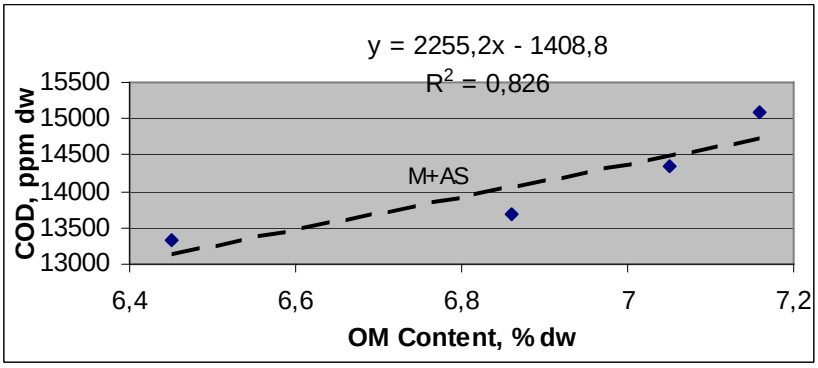
c)


y = 35053x - 142738
R2 = 0,7929
M-BS+AS
TOC, ppm dw
25000
20000
15000
10000
5000
0
4
4,1
4,2
4,3
4,4
4,5
4,6
OM Content, % dw

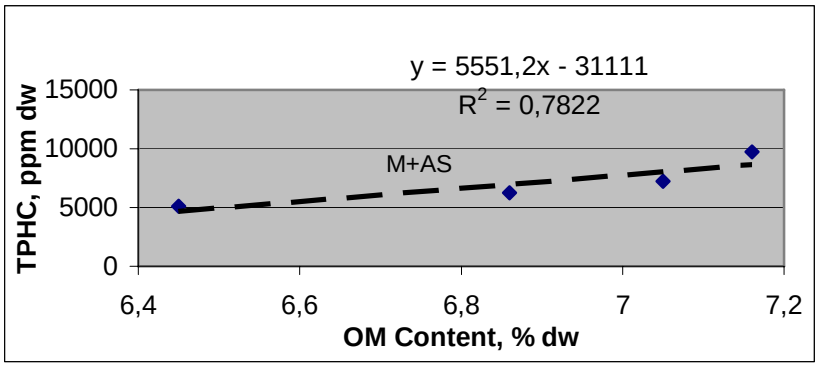
d)


y = 7,6526x - 26286
R2 = 0,98
M-BS+AS
TOC, ppm dw
25000
20000
15000
10000
5000
0
3000
4000
5000
6000
7000
TPHC, ppm dw

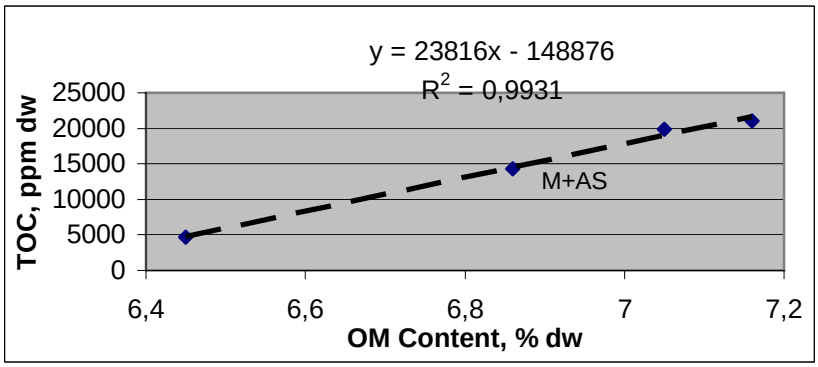
e)


y = 2255,2x - 1408,8
R2 = 0,826
M+AS
COD, ppm dw
15500
15000
14500
14000
13500
13000
6,4
6,6
6,8
7
7,2
OM Content, % dw

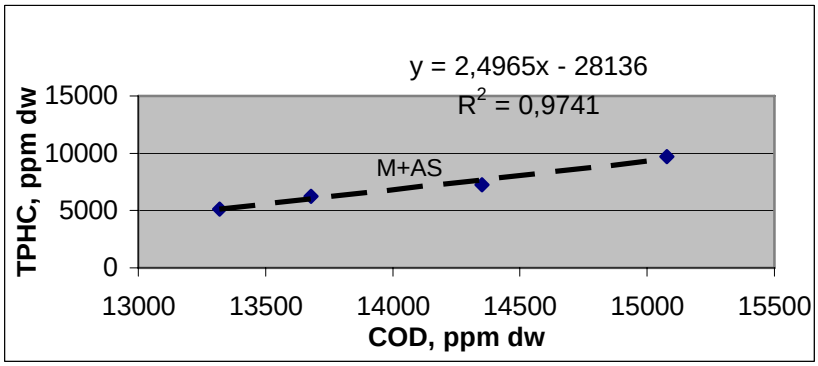
f)


y = 5551,2x - 31111
R2 = 0,7822
M+AS
TPHC, ppm dw
15000
10000
5000
0
6,4
6,6
6,8
7
7,2
OM Content, % dw

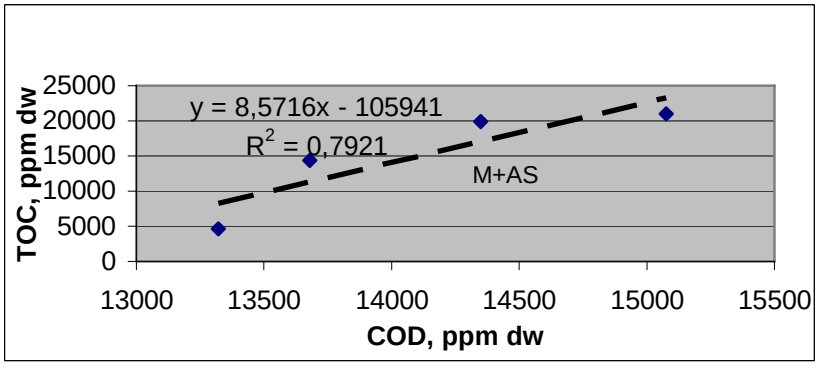
g)


y = 23816x - 148876
R2 = 0,9931
M+AS
TOC, ppm dw
25000
20000
15000
10000
5000
0
6,4
6,6
6,8
7
7,2
OM Content, % dw

h)


y = 2,4965x - 28136
R2 = 0,9741
M+AS
TPHC, ppm dw
15000
10000
5000
0
13000
13500
14000
14500
15000
15500
COD, ppm dw

i)


y = 8,5716x - 105941
R2 = 0,7921
M+AS
TOC, ppm dw
25000
20000
15000
10000
5000
0
13000
13500
14000
14500
15000
15500
COD, ppm dw

j)

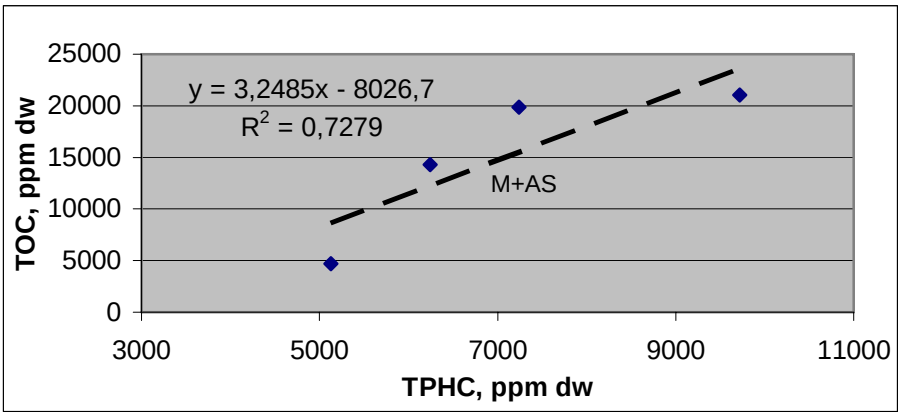
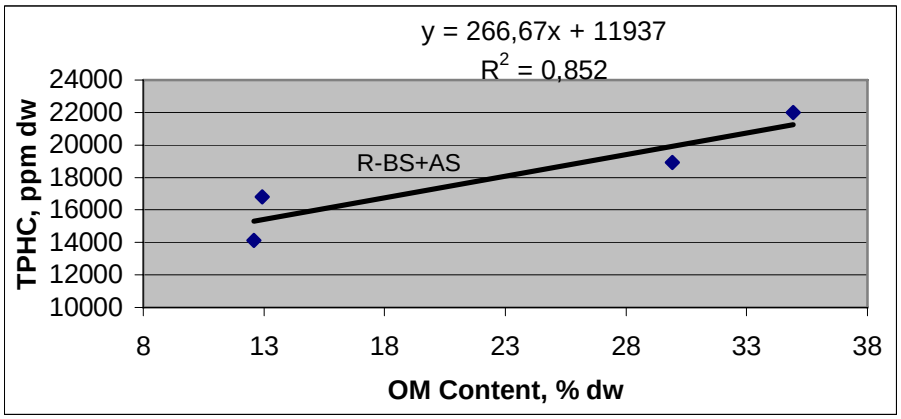


Figure 6.24 The correlation in variation of contaminant concentrations for Menemen soil in activated sludge enhancement (a) OM vs. COD (b) OM vs. TPHCs (c) OM vs. TOC (d) TPHCs vs. TOC (e) OM vs. COD_d (f) OM vs. TPHCs (g) OM vs. TOC (h) COD_d vs. TPHCs (i) COD vs. TOC (j) TPHCs vs. TOC (– – – Menemen, ——— Menemen-BS).

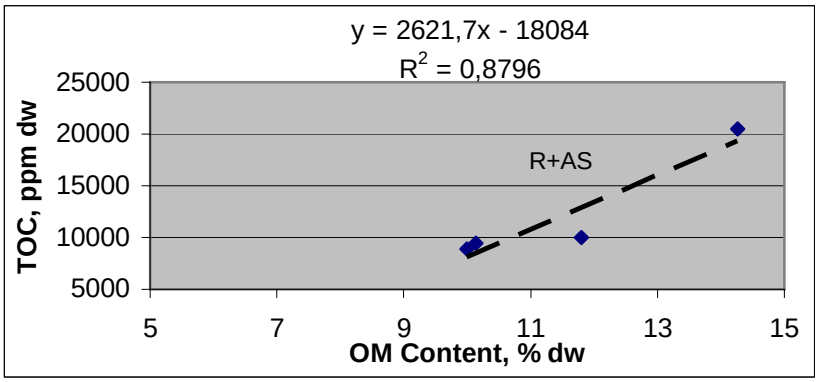
Correlations among organic matter content, total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand has seen in the set of experiments with activated sludge enhanced Menemen soil with the presence of biosurfactant. The same correlations also have seen in the case without biosurfactant in Menemen soil remediation.

- **Trials with Raman Soil**

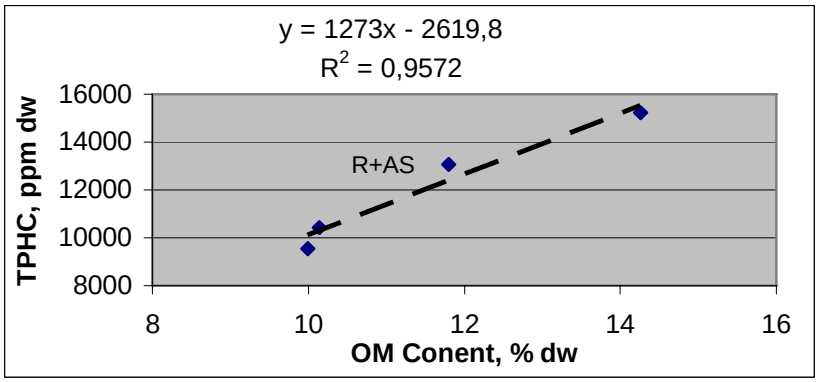
a)



b)



c)



d)

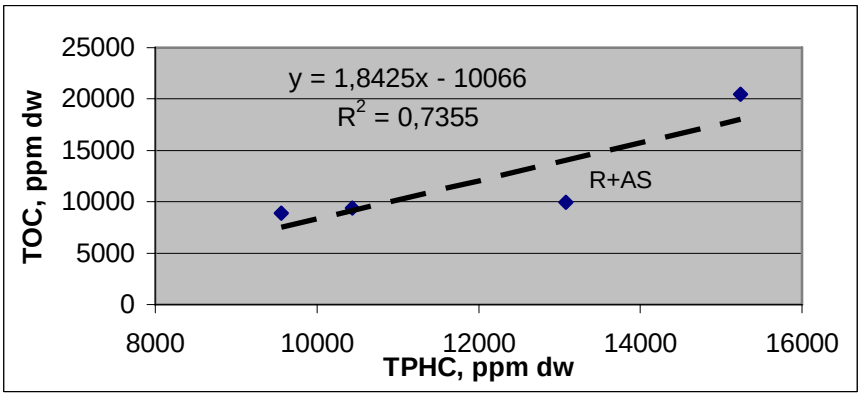


Figure 6.25 The correlation in variation of contaminant concentrations for Raman soil in activated sludge enhancement (a) OM vs. TPHCs (b) OM vs. TOC (c) OM vs. TPHCs (d) TPHCs vs. TOC (– – – Raman, ——— Raman-BS).

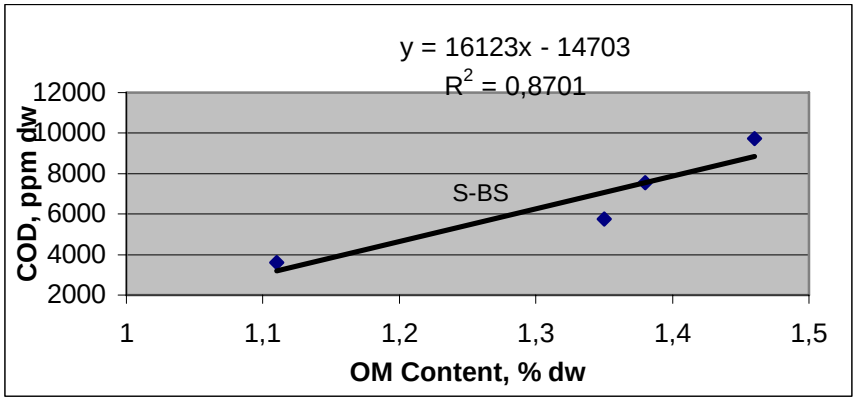
Only a strong correlation between organic matter content and total petroleum hydrocarbons in Raman soil remediation with activated sludge enhancement and presence of biosurfactant. But in the same case without biosurfactant, organic matter

content correlates with water soluble total organic carbon and total petroleum hydrocarbons.

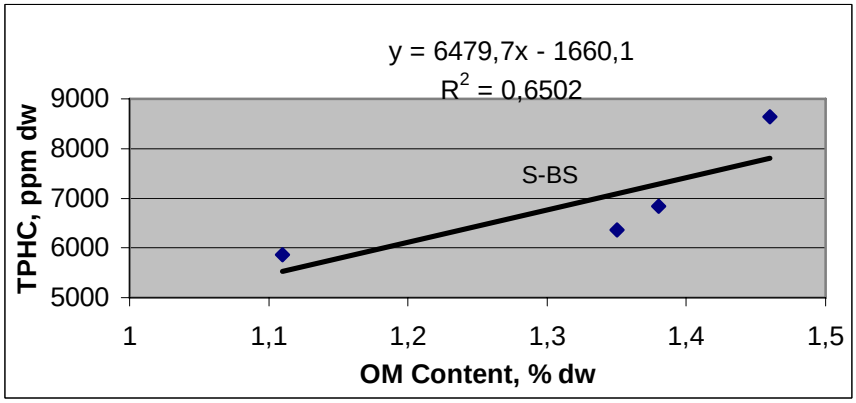
6.2.2.3 No Amendment Soil Remediation with Local Microorganisms (Natural)

- **Trials with Sand**

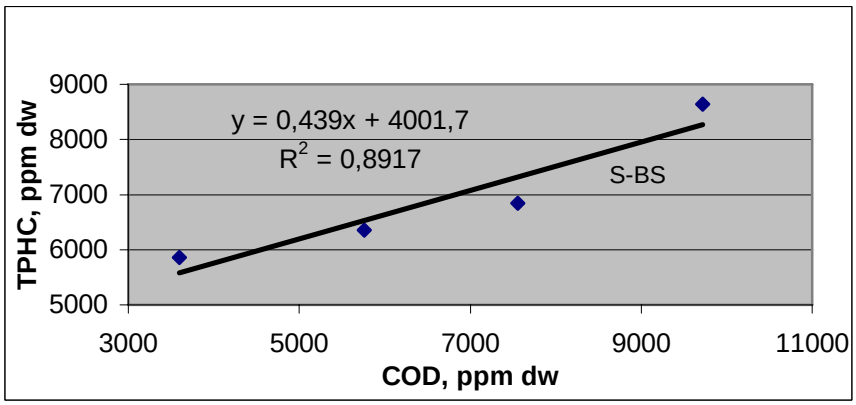
a)



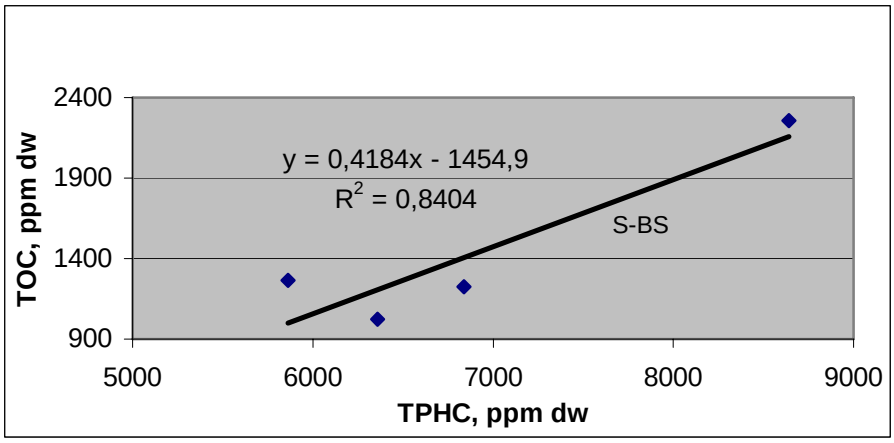
b)



c)



d)



e)

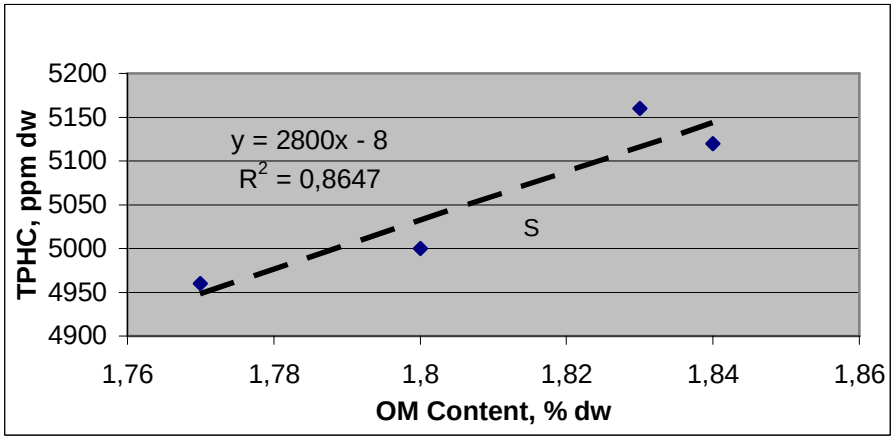
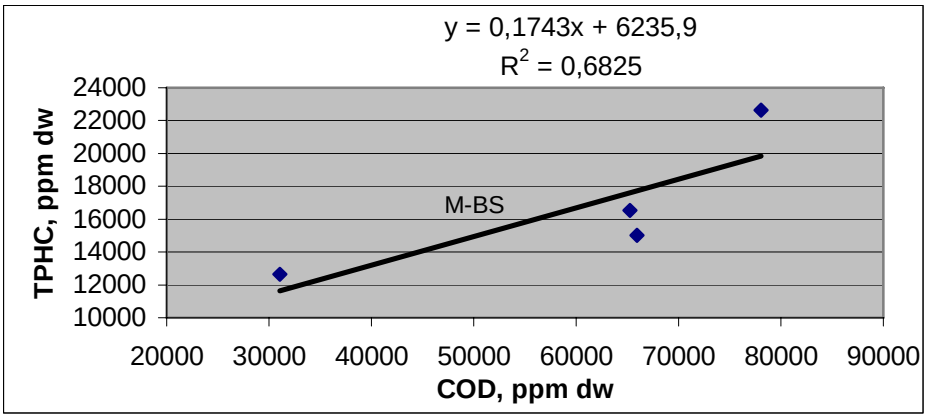


Figure 6.26 The correlation in variation of contaminant concentrations for Sand in natural (a) OM vs. COD (b) OM vs. TPHCs (c) COD vs. TPHCs (d) TPHCs vs. TOC (e) OM vs. TPHCs (– – – Sand, ——— Sand-BS).

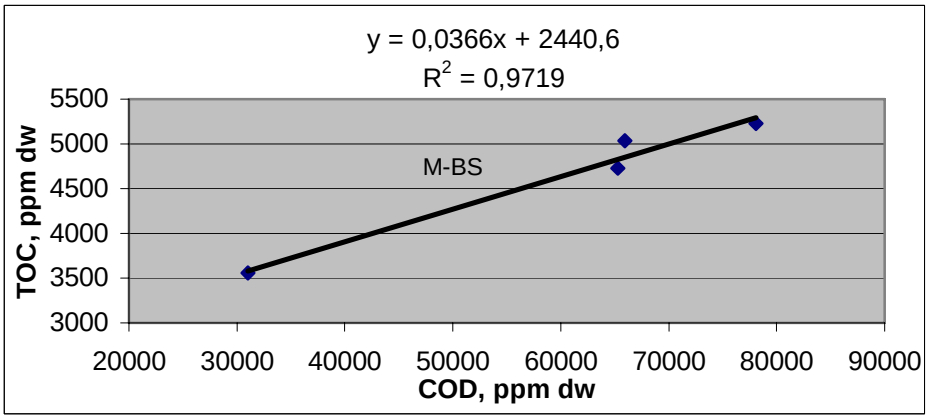
When only local microorganisms are present in the soil, organic matter content, total petroleum hydrocarbons, water soluble total organic carbon and chemical oxygen demand have correlated with the presence of biosurfactant in the sand. If biosurfactant is not present only a organic matter content - total petroleum hydrocarbons correlation can be seen in the same set with sand.

- **Trials with Menemen Soil**

a)



b)



c)

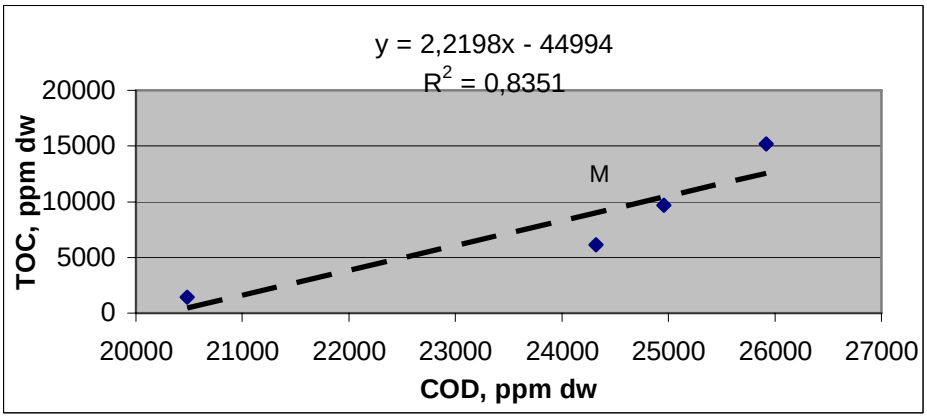
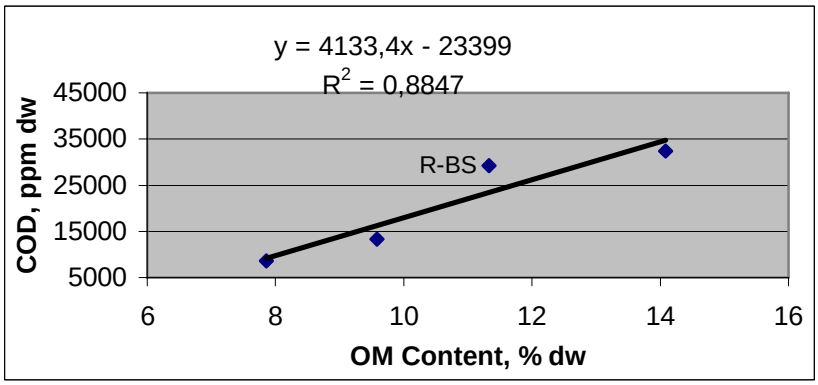


Figure 6.27 The correlation in variation of contaminant concentrations for Menemen soil in natural (a) COD vs. TPHCs (b) COD vs. TOC (c) COD_d vs. TOC (– – – Menemen, ——— Menemen-BS).

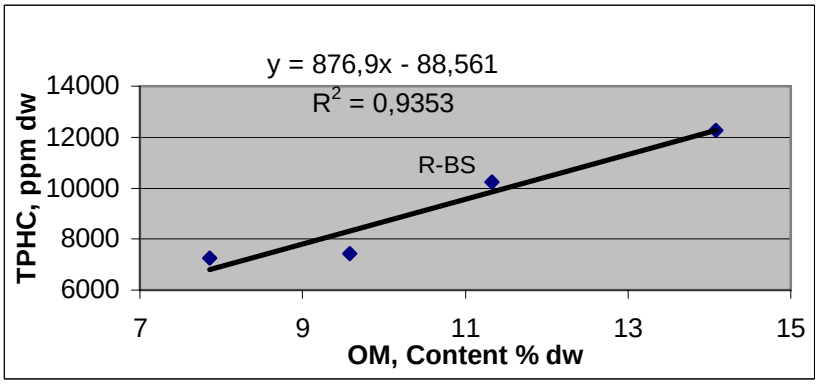
In engine oil treatment in Menemen soil with local microorganisms with the presence of biosurfactant water soluble chemical oxygen demand is correlated with total petroleum hydrocarbons and water soluble total organic carbon, while chemical oxygen demand is only correlated with water soluble total organic carbon without any biosurfactant in soil medium.

Trials with Raman Soil

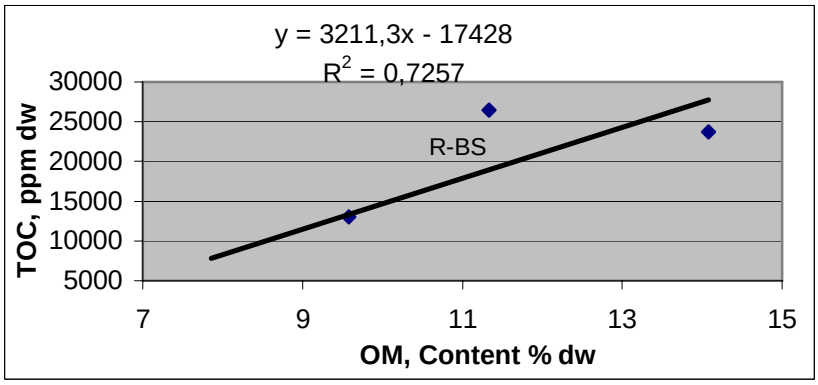
a)



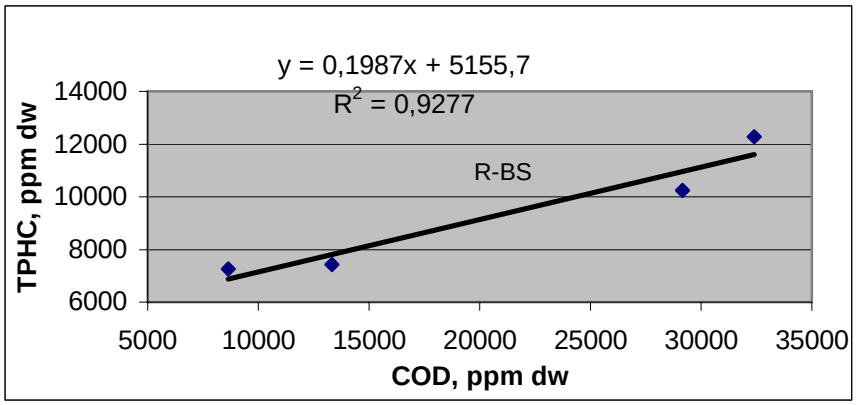
b)



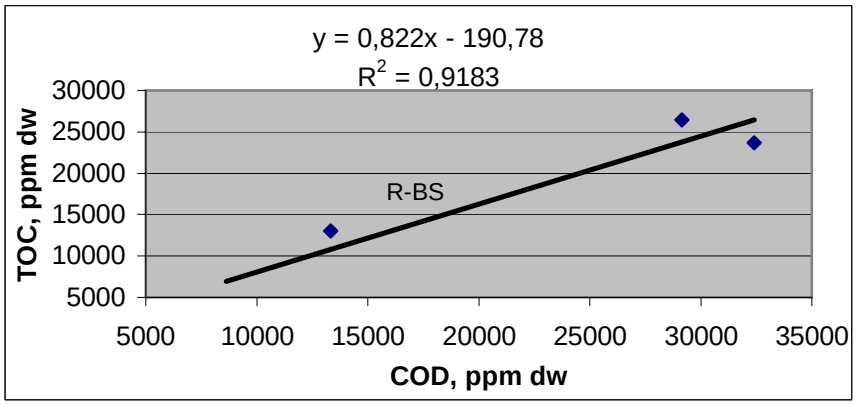
c)



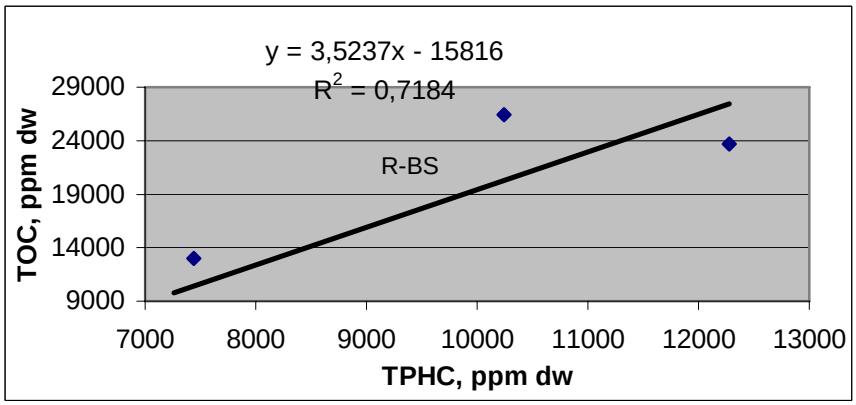
d)


y = 0,1987x + 5155,7
R2 = 0,9277
R-BS
TPHC, ppm dw
14000
12000
10000
8000
6000
5000
10000
15000
20000
25000
30000
35000
COD, ppm dw

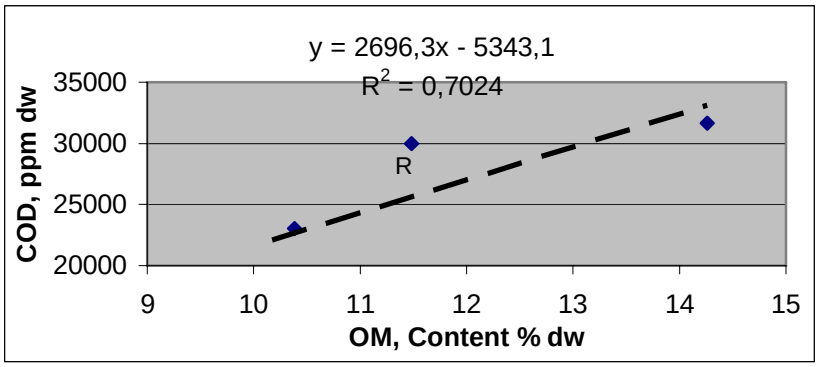
e)


y = 0,822x - 190,78
R2 = 0,9183
R-BS
TOC, ppm dw
30000
25000
20000
15000
10000
5000
5000
10000
15000
20000
25000
30000
35000
COD, ppm dw

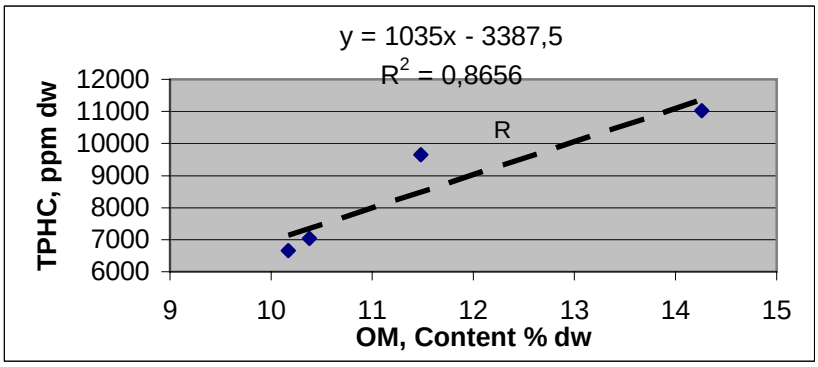
f)


y = 3,5237x - 15816
R2 = 0,7184
R-BS
TOC, ppm dw
29000
24000
19000
14000
9000
7000
8000
9000
10000
11000
12000
13000
TPHC, ppm dw

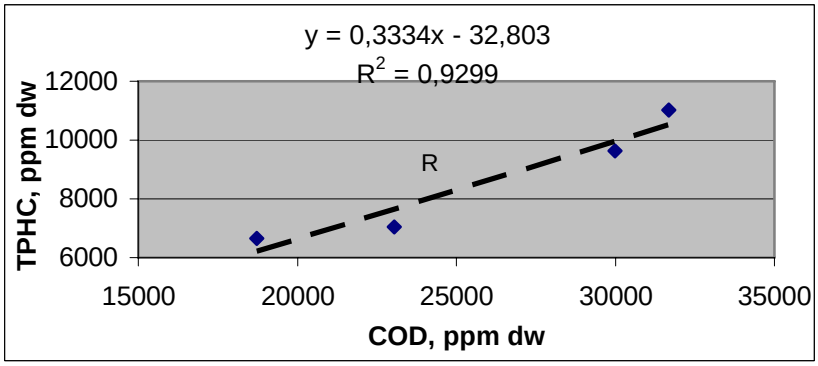
g)


y = 2696,3x - 5343,1
R2 = 0,7024
R
COD, ppm dw
35000
30000
25000
20000
9
10
11
12
13
14
15
OM, Content % dw

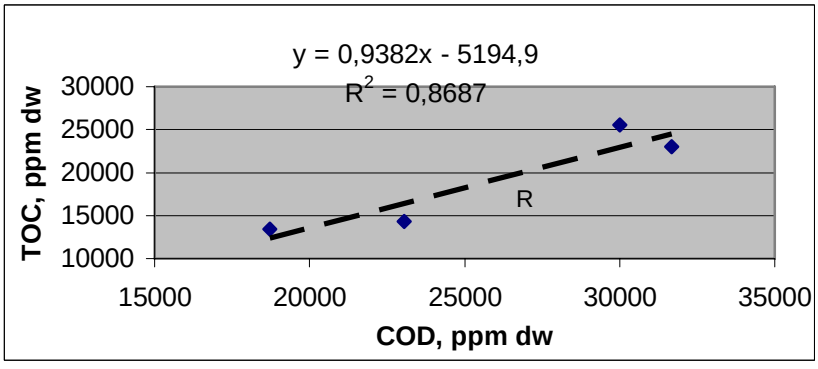
h)


y = 1035x - 3387,5
R2 = 0,8656
R
TPHC, ppm dw
12000
11000
10000
9000
8000
7000
6000
9
10
11
12
13
14
15
OM, Content % dw

i)


y = 0,3334x - 32,803
R2 = 0,9299
R
TPHC, ppm dw
12000
10000
8000
6000
15000
20000
25000
30000
35000
COD, ppm dw

j)


y = 0,9382x - 5194,9
R2 = 0,8687
R
TOC, ppm dw
30000
25000
20000
15000
10000
15000
20000
25000
30000
35000
COD, ppm dw

k)

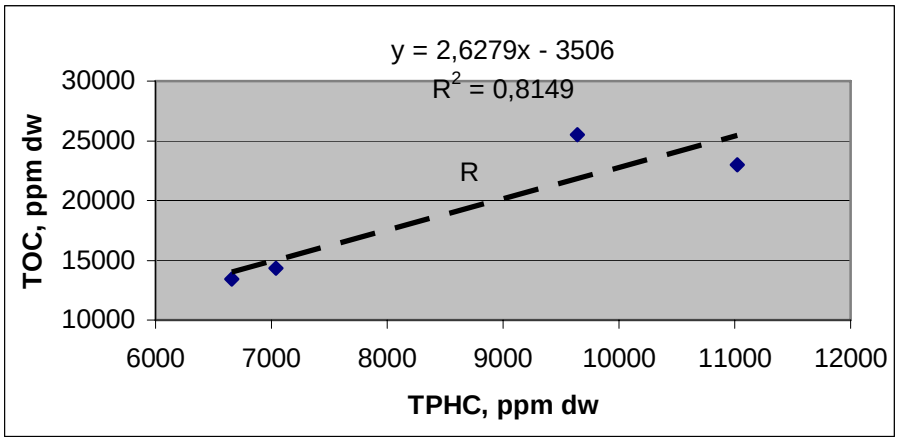


Figure 6.28 The correlation in variation of contaminant concentrations for Raman soil in natural (a) OM vs. COD (b) OM vs. TPHCs (c) OM vs. TOC (d) COD vs. TPHCs (e) COD_d vs. TOC (f) TPHCs vs. TOC (g) OM vs. COD (h) OM vs. TPHCs (i) COD vs. TPHCs (j) TPHCs vs. TOC (– – – Raman, ——— Raman –BS).

Concentrations of almost all parameters are strongly correlated with each other when Raman soil has treated with local microorganisms, both presence or absence of biosurfactant in the soil medium.

6.2.3 Determination of the Remediation Achievements According to the Type of Soil, Contaminant Parameters, and the Presence of Biosurfactant

To determine the achievements according to the type of soil, contaminant parameters, and the presence of biosurfactant; a calculation table is generated by using the removal efficiency of contaminant parameters. In the table, reductions found in all parameters are used as points, and the sum of points and the total scores of the lines and the columns for each group of data are used to evaluate the achievements (Table 6.7). The reductions below zero are accepted as zero.

Table 6.7 Removal percentages of experiments

Organic Matter	**Compost-BS**	**Compost**	**Activated Sludge-BS**	**Activated Sludge**	**Natural-BS**	**Natural**	***Total Points***
Sand	47	44	58	9	24	4	*186*
Menemen	15	10	34	10	62	60	*191*
Raman	18	20	64	30	44	29	*205*
Total Points	**80**	**74**	**156**	**49**	**130**	**93**	***582***
COD_d	**Compost-BS**	**Compost**	**Activated Sludge-BS**	**Activated Sludge**	**Natural-BS**	**Natural**	***Total Points***
Sand	26	-2/0	40	-26/0	63	20	*149*
Menemen	35	17	95	11	-110/0	16	*174*
Raman	27	-40/0	-5/0	8	73	41	*149*
Total Points	**88**	**17**	**135**	**19**	**136**	**77**	***472***
TPHCs	**Compost-BS**	**Compost**	**Activated Sludge-BS**	**Activated Sludge**	**Natural-BS**	**Natural**	***Total Points***
Sand	27	37	81	34	32	3	*214*
Menemen	48	49	45	47	-31/0	21	*210*
Raman	83	50	36	37	41	40	*287*
Total Points	**158**	**136**	**162**	**118**	**73**	**64**	***711***
TOC_d	**Compost-BS**	**Compost**	**Activated Sludge-BS**	**Activated Sludge**	**Natural-BS**	**Natural**	***Total Points***
Sand	72	49	87	84	44	-13/0	*336*
Menemen	54	8	78	89	-33/0	77	*306*
Raman	-73/0	15	26	57	80	42	*220*
Total Points	***126***	***72***	***191***	***236***	***124***	***119***	***862***
Total Score	***452***	***299***	***644***	***416***	***463***	***353***	

As can be calculated from Table 6.7, the sum of reductions for sand, Menemen, and Raman soils for three trials can be found as 885, 881, and 861 respectively. Even though those scores are close to each other, it can be seen that sand is the most appropriate soil type for bioremediation applications. This can be attributed to the lower organic matter content (%0,63) and larger pores of sand that allows penetration of water which carries soluble materials and microorganisms homogeneously.

The parameters indicating contamination are also evaluated according to overall remediation activities. The sum of the points of organic matter content and TPHCs

reduction are the highest in Raman soil, while COD_d total points are the highest in Menemen soil, and the TOC_d total points are the highest in sand soil.

When the enhancements are compared with each other, it can be said that enhancement with activated sludge addition is more effective than the compost addition. Furthermore, the natural soil trial with neither compost nor activated sludge addition has a score higher than compost addition. This result is in contradiction with the general expectations. Since the number of microorganisms contained by activated sludge is high and the types are various, it is effective for all soil types. Here, it should be indicated that compost addition is very effective in TPHCs reduction in the both cases with and without presence of biosurfactant. This may be because of the type of microorganisms obtained from the compost mass.

The experimental results indicate that the presence of biosurfactant Rhamnolipid always increase the remediation efficiency. If the efficiencies with and without biosurfactant is compared in all the three trials, it is seen that the effect of presence of biosurfactant is most significant in activated sludge application and it is followed by compost application. The biosurfactant effect is the least in natural soil trial.

When are examined, the figures reflecting variation of contaminant concentrations in section 6.2.1.1, it can be seen that COD_d and TOC_d concentrations are mostly rising in the first week of the experiments. Here, it should be indicated that those parameters in the experimental matrix are measured as “water soluble-dissolved” state. In the same section, it is also seen that the pH values are decreasing in the same time interval of the experiments. The increase of COD_d and TOC_d and the decrease of pH values may be related with each other, because during the degradation of organic materials, microorganisms produce enzymes, which may help dissolving carbonaceous material and pH decreases.

CHAPTER SEVEN
CONCLUSIONS AND RECOMMENDATIONS

The findings of the study can be listed as follows:

- Engine oil contaminations can be remediated biologically in sandy soils much easily than the soils have higher organic matter contents and smaller pores.
- Activated sludge enhancement gives better results than compost enhancement and natural treatment.
- Compost addition is very effective on reduction in parameter of TPHCs
- Rhamnolipid is used as biosurfactant. Compared with the set of trials without any biosurfactant, it is seen that remediation efficiency is increased with the presence of Rhamnolipid which is applied in the level of CMC.
- The positive effect of biosurfactant is more apparent in activated sludge enhancement and in sandy soil remediation.
- COD_d and TOC_d concentrations may increase and pH may decrease in the first days of the bioremediation, because of the enzymes exposed during the microbial activities. So, organic matter content and TPHCs are recommended parameters to follow the change of contaminant concentration during the remediation studies.
- The best enhancement have been observed in Raman soil where 83% removal has been observed in compost + biosurfactant addition experiment as compared to 40% removal in natural decay condition. 43% increase in removal efficiency during 4 weeks time period is very high. Therefore here it is recommended to apply this alternate for solving the soil pollution problem. Before the application larger scale experimental study would be beneficial.

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