Effects of Biosurfactants on Remediation of Soils Contaminated with Pesticides

By

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ABSTRACT

Pesticides have played a significant role in increasing food production, and in view of growing worldwide food demand. Nevertheless; some of them have been classified as persistent toxic chemicals. This has resulted in serious concern about environmental contamination. Once a pesticide or toxic chemical find its way in the environment, a major part of it comes in contact with soil.

There are several possible sources of pesticide contamination; at manufacturing, storage, or user sites. The most serious examples of pesticide contamination are typically the result of poor production and waste management practices of pesticide manufacturing, formulation, and application facilities. Improper storage, handling, and disposal also have resulted in pesticide contamination at these sites and at landfills.

Today, many remediation technologies are used to remove the pesticides from the soil. One of the soil treatment methods is enhanced biodegradation. Bioremediation of the soil has often proven to be a cheap solution for contaminated soil problem.

This research was conducted to investigate the effectiveness of biologically produced surfactants (biosurfactants) on the biodegradation of pesticide-contaminated soil and evaluate the potential for biosurfactant-enhanced bioavailability of pesticide in soil.

In order to determine the effectiveness of biosurfactants on pesticides, sophorolipid and rhamnolipid type biosurfactants were used. These biosurfactants were chosen since they are well characterized and their stimulating effect on the biodegradation of hydrophobic substrates was described in the literature. In this study, endosulfan and trifluralin were selected as pesticides. The study was performed in two stages in laboratory conditions. In the first part of the experiment, degradation of endosulfan-contaminated soil was studied by the presence of sophorolipid and in the second part of the experiment; rhamnolipid (JBR 425) was used on the removal of trifluralin-contaminated soil. Throughout the experiment, three different concentrations of sophorolipid and rhamnolipid were applied to soil which, are 0.98, 9.75 and 195 ppm for sophorolipid and 1.6, 100 and 1000 ppm for rhamnolipid.

The effectiveness of synthetic or microbial surfactants on biodegradation of chemicals has been investigated by many researchers. However, studies about the

biosurfactant enhanced soil remediation for the pesticide contaminants are limited. Besides that, the outcome of surfactant applications has been highly system-specific, with conflicting results reported in the literature.

Therefore, despite the general trends outlined in literature, the effect of biosurfactants on the biodegradation of organic compounds is poorly understood. Opposed effects are frequently observed. This study is the first M.Sc. thesis study about the use of biosurfactant enhanced bioremediation of pesticides in Turkey.

The results from first part of our study obtained from sophorolipid, were not satisfactory since the degradation patterns for endosulfan were not affected by the presence of sophorolipid. According to the second experiment results, removal of trifluralin ranged from 24-35 %, with the increase in rhamnolipid concentrations. Addition of rhamnolipid (JBR 425) into the soil was found to increase the degradation rate of trifluralin by 13 % as compared to the control soil column. Additional time would probably increase the rate of degradation and bioavailability, as a result of providing the adaptation of microorganisms in contaminated soil media and formation of more bioavailable metabolites.

ÖZ

Pestisitler, artan gıda üretimi ve ürün taleplerini karşılama açısından önemli bir yere sahiptir. Fakat bazı pestisitler, özellikle çevrede parçalanması güç toksik kimyasal maddeler olarak sınıflandırılanlar, çevre kirliliği açısından büyük tehlike oluştururlar. Bu tür pestisitler çevrede, özellikle toprak ortamında uzun süre kalarak yüzeysel sular, yeraltı suları ve hava gibi diğer alıcı ortamlara yayılırlar.

Pestisit kirliliği, bu maddelerin üretim aşamasında, depolanmasında veya kullanıldığı alanlarda ortaya çıkar. Kirliliğe yol açan en önemli faktörler, pestisitlerin düşük kalitede üretimleri, üretim sahalarında, formülasyonlarında veya tatbik edildiği alanlarda, pestisit kontrolüne yönelik uygulamaların yetersiz olmasıdır. Bunun dışında, pestisit atıkların uygunsuz şekilde depolanması ve çevreye bırakılması da pestisit kirliliğine yol açan diğer faktörlerdendir.

Günümüzde pestisitleri toprak ortamından uzaklaştırmak amacıyla bir çok teknoloji uygulanmaktadır. Bunlardan bir tanesi de "hızlandırılmış ayrıştırma" metodudur. Toprağın biyolojik olarak arıtılması diğer teknolojilere oranla ekonomik açıdan daha düşük maliyet sağlayan bir çözümdür. Bu çalışmanın amacı, biyolojik olarak üretilen surfaktanların (biyosurfaktan) pestisitlerin biyolojik olarak parçalanmasındaki etkilerini incelemektir.

Bu konuyla ilgili olarak daha önce pek çok araştırmacı sentetik ve biyolojik surfaktantların ayrışma üzerine etkilerini çalışmışlardır. Fakat, biyosurfaktanların pestisitlerin biyolojik olarak arıtılmaları üzerindeki etkilerine dair yeterli sayıda çalışma mevcut değildir. Ayrıca biyosurfaktan uygulamalarına yönelik yapılan çalışmalarda elde edilen sonuçlar ortam şartlarına göre değişken olup birbiri ile tutarsız sonuçlar da literatürde yer almaktadır. Bu nedenle, biyosurfaktanların organik maddelerin giderilmesindeki etkilerini tahmin etmek güçtür. Bu proje, pestisitlerin biyolojik yolla toprak ortamından uzaklaştırılmasında biyosurfaktanların etkilerini araştırmaya yönelik Türkiye'de yapılmış ilk yüksek lisans tez çalışmasıdır.

Biyosurfaktanın pestisitlerin ayrışması üzerindeki etkisini saptamak amacıyla sophorolipid ve rhamnolipid biyosurfaktanlar kullanılmıştır. Bu biyosurfaktanların seçilmesindeki sebep, iyi karakterize edilmiş olmaları ve hidrofobik maddelerin biyodegredasyonunda hızlandırıcı etkiye sahip olmalarıdır.

Bu çalışmada kirletici olarak endosulfan ve trifluralin pestisitleri kullanılmıştır. Proje iki aşamalı olup laboratuar koşullarında gerçekleştirilmiştir. Projenin ilk

bölümünde sophorolipid varlığında endosulfan ile kirletilmiş toprağın degradasyonu çalışılmış, ikinci kısımda ise trifluralin ile kirletilmiş toprağın arıtılmasında rhamnolipidin (JBR 425) etkisi incelenmiştir. Çalışma süresince toprağa üç farklı konsantrasyonda; 0.98, 9.75 ve 195 ppm sophorolipid ve 1.6, 100 ve 1000 ppm rhamnolipid eklenmiştir.

Sophorolipid ile ilgili elde edilen ilk çalışmanın sonuçları tatmin edici değildir. Endosulfan için ayrışma hızı sophorolipid varlığından etkilenmemiştir. İkinci çalışmanın sonuçlarına göre, biyosurfaktan konsantrasyonunun arttırılmasıyla trifluralin % 24-35 oranında giderilmiştir. JBR 425 biyosurfaktanın toprağa uygulanması sonucu, kontrol toprak örneğine göre pestisit gideriminde % 13 oranında bir artış görülmüştür. Mikroorganizmaların ortama adaptasyonlarının tam olarak sağlanması ve böylelikle ortamda biyolojik olarak parçalanmaya elverişli ürünlerin oluşacağı düşüncesi, uygulanan bekletme süresinin uzatılmasının yararlı olacağı izlenimini vermektedir.

We approve the thesis of Özlem ZENGİNYÜREK

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INTRODUCTION

1.1 Problem Statement

Increasing type and the amount of chemicals and the diversity of the sources of pollution have lead to multiple impacts on humans and the environment. Substances hazardous to human health and ecosystems are still widely used, however, most of the research and registration aim to identify toxic compounds and they seek proper substitution.

Pesticides stand out as one of the major developments of the twentieth century. Chemicals for crop protection and pest control, known as pesticides are used to destroy, repel or otherwise control insects, weeds, and pest. Enormous quantities of pesticides are currently used in developing countries. However, the use of pesticides is a great concern to human and environment. These pesticides range in persistence from compounds that degrade rapidly and are broken down in hours or days, to some of the most complex and persistent molecules. The environmental impact of pesticide use is related to several fundamental properties. Firstly, pesticides are toxic compounds capable of effecting target and also non-target organisms. Secondly, many pesticides need to be resistant to environment degradation so that they persist in treated area and thus their effectiveness is enhanced. This property also promotes long term effects in natural ecosystem. Many pesticides do not reach their targets but instead end up on crops, trees, or animals, if they are persistent, usually end up either in soil or aquatic sediments in freshwater. Pesticides in fast-flowing waterways become progressively carried down to the mouth of rivers, estuaries or bays where they can affect many bottom-living organisms. They can also volatilize into the atmosphere from water surfaces and persist in the sediment for many years by adsorbing onto floating particles. In addition; the use of pesticides, especially more soluble ones, has extensively potential for contaminating groundwater. More commonly, pesticides contaminate the groundwater by fallout from aerial sprays, through drainage from soil and water erosion, or through disposal of pesticide containers or effluent from pesticide factories. Once a groundwater is contaminated, analyzing problem and providing alternative water supplies can be quite expensive and contamination may last for many years. Because

cold temperatures and low microbial activity in groundwater cause pesticide degradation to occur more slowly than at the soil surface. According to the studies conducted in General Directorate of State Hydraulic Works (DSI), total surface and groundwater quantity that can be consumed considering technical and economical aspects is 110 km^3/year. Of this amount, 95 km^3 is supplied by surface water originating within Turkey, 3 km^3 by surface water entering from foreign countries and 12.3 km^3 is supplied by groundwater. Therefore, the overall aim is to be protecting the groundwater and also surface water sources from the pesticide contamination. In recent years drinking water quality has become a major issue for public and political debate. Water quality issues in the public eye include nitrates, lead, aluminum, trihalomethanes (THMs) and pesticides. According to EPA and Turkish drinking water regulatory standards, maximum admissible pesticide concentration is 0.10 μg/L and total pesticide concentration is 0.50 μg/L [32, 35]. In addition, maximum acceptable pesticide concentration in soil is 2 ppm according to the contaminated soil regulatory standards in Turkey [36].

Contaminated sites have a potential risk for the ground and surface water contamination. In order to prevent these sources from any contamination, soil contaminated with any hazardous substances has to be treated first. However, the technologies used in the remediation of sites contaminated with hazardous wastes have been deemed expensive and inefficient [25]. The cost and ineffectiveness of current remediation approach warrant the investigation of alternative clean-up strategies. Alternative approach such as bioremediation may be more effective and less costly than conventional approaches. On the other hand, this technology suffers from several bottlenecks, one of which is the low availability of hydrophobic organic contaminants to the microorganisms. This poor bioavailability is caused by low mass transfer rates of the contaminants to these microorganisms from sites where they are inaccessible. Several bacteria produce biosurfactants that may be used to enhance biodegradation rates of hydrophobic organic contaminants during soil remediation. Because of many advantages over the synthetic counterpart, biosurfactants are widely used in various industrial processes such as pharmaceutical, cosmetic, petroleum, food production, enhanced oil recovery and cleaning of oil tanks, and soil remediation.

1.2 Objectives of the Study

The study aimed to investigate whether biosurfactant stimulate the biodegradation of pesticides in soil or not. Biosurfactant especially rhamnolipid and sophorolipid type-biosurfactants have been previously shown to both increasing the biodegradation of slightly soluble organics (i.e., naphthalene, hexadecane) and metals. In addition, they have long been used in the oil industry to enhance oil recovery [27]. For this reason, addition of biosurfactants to soil contaminated with pesticides would increase the pesticide degradation.

The purpose of the study is to investigate the effects of biosurfactants in microbial degradation of soil contaminated with endosulfan and trifluralin types of pesticides and determine the enhancement level of biosurfactants in degradation of pesticides. Endosulfan is one of the organochlorinated insecticide used to control of large spectrum of insect pests on fruits and vegetables. Trifluralin is a group of di-nitroaniline herbicide which is used to destroy or control plants. However, these pesticides tend to accumulate in the environment as it is not readily consumed by soil microorganisms and residuals are detectable in crops at harvest time. Because the use of endosulfan and trifluralin are unrestricted, they could be a potential problem in the future.

The main goal of the study is to investigate whether there is any effect of biosurfactant on the pesticide removal in soil media and if so to determine which types of biosurfactant and which biosurfactant concentrations are the best for assessing biodegradation of the pesticides. To address these questions, two experiment systems were created through top soil. In the first experiment endosulfan biodegradation was studied in the presence of sophorolipid type of biosurfactant. In many studies on the environmental applications of biosurfactants, sophorolipid has been found effective in contaminated soil and groundwater clean-up studies. In the second experiment, mineralization of trifluralin pesticide was studied by using JBR 425 (rhamnolipid) type of biosurfactant.

Chapter 2

PESTICIDE POLLUTION AND CONTAMINATED SOIL REHABILITATION

2.1 Overview of Pesticides Contamination

Enormous quantities of pesticides are currently used in agricultural activities. Some of these pesticides degrade rapidly, and are broken down in hours or days while the others are most complex and persistence molecules. We still do not know the full degradation pathways ultimate fate of many pesticides in the field. Many pesticides do not reach their targets but instead end up on crops, trees, animals, soils, or surface and groundwater sources.

By far the greater quantity of pesticides applied to crops end up in the soil, either through aerial drift, runoff from plants, or death of the plants. Depending on the nature of the pesticide it may be broken down rapidly, usually by soil microorganisms, or become bound onto soil fractions, such as organic matter or clay minerals, and persist weeks, months, or even many years. Some of even least volatile pesticides volatize from the soil surface or from deeper soil by “wick” process and reach atmosphere, where they may be adsorbed onto atmospheric particles. They may wash out from the atmosphere in precipitation to contaminate untreated soils. Pesticides are also lost from soils by wind and water erosion in quite large quantities.

Pesticides can reach water as a result of direct treatment to control pests but more commonly they contaminate aquatic systems by fallout from aerial sprays, through drainage from soil and water erosion, or through disposal of pesticide containers or effluent from pesticide factories. Some pesticides can persist in the sediment for many years and are periodically recycled into the water when the sediment is disrupted [1].

Because of the long term leaching characteristics of some pesticides, they play an important role for the environment. Clearly some of the environmental impacts of pesticides are serious such as they may reach the drinking water sources, sometimes beyond accepted safety levels. Therefore, we must progressively explore alternatives to

pesticides that are more ecologically acceptable and keep the use of pesticides at levels, which create no environmental or human problems.

Overview of Pesticide Use in the Agricultural Sectors

In developed countries, environment and health are very important subjects. Therefore; the use of pesticide in the country is quite considered in terms of the environment and health. Thus, the evaluation of pesticide use is necessary for the understanding the situation of the pesticides in the country.

It has been known that the main pesticide usage of countries is designated as the effective compound per hectare area. In Table 2., pesticide consumption in Turkey, in Table 2.2, pesticide consumption in European Union member states are given.

Table 2.1 Pesticide consumption as effective compound in Turkey

Groups of Pesticide	Consumption of pesticide kg per year				
	1979	1987	1994	1996	1998
Insecticides	2 287 658	3 303 446	2 046 991	3 027 380	6 509 542
Acaricides	203 107	230 360	192 279	223 857	316 119
Lubricant	1 594 526	2 147 106	1 977 281	2 871 160	1 731 932
Fumigants and Nematicides	315 655	322 227	530 738	1 076 661	1 244 698
Rodenticides and Molluscicides	5 600	2 124	2 509	3 268	2 291
Fungicides	1 537 315	2 611 960	2 201 406	2 951 191	2 625 626
Herbicides	2 451 977	3 495 044	3 902 588	3 643 971	2 499 205
TOTAL	8 395 848	12 112 267	10 871 792	13 979 488	14 929 413

Source EU Project: ERBIC18CT970167 "Development of a simple technology in drinking water treatment for nitrate and pesticide removal"

It can be seen from the Table 2.1 that the consumption of insecticides consisting of high acute toxicity compounds increases to 43.60% of the total consumption in 1998 whereas this ratio is of 20% in the period of 1979 to1996. The compounds having high acute toxicity values pose a threat to the environment. Since these compounds have a high volatilization characteristic and a tendency for leaching the surface and groundwater, they cause air and water contamination. Besides, unconscious usage of these compounds results in the leaching of the toxic residues to the crops.

Additionally, the compounds having a low acute toxicity but high chronic toxicity characteristics have also lead to environment and health problems if exposed permanently.

Table 2.2 Intensity and efficiency of pesticide use in Turkey and the EU Member States: 1993 - 1995.

Countries	**Intensity of Pesticide Use (Pesticide Use in kg active ingredients per hectare)**
France	5.6
Italy	9.3
U.K	6.4
Spain	2.3
Germany	2.6
Belgium	13.8
Sweden	1.2
Portugal	6.0
Netherlands	13.5
Greece	4.4
Denmark	1.7
Austria	4.0
Ireland	16.3*
Finland	1.2
Luxembourg	4.4
TURKEY	**0.5 (0.6)****

* Recent evidence suggests that this figure is far too high. A more realistic estimate seems to lie in the order of 5 to 8 kg per hectare
** in 1998

Sources: European Commission/DG XI, July 1999

The intensity of pesticide use provides relevant information with respect to the potential negative effects to the environment. In general, a higher intensity will lead to a higher threat, as more pesticides are being used per hectare. From the data in Table 2.2, it can be seen that Belgium, the Netherlands and Italy have high intensity of pesticide use. The consumption of pesticide in Turkey is very low compared with Member States. However, it must be considered that the consumption of pesticide in Turkey is very heterogeneous and the amount of pesticide utilized in the Mediterranean and Aegean is more than 2/3 of total pesticide consumed in Turkey [12].

Pesticides have been classified according to their volatilisation, mobility and persistence characteristic and groundwater pollution potential. These properties are classified in Tables 2.3 and 2.4.

Pesticides can be classified in three groups as their volatilization

Category 1	High volatile
Category 2	Medium volatile
Category 3	Low volatile

Table 2.3 Classification of commonly used pesticides according to their volatilization

Category 1	**Category 2**	**Category 3**
Azinphos- Methyl	Carbanyl	Aldicarp
Captan	Carbosulfan	Atrazine
Chlorpyifos	Dicofol	Benomyl
Deltamethrin	Profenos	Bromacil
Diazinon		Bromophophylate
Endosulfan		Cypermethrine
EPTC		2,4 D
Fenthion		Dichlorvos
Methyl Bromid		Ethoproshos
Propanil		Fenarimol
Terbutryn		Fenitrothion
Trifluralin		Linoron
		Malathion
		Mancazeb
		Maneb
		Methiocarb
		Metolachlor
		Methomyl
		Methly-parathion
		Monocorotophos
		Phosalone
		Propineb
		Triadimefon
		Trichlorfon

Table 2.4 Classification of pesticides with regard to their persistence

Group 1	T ½ > 100 d	Very high persistence
Group 2	31 d < $T_{1/2}$ < 100 d	High persistence
Group 3	16 d < $T_{1/2}$ < 30 d	Normal persistence
Group 4	6 d < $T_{1/2}$ < 15 d	Low persistence
Group 5	T ½ < 5 d	Very low persistence

Source: EU-Project ERBIC18CT970167 "Development of a simple technology in drinking water treatment for nitrate and pesticide removal"

According to Table 2.4 endosulfan with a half-life of 30-70 (60 days for α-isomer) and trifluralin having a half-life of 57 to 126 days generally belong to Group 2 [10].

Among these pesticides, trifluralin and endosulfan were chosen as a model pesticide because of their great consumption values and being potential threat for surface and ground water. Pesticides selection criterias were explained in Chapter 3.

2.2 Fate of Pesticides in the Environment

Pesticides that become incorporated into the soil may be destroyed, inactivated or removed from the environment by a number of means. Such environmental mechanisms of pesticide fate and transport are as follows:

- Volatilization
- Leaching of the chemicals through and out of the surface soil
- Chemical reactions
- Adsorption of the compound by soil colloids
- Photochemical destruction
- Plant removal from the soil
- Biological detoxication

The specific mechanism depends upon the chemical in question, the soil type and environmental conditions. Some pest control agents disappear largely by means of volatilization; others are readily removed from the surface horizons by leaching while some are destroyed largely or entirely by microbial agencies. [46]

2.2.1 Volatilization

Volatilization is a process by which a chemical compound is released to the atmosphere in the form of a vapor or gas. Few pesticides are known to be volatile. Most of these belong to the lower molecular weight halogenated aliphatic compounds (e.g., ethylene dibromide, dibomochloropropane, and methy bromide) The rate of volatilization for an individual compound is controlled mainly by the Henry's law constant, which is the ratio of the concentration of contaminant in the liquid equilibrium phase. Volatilization is affected by the moisture level of the soil, wind speed, temperature, soil organic matter content and by the pesticide formulation.

2.2.2 Leaching

The rate and extent of loss by leaching is associated with the amount of rainfall and irrigation; the compound ultimately moving downward and into the groundwater.

A major factor controlling the downward migration of the pesticides is the solubility of chemical compounds in water.

Leaching of pesticides is caused mainly by percolation of stormwater through the contaminated soil media, which causes the dissolved portion of the organic and inorganic compounds to enter the ground water aquifer and be carried away.

Leaching, run-off and soil erosion can be the prelude to pollution of ground water, streams and rivers. Run off occurs when water accumulates on the land surface at a rate faster than it can infiltrate the soil. Pesticide can be moved by run off when they are either dissolved in the water or bound the eroding soil particles. Herbicides in runoff can cause direct injury to non-target plants. Insecticides and nematicides that are carried by run off into surface waters such as stream and ponds can be harmful to a variety of aquatic organisms. Pesticides residues in surface waters can cause injury to crops, livestock, or human if the contaminated water is used down streams

2.2.3 Chemical Reactions

Chemical transformations can be classified as hydrolysis, oxidation, and reduction. These reactions may be catalysed by the presence of metal ions, metal oxides, clay surfaces, organic compounds, and organic surfaces. The pH of solutions and the effective pH of clay surfaces, which may be quite different from the surrounding aqueous environment, can significantly influence rates of degradation.

2.2.4 Soil Adsorption

The tendency of a pesticide to leach also depends on how strongly it adsorbs to soil. Adsorption refers to the attraction between a chemical and soil particles. Adsorbent materials in soils and sediments can be divided into clay minerals and soil organic matter. Adsorption is more pronounced in soils with high clay content and high organic matter. Compounds that are strongly adsorbed onto soil are not likely to leach, regardless of their solubility. They are retained in the root zone where they are taken up

by plants or eventually degraded. Compounds that are weakly adsorbed, on the other hand, will leach in varying degrees depending on their solubility. The extent of adsorption is related to the individual colloid, the specific chemical, moisture, pH, temperature and the type of formulation. As a rule, adsorption decreases with increasing pH and temperature.

A pesticide's tendency to be adsorbed by soil is expressed by its adsorption coefficient

$$K_{OC} = K_d * \%\ Organic\ carbon\ in\ soil$$

First term is expressed as adsorption coefficient (Kd) and can be calculated by mixing soil, pesticide, and water, then measuring the concentration of pesticide in solution after equilibrium is reached.

$$K_d = \frac{Concentration\ of\ \ chemical\ adsorbed}{Concentration\ of\ \ chemical\ dissolved}$$

A wide range exists in pesticide partition coefficients. DDT, for instance, has a Kd value roughly 20 times as high as that for aldicarp and 1.5 times as high as that for atrazine. This clarifies why aldicarp and atrazine have been found in ground water in agricultural areas while DDT has not.

High Koc values indicate a tendency for the chemical to be adsorbed by soil particles rather than remain in the soil solution. Adsorption coefficients less than 500 indicate a considerable potential for losses through leaching.

2.2.5 Photochemical Transformations

Photochemical degradations occur in air and water but are probably of little or no significance in soil. Before a substance can undergo a photochemical reaction, it must have the ability to absorb energy from the appropriate portion of the spectrum. When energy is absorbed from UV light, electrons in the molecule are excited and the resulting event cause a breakage of existing chemical bonds or the formation of new ones.

2.2.6 Plant Removal of Pesticides from the Soil

Not only may pesticide disappearance from the soil result from non-biological and microbial agencies but non-cultivated and cultivated plants may assimilate through their roots a variety of herbicides and insecticides and thereby lower the chemical concentration in the ecosystem. The fact that food or feed crops take up the pesticides or their toxic derivatives from the soil raises another potentially serious problem since the assimilated substances may be translocated from the roots into aerial portion of the plant. The latter, in turn, it might be consumed by animals or man.

2.2.7 Microbial Degradation of Pesticides

Microbial degradation process involves similar biochemical reactions. These include dehalogenation, oxidative reactions such as epoxidation, dealkylation, reduction, ester hydrolysis and condensate or conjugate formation. Most pesticide-degrading soil microorganisms have been isolated from soil. The types and rates of microbial degradation are determined by the pH, temperature, redox potential, nutrient availability and the general microbial ecology of a given system. If the pesticide can be used as an energy or nutrient source, it will disappear from the soil slowly or rapidly, the rate depending upon the compound, the method of application, the extend and degree of adsorption, the rate of growth of the active species, various environmental factors and possible toxicity of the substrate to microorganisms using it [46].

2.3 Pesticide Contaminated Soil Rehabilitation

During the past couple of years, a great number of contaminated sites were identified in several countries in the world. Significant problems were encountered on the property of industrial developments (e.g., gasification plants, cooking plants, chemical industries) where waste substances were inadequately stored or even dumped. Besides, underground contamination is often generated by leakage of pipes and tanks. (e.g., at refineries, airports, gas stations).

Treatment of contaminated soil is long-term process. With regard to treatment technology a variety of mechanical, physical, chemical and biological methods are currently applied, but the technology, which has been applied so far, is still in a rather infant state.

The technology of soil protection and soil remediation is currently developing to a new scientific branch of cross-disciplinary character. Knowledge and experience of many disciplines must merge to generate solutions, as they are so urgently needed. The involvement of chemists, microbiologist, soil scientists, geologist, civil, chemical and environmental engineers is necessary in solving contaminated soil problems.

Remedial action techniques are given below

- Thermal techniques
- Extractive techniques, flotation
- Biological techniques
- Air stripping, soil vapor extraction
- Other remedial action techniques

2.3.1 Remedial Options at Pesticide Sites

The technologies available for remediation can be grouped as three basic approaches:

2.3.1.1 Immobilization Technologies

The purpose of these technologies is minimize or prevent contaminant migration. These technologies are physical barriers to reduce the flow of contaminated ground water or water through contaminated media. Additionally, chemical reaction, physical interactions, or both can be used to retain or stabilize a contaminant and prevent its migration or interaction into the environment. Immobilization technologies function only to limit the environmental mobility of pesticides with no detoxification or volume reduction. Technologies used for the immobilization of pesticide-contaminated media are categorized as containment technologies, Stabilization/Solidification (S/S) technologies, and vitrification [14].

2.3.1.2 Destruction Technologies

These technologies contain thermal, chemical, or biological processes to reduce or eliminate toxicity and may result in significant volume and mobility reductions. Pretreatment activities such as concentrating contaminants or contaminated materials are often required to prepare media for processing with the final destruction technologies.

The destruction technologies for remediation of pesticide-contaminated soils, sludge, and sediments are broadly divided into the categories listed below:

- Thermal Destruction Technologies
- Chemical Destruction Technologies
- Biological Destruction Technologies
- Vitrification

Destruction technologies are advantageous because pesticides are removed permanently by reducing or eliminating toxicity and mobility of contaminants.

Treatment trains for ex-situ applications typically contain several material handling steps (e.g., excavation, dewatering, dredging, conveying, and screening) that are required to prepare and deliver the contaminated media for destruction treatment. Separation/concentration of the contaminants may be required as an initial pretreatment to increase the treatment effectiveness of some destruction technologies or reduce the total volume of materials to be treated [14].

For in-situ bioremediation and chemical treatment, the media may need to be plowed periodically to ensure aeration and/or proper contact between the contaminants and the reactants. In-situ treatment requires proper drainage and recirculation systems to ensure continuous contact between the contaminants and the reactants.

Biological Destruction Technologies

In microbial destruction technology, microorganisms are used to convert the organic contaminants into the simpler and less toxic products in the presence of oxygen and nutrients. In some cases, adding of microbial culture can be necessary if the native media does not contain sufficient amount of microbes. The biological treatment process can be performed by ex-situ or in-situ.

a. Ex-Situ Bioremediation

Ex-situ bioremediation process can be applied by following types:

Slurry-phase Bioremediation

In this process, excavated soil or sludge are mixed with water in a reactor to create slurry, which is agitated mechanically. Some parameters such as pH, oxygen and temperature are controlled and if necessary nutrients are added to reactor. This type of bioremediation is suitable for high concentration of organic contaminants in soil and sludge. However, inorganic contaminants or pesticides containing inorganic compounds can hinder microbial activity. In this case, stabilization may be necessary for suitable treatment. Depending on the contaminant characteristics, air pollution control measures may be necessary.

Solid-phase Bioremediation

In this process excavated soil or sediments are treated without the addition of water. This type of bioremediation can be performed by two forms; landfarming and composting.

In landfarming, contaminated soil is placed in a lined bed to which nutrients are added. This process has been widely used technologies. The bed is covered with clay and plastic liners, furnished with irrigation, drainage, and soil-water monitoring systems. Composting process depends on mixing of contaminated soil with a bulking agent (wood chips, straw, bark, manure), pilling and aerating in a contained system. Carbon additives provide a source of metabolic heat. However, this process has some disadvantages in that bulking agents added to the system cause to increase the volume of treated material. Irrigation techniques can optimize moisture for biological growth and an enclosed system accomplishes volatile emission control.

b. In-Situ Bioremediation

In-situ bioremediation of soil, groundwater and sediments aims at the stimulation of the biological degradation of the contaminants in the subsurface environment. Usually a recirculation system for ground water is installed. Contaminated groundwater is treated above ground, after which oxygen and, if necessary, nutrients are added to the water that infiltrates the soil, in order to stimulate the indigenous microorganisms to degrade contaminants.

In this technology, proper liquid drainage collection and a recirculation system are required to ensure proper contact as well as sufficient aeration to support aerobic microbial growth. Figure 2.1 presents a schematic diagram of an in-situ biodegradation process.

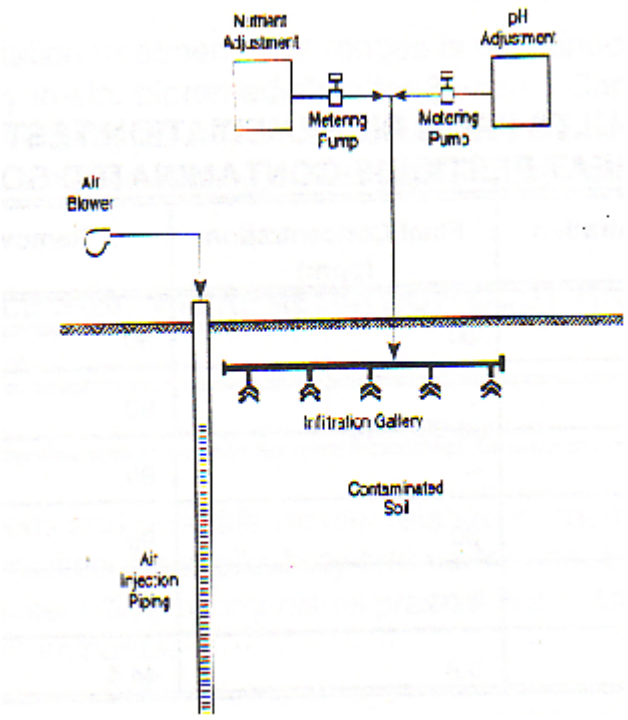


Figure 2.1 Schematic for in-Situ bioremediation using injection for pesticide-contaminated soils.

The advantage of this technology is that this process can destroy organic contaminants in place without the high costs of excavation and materials handling under appropriate conditions. It can also diminish the release of volatile contaminants into the air. However, in-situ bioremediation process normally requires time to accomplish remediation goals. The technology is applicable for soil, sediments, sludges contaminated with organic pesticides

2.3.1.3 Separation/Concentration Technologies

These technologies use physical and chemical processes to separate contaminants from their media matrix for further treatment and possibly to reduce the volume of contaminated materials. These technologies do not alter the fundamentals nature of the contaminant toxicity or mobility, but rather collect contaminants into the concentrated form and smaller volume or transform them into a different medium (such as by soil washing) that is easier to handle for further treatment and disposal. Typically,

separation/concentration technologies prepare pesticides for further remediation by destruction or immobilization technologies.

Remediation strategies for pesticide-contaminated sites may incorporate several distinct technologies assembled into a treatment train to attain specific site cleanup goals. Combining technologies sequentially or in parallel is often the best way to achieve site-specific objectives and acceptable residual contaminant levels.

The Separation/Concentration techniques are mass transfer processes that are necessary to produce isolated or concentrated streams that can be treated by destruction or immobilization technologies. These technologies are capable of limiting environmental mobility of pesticide contaminants by separating the toxic components into a controlled phase for further management; however, no destruction or reduction of toxicity is attained. The Separation/Concentration technologies for potential remediation of pesticide-contaminated soils, sludges and sediments can be classified as follows:

In-Situ Technologies:

- Soil Flushing
- Soil vapor Extraction (SVE)
- Stream Extraction
- Radio Frequency (RF) Heating

Ex-Situ Technologies:

- Soil Washing
- Thermal Desorption
- Solvent Extraction

The decision to select and implement separation/concentration techniques for remediation of soils, sludge and sediments rests primarily on action levels established for the site, acceptable residuals management and further need for treatment of concentrated pesticide wastes.

Chapter 3

SELECTED PESTICIDES: ENDOSULFAN AND TRIFLURALIN

3.1 Pesticide Selection Criteria

There are some criteria for selection of pesticides.

These are;

1. Quantities applied in Turkey
2. Toxicity
3. Low biodegradability (long half-life)
4. Octanol-water partition coefficient (Kow)
5. Analytical method

Moderate Kow values are considered in the selection of pesticides. Because high Kow value represents strong adsorption of pesticide by soil. This means that the probability of leaching of pesticide into the groundwater will be less as compared to the pesticide having low Kow value. Due to its strong adsorption property to soil, there will be less chance to volatilize to atmosphere and leaching to surface water. On the other hand, pesticides having low Kow values show a great tendency to leach to the groundwater.

In this study, endosulfan and trifluralin were selected as pesticide. Trifluralin is the most widely used pesticide in Turkey. It is adsorbed by soil strongly. Additionally, due to its long half- life, it has a higher potential of reaching surface or groundwater because it is exposed to the hydrologic forces for a longer period of time. This pesticide is more susceptible to surface loss.

Endosulfan is also widely used pesticide in Turkey. Endosulfan, due to its persistence, has a higher potential for leaching to groundwater as compared to trifluralin.

3.2 Endosulfan

3.2.1 General Information

Endosulfan is a chlorinated hydrocarbon insecticide of the cyclodiene subgroup which acts as a contact poison in a wide variety of insects and mites. It can also be used as a wood preservative. It is used primarily on food crops like tea, fruits, vegetables and on grains. Technical endosulfan is a mixture of endosulfan isomers (80% α-isomer/ 20% β-isomer)

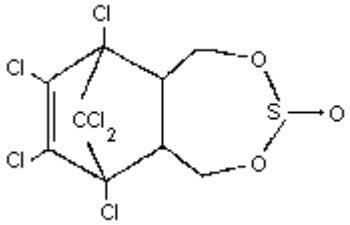
Formulations of endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid, and smoke tablets.

3.2.2 Toxicological Effects

Endosulfan is a highly toxic substance and carries the signal word DANGER on the label. Undiluted endosulfan is slowly and incompletely absorbed into the body whereas absorption is more rapid in the presence of alcohols, oils and emulsifiers.

Stimulation of the central nervous system is the major characteristic of endosulfan poisoning [17].The oral LD50 in rats ranges from 18 - 220 mg/kg. Some other oral LD50 values are: mice 7.36 mg/kg, hamsters 118 mg/kg, cats 2 mg/kg, and dogs 76.7 mg/kg. The dermal LD50 for rats is 74 mg/kg while for rabbits figures from 200 to 359 mg/kg are recorded. As noted before, the solvents and emulsifiers used to dissolve endosulfan influence its toxicity. Rats have an inhalation LC50 of 8.0 mg/m3 for four hours. Dogs are less tolerant than rats to this compound and rats are nearly twice as susceptible to endosulfan when they have been deprived of protein.

Table 3.1 Chemical and physical properties of endosulfan

Chemical Name:	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide
Empirical formula:	$C_9H_6Cl_6O_3S$
Structure:	
Rel. molecular mass:	406.95 g
Density:	1.735 g/cm^3 at 20°C
Relative gas density:	14.1
Boiling point:	106°C at 0.9 hPa (partial decomposition)
Melting point:	Technical 70-100 α -isomer 108-109°C β -isomer 206-208°C
Log Koc	3.31
Log Kow	3.55
Henries Law Constant	1.01 x 10^{-4} atm.m^3/mol at 25°C
Vapour pressure:	< 1 x 10^{-3} Pa
Solubility:	in water 1.4 mg/l; in toluene 20 g/100 g; in hexane 2.4g/100g in benzene 33g/l; in xylene 45g/l; in chloroform 50g/l; in methanol 11 g/l.

Chronic toxicity: In rats, oral doses of 10 mg/kg/day caused high rates of mortality within 15 days, but doses of 5 mg/kg/day caused liver enlargement and some other effects over the same period [30]. This dose level also caused seizures commencing 25 to 30 minutes following dose administration that persisted for approximately 60 minutes [30]. There is evidence that administration of this dose over 2 years in rats also caused reduced growth and survival, changes in kidney structure, and changes in blood chemistry [30, 42].

Carcinogenic effects: There are no reports of cancer in humans exposed to endosulfan. The EPA has placed endosulfan in the "not classifiable" category due to the lack of data on its carcinogenicity.

Fate in humans and animals: Endosulfan is rapidly degraded and eliminated in mammals with very little absorption in the gastrointestinal tract. Cattle fed 0.15 mg/kg for 60 days had no residues in the fat. The metabolite, endosulfan sulfate, seems to show similar acute toxicity to the parent compound. The beta isomer is cleared from blood plasma more quickly than the alpha isomer. Most of the endosulfan seems to leave the body within a few days to a few weeks.

3.2.3 Environmental Fate

3.2.3.1 Breakdown in Soil and Groundwater

Endosulfan is moderately persistent in the soil environment with a reported average field half-life of 50 days [30]. The compound is broken down in soil by fungi and bacteria [17]. Endosulfan does not easily dissolve in water, and has a very low solubility [17, 30]. It has a moderate capacity to adhere or adsorb to soils [30]. Transport of this pesticide is most likely to occur if endosulfan is adsorbed to soil particles in surface runoff. It is not likely to be very mobile or to pose a threat to groundwater. It has, however, been detected in California well water [33].

3.2.3.2 Breakdown in Water

In raw river water at room temperature and exposed to light, both isomers disappeared in four weeks. A breakdown product first appeared within the first week. The breakdown in water is faster (5 weeks) under neutral conditions than at more acidic conditions (5 months) [33]. Under strongly alkaline conditions the half-life of the compound is one day. Large amounts of endosulfan can be found in surface water near areas of application [42]. It has also been found in surface water throughout the country at very low concentrations [33].

Endosulfan and endosulfan residues have been found in numerous food products at very low concentrations. They have been detected in vegetables (0.0005 - 0.013 ppm), in tobacco, in various seafoods (0.2 ppt - 1.7 ppb), and in milk.

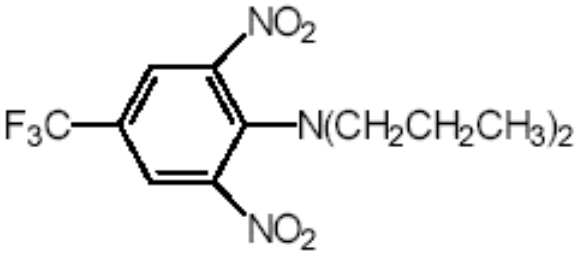
3.3 Trifluralin

3.3.1. General Information

Trifluralin is a selective, pre-emergence dinitroaniline herbicide used to control many annual grasses and broadleaf weeds in a large variety of tree fruit, nut, vegetable, and grain crops, including soybeans, sunflowers, cotton, and alfalfa. Pre-emergence herbicides are applied before weed seedlings sprout. Trifluralin should be incorporated into the soil by mechanical means within 24 hours of application. Granular formulations may be incorporated by overhead irrigation.

Formulation: Granular formulations may be incorporated by overhead irrigation. Trifluralin is available in granular and emulsifiable concentrate formulations. The technical material is approximately 96% pure and the emulsifiable concentrate is about 45% pure.

Table 3.2 Physical and chemical properties of trifluralin

Chemical Name:	a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine [1]
Empirical formula:	$C_{13}\ H_{16}\ F_3\ N_3\ O_4$
Structure:	
Rel. molecular mass:	335.50
Density:	1.294 at 25^{o}C
Boiling point:	139-140 degrees C (282-284 degrees F) at 4.2 mm Hg; 96-97 degrees C at 0.18 mm Hg
Melting point:	48.5-49°C
Log Koc	2.94-4.49
Log Kow	5.07, 5.28
Henries Law Constant	4.84 x 10^{-5} atm.m^3/mol at 23°C
Vapour pressure:	13.7 mPa @ 25 C
Solubility:	Water < 1 mg/L Acetone > 50 g/100 ml Methanol 2 g/100 ml Xylene 81 g/100 ml

3.3.2 Toxicological Effects

Acute toxicity: Pure trifluralin is practically nontoxic to test animals by oral, dermal, or inhalation routes of exposure. The oral LD50 for technical trifluralin in rats is greater than 10,000 mg/kg, in mice is greater than 5000 mg/kg, and in dogs, rabbits, and chickens, is greater than 2000 mg/kg. However, certain formulated products that contain trifluralin may be more toxic than the technical material itself. For example, the oral LD50 for Treflan TR-10 in rats is greater than 500 mg/kg. The dermal LD50 for technical trifluralin in rabbits is greater than 2000 mg/kg. The 1-hour inhalation LC50 for technical trifluralin in rats is greater than 2.8 mg/L [48]. Nausea and severe gastrointestinal discomfort may occur after eating trifluralin. Trifluralin does not cause skin irritation. When applied to the eyes of rabbits, trifluralin produced slight irritation, which cleared within 7 days. Skin sensitization (allergies) may occur in some individuals. Inhalation may cause irritation of the lining of the mouth, throat, or lungs.

Chronic toxicity: Prolonged or repeated skin contact with trifluralin may cause allergic dermatitis. The administration of 25 mg/kg/day to dogs for 2 years resulted in no observed toxicity [48]. In another study of beagle dogs, toxic effects were observed at 18.75 mg/kg/day. These included decreased red blood cell counts and increases in methemoglobin, total serum lipids, triglycerides, and cholesterol [43]. Trifluralin has been shown to cause liver and kidney damage in other studies of chronic oral exposure in animals.

Carcinogenic effects: In a 2-year study of rats fed 325 mg/kg/day, the highest dose tested, malignant tumors developed in the kidneys, bladder, and thyroid [44]. However, more data are needed to characterize its carcinogenicity.

Fate in humans and animals: Trifluralin is not readily absorbed into the bloodstream from the gastrointestinal tract; 80% of single oral doses administered to rats and dogs were excreted in the feces.

3.3.3 Environmental Fate

3.3.3.1 Breakdown in Soil

Trifluralin is of moderate to high persistence in the soil environment, depending on conditions. It is strongly adsorbed on soil and shows negligible leaching. Organic matter and clay content of the soil influence the application rate necessary for herbicidal activity.

Trifluralin is subject to degradation by soil microorganisms. Trifluralin remaining on the soil surface after application may be decomposed by UV light or may volatilize. Reported half-lives of trifluralin in the soil vary from 57 to 126 days to 6 to 8 months [10, 21]. After 6 months to 1 year, 80 to 90% of its activity will be gone. It is strongly adsorbed on soils and nearly insoluble in water. Because adsorption is highest in soils high in organic matter or clay content and adsorbed herbicide is inactive, higher application rates may be required for effective weed control on such soils [48].

3.3.3.2 Breakdown in Water

Trifluralin is nearly insoluble in water [21]. It will probably be found adsorbed to soil sediments and particulates in the water column.

Chapter 4

BIOSURFACTANTS

4.1 BACKGROUND AND LITERATURE REVIEW

Contaminated soil and sediment system often contain hydrophobic organic compounds (HOCs), which were introduced into the environment through industrial discharges, agricultural uses, or improper waste disposal practices. The prevalence and persistence of these chemicals in the environment pose a chronic threat to the health and safety of humans and wildlife.

Long-term persistence of these materials in soils is directly related to poor mobility of the contaminants and to resistance of the contaminant to microbial degradation. Many of these organic contaminants are sorbed onto clays or organic matter in soils. Through a combination of sorption processes, the contaminant may move deep into soil pores and/or clay mineral lattice structures, effectively immobilizing the contaminant. Inability of sorbed contaminants to partition back into the aqueous phase severely limits microbial degradation of contaminants in soil treatment systems. Correspondingly, effective biotreatment for those compounds is impaired because the bacteria are unable to contact the sorbed compound. As a result of these processes, immobilization is a significant problem to overcome in site restoration.

Accidental and intentional release of hazardous wastes threatens environmental sustainability and human health. In most regions in the world have many industrial centres where accidental or intentional releases of hazardous substances to soils and subsurface environments are common. As a result the region has numerous sites that require cleanup of soils and aquifers under various federal and state programs. Many of the contaminated sites in these regions are located in areas that have shallow water tables and course-textured, permeable soils making the groundwater more susceptible to contamination. Although the capacity of soils to detoxify waste has been well documented, this capacity is limited however, and natural detoxification processes often require years to restore impacted sites. In the United States alone, it has been estimated that hazardous waste site restoration costs may approach 1.7 trillion dollars over the next 30 years. These estimates have raised serious concerns regarding the ability to pay for site restoration. Yet in the U.S, 40 million people live within 6.5 km of a

contaminated soil site. Therefore, it is likely that support will continue to grow for site clean-up and restoration. Consequently it is imperative that less expensive and more efficient remediation approaches be developed.

One of the important problems about contaminated land is pesticide pollution. Concerns about pesticide pollution have prompted global efforts to find alternative biological control technologies.

Biological treatment methods have been often considered as the most complete, environmentally acceptable and cost-effective treatment options. The presence of refractory or toxic pollutants in the soil or water often hinders treatment of these wastewater and soil through biological processes. These contaminants are often co-metabolised, thus not completely mineralised due to their low aqueous solubilities, and strong sorption properties. Low dissolution rates, which limit the bioavailability of these compounds to degradative organisms, and toxicity, directly inhibiting biodegradation, extend the persistence of these pollutants.

Surfactants constitute an important class of industrial chemicals that are widely used in almost every sector of modern industry. However, very few studies have addressed the effects of biosurfactants on the bioavailability of soil-sorbed substrates. Biosurfactants may influence these systems in several ways. First, soil solution biosurfactant concentrations above the critical micelle concentration (CMC) may enhance the overall rate of nonpolar organic compounds (NOC) degradation by: 1) enhancing the apparent solubility of NOC resulting in higher aqueous phase concentrations and thus higher rates of degradation, 2) altering the distribution of the contaminant between sorbed and solution phases, or 3) enhancing the mass transfer rate of the contaminant from the sorbed to the solution phase. Alternatively, if the micelle-associated contaminant is inaccessible to microorganisms, if the biosurfactant is toxic, or if the biosurfactant is preferentially degraded, then reduced NOC biodegradation maybe observed. Preliminary experiments have shown that treholose micelle-water partition coefficients for toluene, xylene, and trimethyl benzene were higher than those observed for soil organic matter. Therefore, it is anticipated that the presence of biosurfactant will enhance the overall rate of NOC biodegradation via enhanced desorption [37]. Once this has been demonstrated at the laboratory scale, the results of this research will provide the basis for developing economically and technically feasible remediation techniques based on flushing the contaminated area with biosurfactant or stimulating biosurfactant production in situ. The proposed experiments are

comprehensive and will provide sufficient information to elucidate the mechanisms responsible for surfactant-enhanced NOC biodegradation, ultimately leading to the development of improved bioremediation strategies. The interdisciplinary nature of the research requires expertise in transport phenomena, surface chemistry, microbiology, organic chemistry, and environmental engineering.

A number of studies conducted to investigate the ability of surfactants to enhance the recovery of organic compounds from the soils however, there is no enough project, research report, article or other types of publications containing the pesticide removal in the presence of biosurfactants.

There are some case studies about the effect of surfactants on the hydrophobic organic compound (HOC) solubilization and desorption. Many of the early surfactants have been examined for the capacity of micellar solutions to solubilize strongly-sorbed contaminants, such as polychlorinated biphenyls (PCBs) and polycylic aromatic hydrocarbons (PAHs), in either batch (soil washing) or column studies (surfactant flushing).

Liu et. al. (1991) tested six nonionic and two anionic surfactants on their ability to solubilize PAHs, (anthracene, phenanthrene, and pyrene) in soil-water suspension. The anionic surfactants (a lignin sulfonate and a dodecyl benzene sulfonate) as well as the polyoxyalkylated fatty acid esters (a nonionic) were poor solubilizer of the three PAHs tested. Most of the nonionic surfactants performed well. The dodecylethoxylate (Brij-30), the octylphenylethoxylates (lgepal CA-720 and Trion X-100), and the nonylphenylethoxylate (Hyonic NP-90) at concentration of 1% solubilized more than 56% of the phenanthrene added to soil. However, it should be noted that these experiments involved soils that were spiked with the PAHs in the laboratory, and the outcome may have been different if an actual environmental soil sample (in which the contaminant has aged for extended periods) had been utilized.

Scheibenbogen et. al. (1994) examined the use of extracellular biosurfactants produced by *Pseudomonas aeruginosa* UG2 to enhance washing of hydrocarbons in soil columns. The results showed that, UG2 biosurfactants effectively removed both aliphatic and aromatic hydrocarbon mixture (pentadecane, hexadecane, octadecane, pristane, naphthalene, phenanthrene and pyrene) from unsaturated soil columns. The total hydrocarbon removed by UG2 biosurfactant solutions ranged from 23 to 59 %, with increase in removal being a function of higher surfactant concentrations.

Bai, Brusseau and Miller have also investigated the potential of an anionic monorhamnolipid biosurfactant produced by *P. aeruginosa* to remove residual hexadecane from sand columns by flushing process. The CMC of rhamnolipid has been determined as 50 mg/L from a plot of surface tension vs. biosurfactant concentration. The solubility of hexadecane has been found to increase in the presence of varying concentration of rhamnolipid. Of the rhamnolipid concentrations tested, which ranged from 40 to 1500 mg/L, the optimal concentration for residual removal was 500 mg/L, approximately ten times the critical micelle concentration (CMC). The recovery of hexadecane from column packed with larger diameter (20/30 mesh) has been much higher (approximately 84 % after 120 pore volumes) than recovery from the 40/50 mesh sand column (22%)

Deitsch and Smith (1995) examined the use of Trion X-100 to enhance the desorption of trichloroethylene from contaminated field samples and concluded that the surfactant was able to enhance desorption by both increasing the concentration gradient at the solid-liquid boundary (through solubilization of the contaminant) and by increasing the mass transfer coefficient between the solid and aqueous phases.

In addition to HOC solubilization and desorption, there are also several studies to examine the use of surfactants to aid the biodegradation of sorbed-phase contaminants through bioavailability enhancement. Some researchers have reported success while others reported either no enhancement or even inhibition in the presence of surfactants.

Laha and Luthy (1991) studied the effect of nonionic surfactants on bioavailability enhancement for PAH degradation in soil-water slurries. The researchers found that surfactants at low concentrations did little to enhance the rate of degradation, while higher concentrations (500 to 1000 mg/L) were inhibitory to phenanthrene mineralization. The inhibitory effect was, however, reversible upon dilution of the surfactant. Several possibilities were considered for the inhibitory effect of surfactant, including surfactant toxicity, reduction of free PAH concentration in the aqueous phase, preferential use of the surfactant over the PAH as a substrate, interference of micelles with cell activity, and limited bioavailability of micellized PAHs. Subsequent investigation prompted Laha and Luthy (1992) to speculate that the inhibitory effect was not due to surfactant toxicity but rather due to the phenanthrene being unavailable for degradation. They concluded that the observed inhibition was not so much due to PAH micellar exit rate limitation but rather due to surfactant-bacteria interactions.

Although the nonionic surfactants are less inhibitory to bacterial cells than their ionic counterparts, high concentrations of surfactants can potentially interfere with microbial metabolism.

Aronstein *et al.* (1991) and Aronstein and Alexander (1992) examined the effect of nonionic ethoxylate alcohol surfactants (Alfonic 810-60 and Novel II 1412-56) at low concentrations (sub-CMC) on the desorption and biodegradation of phenanthrene and biphenyl from soils containing 8% and 33% organic matter. The soil samples were not obtained from a contaminated site; rather, the target compounds were added to clean soils in the laboratory. Both surfactants increased the desorption rate of phenanthrene but did not affect the desorption of biphenyl. Yet, both surfactants enhanced the aerobic biodegradation rate of both contaminants. From these results, the researchers concluded that: 1) surfactant concentrations that are too low or too high can either fail to increase desorption or actually decrease the desorption rate; high surfactant concentration can also impede biodegradation due to toxicity effects; and 2) as evident from the biphenyl experiments, biodegradation rate can be enhanced even though the surfactants failed to increase the equilibrium aqueous-phase concentration of biphenyls through increased desorption. These findings suggest that, through biological interactions, surfactants at low concentration may promote contaminant biodegradation even though desorption may not be appreciable. The advantages for using surfactants at low concentrations include lower cost, reduced microbial inhibition by the surfactant, and a lower oxygen demand exertion due to surfactant biodegradation.

Awasthi and Kumar (1999) studied the biodegradation of soil-applied endosulfan in the presence of a lipopeptide biosurfactant identified as surfactin. In this study biodegradation of endosulfan isomers in soil-applied and flask-coated conditions was investigated, by an isolated bacterial coculture. Biosurfactant was prepared from a strain of Bacillus subtilis (MTCC 1427) coculture. Results showed that biodegradation of endosulfan isomers by the isolated bacterial coculture was enhanced in the presence of biosurfactants. At the end of the study, alpha and beta endosulfan were degraded by 75 % and 68 %, respectively in 20 days. Addition of biosurfactant to the incubation mixture also increased the rate of biodegradation by about 45 % and mobilized the residual endosulfan towards complete degradation. Nevertheless, parallel controls, with or without bacteria/surfactant adding, did not demonstrate any degradation of both isomers. In addition to flask-coated conditions degradation of alpha and beta endosulfan in soil-applied form was 62 % and 45 %, respectively. These results have shown that

addition of biosurfactant lead to an enhanced degradation of endosulfan isomers in both soil- applied and flask-coated conditions. Moreover, flask-coated conditions have found 20-30 % more effective than soil-applied form in biodegradation of endosulfan.

Another study was examined by Kewin and Robinson (1996) about the mineralization enhancement of non-aqueous phase and soil-bound PCB using biosurfactant. In this study the impact of a biologically produced surfactant (rhamnolipid RI) on the mineralization of a target PCBs (4,4-chlorobiphenyl) and bioavailability of non-aqueous and soil-bound phases upon biosurfactant treatment was evaluated. In order to enhance the mineralization of PCBs, culture of Alcaligenes eutrophus A5 was prepared. Study was performed in closed vessels containing biosurfactant having a CMC of 54 mg/L and cell suspension. Four surfactant concentrations (4.0, 1.0, 0.2, 0.02 g/L) were used. It was found that high biosurfactant concentrations (above CMC), the mineralization of PCB has been higher than those at or below the CMC. In addition, the solubility of 4,4'CB has been shown to increase in the presence of varying concentration of rhamnolipid, the average mineralization rate of 4.4'CB was 45 time in comparison to that measured in controls which did not contain biosurfactant. Elevated mineralization arisen from aqueous solubility enhancement of the PCB in the presence of biosurfactant. These results showed that addition of biosurfactant followed by pure culture in biological treatment was a promising technique for the removal of non-aqueous phase and soil-bound PCBs.

4.2 General Classification of Surfactants

After improving in microbial synthesis of biosurfactants, surfactants can be classified in two groups;

1. Chemically synthesized surfactants- (chemical/synthetic surfactants)
2. Microbial surfactants-biosurfactants

4.2.1 Synthetic Surfactants

There are thousand of surfactants in use commercially, however, the majority of them have common structural features and can be divided into three main categories depending on the charge of the polar head group (cationic, anionic, or nonionic). The hydrophilic portion of a surfactant may ionise or it may not. Surfactant molecules having ionizing hydrophilic portions are ionic surfactants, however those having non-ionizing hydrophilic portions are called as non-ionic surfactant. Polar group of non-ionic surfactant molecules is not electrically charged. An ionic surfactant molecule that can dissociate to yield a surfactant ion, whose polar groups is negatively charged, is called as anionic surfactant; and that whose polar group is positively charged is known as cationic surfactant. However, a surfactant molecule that may contain both negatively and positively charged groups and the ionic character of the polar group of the surfactant molecules depends on solution pH, these types of surfactants are known as zwitterionic or amphoteric surfactant [26].

Only about 10% of commercially used surfactants are cationic, most of which are quaternary ammonium compounds (general structure: R_4N^+). Polyamines and their salts, quaternary ammonium salts, and amine oxides are examples of cationic surfactants. Cationic surfactants tend to be toxic and are therefore not widely used in environmental applications. Cationic surfactants tend to sorb to anionic surfaces and so can be severely retarded in groundwater systems.

Anionic surfactants represent the major fraction of the surfactants used commercially today. Common hydrophilic functional groups are sulfonate $(-SO_3)^-$,

sulfate $(-OSO_3)^-$, and carboxylate $(-CO_2)^-$. Sulphonic acid salts, alcohol sulfates, alkylbenzene sulphonates, phosphoric acid esters, and carboxylic acid salts are some examples of anionic surfactants.

Nonionic surfactants represent about one-third of the surfactants in use commercially ($2.73 billion in 1986) [6]. Nonionic surfactants tend to be good solubilizers and are relatively nontoxic. They are usually easily blended with other types of surfactant (i.e., used as cosurfactants) and therefore have found widespread use in petroleum and environmental applications. Examples of nonionic surfactants include polyoxycthylenated alkylphenols, alcohol ethoxylates, alkylphenol ethoxylates, and alkanolamides. Nonionic surfactants have specific advantages over anionic or cationic surfactants in remediation of contaminated soils and sediments due to desirable properties in terms of surfactant charge, micellarization behavior, toxicity, and biodegradability [11].

4.2.2 Microbially Produced Surfactants

In addition to synthetic surfactants formulated for specific commercial application, surfactants are also naturally produced. Many natural organic acids, such as humic and fulvic materials, are surface active and have foaming capabilities. A variety of microorganisms produce biosurfactants, or extracellular secretion with surfactant properties, enabling them to emulsify and uptake substrate (e.g., petroleum related products) which do not readily solubilize in aqueous solutions [9].

Many bacteria, yeast, and fungi produce extracellular or membrane-associated surface active compounds called biosurfactants [16, 2, 23, 26].

Biosurfactant molecules can be either cell wall-associated or extracellular. They can promote cellular attachment to hydrophobic surfaces, affect the distribution of cells between oil and water phases, emulsify water-insoluble substrates, and mediate transport of hydrophobic substrates into the cell. Production of biosurfactants is enhanced by growth of the microorganism on certain water-insoluble substrates such as alkane hydrocarbons and vegetable oils. Biosurfactant synthesis can also be influenced by other environmental conditions such as low availability of nitrogen or divalent cations [28, 41].

The enormous market demand for surfactant is currently met by numerous synthetic, mainly petroleum based, chemical surfactants. These compounds are usually

toxic to the environment and non-biodegradable. They may bio-accumulate and their production, process and products can be environmentally hazardous. Tightening environmental regulations and increasing awareness for the need to protect the ecosystem have effectively resulted in an increasing interest in biosurfactant as possible alternatives to chemical surfactants. Biosurfactants are amphiphilic compound of microbial origin with considerable potential in commercial applications with in various industries due to their low toxicity, biodegradable nature, diversity and effectiveness at extreme temperature, pH, and salinity.

4.2.2.1 Classification of Biosurfactants

Biosurfactants can be classified into five groups:

1. Glycolipids, e.g. threalose, sophorose and rhamnose lipids and mannosylerithritol lipids. They are involved in the uptake of low polarity hydrocarbons by microorganisms.
2. Liposaccharides, e.g. the high molecular weight, water-soluble extracellular emulsifiers produced by hydrocarbon degrading bacteria like Acinetobacter calcoaceticus (emulsans).
3. Lipopeptides, e.g. ornithine lipids and the subtilysin produced by Bacillus subtilis, claimed to be the most effective biosurfactant reported to date because of lowering the surface tension of water.
4. Phospholipids, although they are present in every microorganism, they are very few examples of extracellular production, the most notable one being the biosurfactants produced by Carynebacterium lepus.
5. Fatty acids and neutral lipids, e.g. ustilagic acid, the corynomycolic acids, the lipotheichoic acids (sometimes classified as glycolipids) and the hydrophobic proteins.

4.2.2.2 Advantages of Biosurfactants

Almost all surfactants currently in use are chemically derived from petroleum; however, interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally acceptable nature, the possibility of their

production through fermentation, and their potential applications in the environmental protection, crude oil recovery, health care and food-processing industries [26]. Biosurfactants can be produced using relatively simple and inexpensive procedures and substrates [31, 40].

Biosurfactants with surface active and emulsifying properties can exceed the performance of their surfactant synthetic equivalents in terms of efficiency. Potential environmental advantages of such biologically based surfactants include their biocompatability and hence decreased likelihood of cellular toxicity relative to synthetic surfactants. Other advantages of microbial surfactants compared with synthetic counterparts are as follows;

1. Biodegradability: Biosurfactants are biodegradable, which is a positive ecological aspect. Because of this characteristic, biosurfactants can be readily and fully degraded if released to the environment after its function is completed.

2. Having low or no toxicity: Because biosurfactants are produced by living organisms on environmentally acceptable substrates (hydrocarbons and/or carbohydrates) they are non-toxic or less toxic than chemical surfactants.

3. Acceptable production economics: At present many types of biosurfactant are being utilized but they have been unable to compete economically with their chemically synthesized counterparts in the market, due to high production costs involved. However, this problem can be overcome by improving the efficiency of current bioprocessing methodology and strain productivity, and the use of cost-effective substrates such as using sterilized or pasteurized fermentation broth without any need for extraction, concentration or purification of the biosurfactant may significantly reduce the cost of production.

4. Biocompatability: That many biosurfactants especially those produced by yeast such as sophorolipids are compatible with living tissues allow them to be used extensively in industrial application such as food processing, pharmaceuticals, and cosmetic industries.

5. Availability of raw material: Biosurfactants can be produced from cheap raw material, which are available in large quantities. The hydrophilic and hydrophobic moieties of biosurfactants are synthesized by two metabolic pathways: the hydrocarbon, carbohydrates and/or lipids. These pathways constitute carbon source and may be used separately or in combination with each other.

Because industrial and municipal wastewaters contain organic pollutants, they can be utilized as substrate for the production of biosurfactants: With the use of wastewaters as organic matter source, a double benefit is expected:

a. The wastewaters utilized for the biosurfactant production is treated.
b. Valuable product is emerged.

According to Kosaric, another alternative for cheaper production of biosurfactants is to use municipal waste sludge as substrate in an anaerobic treatment process, followed by partial hydrolysis of anaerobic sludge on which lipogenic microbes can be grown.

With the use of waste organic pollutants as substrate in the production of biosurfactant, several advantages can be achieved. These are followed as;

1. In-situ production of biosurfactant is possible
2. Only one feedstock is used (i.e.; municipal waste sludge)
3. The feedstock is available year-around
4. Energy requirements can be met by the production of methane
5. Process is relatively simple [31].

6. Use in the environmental control: Due to their environmental friendly composition biosurfactants are considered as a feasible approach to resolve certain environmental related problems caused by mankind. Some areas in which biosurfactants are effectively used are bioremediation of contaminated soil and groundwater, biodegradation and detoxification of industrial effluents and control of oil spills.

7. Specificity: Different biosurfactants characterized so far exhibit a rich diversity of chemical structure. Having a wide range of functional characteristics, biosurfactants are often specific in their action. Due to this property, biosurfactants have gain particular interest in detoxification of organic or inorganic contaminants, de-emulsification of industrial emulsions, and other specific food, cosmetic and pharmaceutical applications. [31].

8. Extreme temperature, pH, and salinity tolerance: Compared with synthetic surfactant, biosurfactants show stable activity under extreme environmental conditions such as extreme temperature, pH and salinity values. [41].

4.2.2.3 Production of Biosurfactants

Biosurfactants are produced by microbial biosynthesis using organic matter, containing carbon and oil sources. Most of the biosurfactants are high molecular weight lipid complexes, which are normally produced under highly aerobic conditions. The production of microbial biosurfactants can be achieved in their ex-situ production in aerated bioreactors. When their large-scale application is encountered, their in-situ production or action (production of biosurfactants in the application site directly) would be advantageous. Low oxygen availability in their in-situ production conditions requires maintenance of anaerobic microorganisms and aerobic biosynthesis of biosurfactants [31].

4.2.2.4 Application of Biosurfactants

Biosurfactants are amphiphilic compounds of microbial origin with considerable potential in commercial application with in various industries.

Biosurfactants have potential applications in agriculture, cosmetics, pharmaceuticals, detergents, personal care products, food processing, textile manufacturing, and laundry supplies. At present, biosurfactants are also used in studies on enhanced oil recovery and hydrocarbon bioremediation. The solubilization and emulsification of toxic chemicals by biosurfactants have also been reported.

Several oil spill accidents, reaching petroleum the oceans and deliberate releases of soil have caused considerable contamination. Such accidents have increased attempts to advance various chemicals, procedures and techniques for resisting oil pollution both at sea and along the shoreline. Biosurfactants are such chemicals and applied to such contaminated area due to their ability to emulsify hydrocarbons in the environment by increasing the bioavailability of the compound. Some microorganisms such as *Pseudomonas aeruginosa* SB30 is capable of hydrocarbon degradation by quickly dispersing oil into fine droplets.

4.2.2.5 Potential Limitation of Biosurfactants Applications

Existing problem about biosurfactants are related with their application areas. For environmental applications, large amount of biosurfactants is required due to the bulk use. Therefore amount of biosurfactant used can be expensive. Using non-traditional and relatively cheap raw materials for the production of biosurfactants, such as waste organic substrate, the production costs might be decreased. Another problem about biosurfactant is their purity, which is of particular importance in pharmaceutical, food and cosmetic applications [31]. This problem seems to have very slight effects on the environmental application, because biosurfactants are used as an enhancement tool in the contaminated soil and groundwater bioremediation or oil spill clean-up.

4.3 Characteristics and Functions of Biosurfactants

Surfactants are surface-active compounds capable of reducing surface and interfacial tension at the interfaces between liquid, solids and gases, thereby allowing them to mix or disperse readily as emulsions in water or other liquids [24].

The surfactant molecule is typically composed of a strongly hydrophilic (water loving) group, or moiety, and a strongly hydrophobic (water fearing) moiety [9]. The entire surfactant monomer is often referred to as amphiphilic because of its dual nature. The hydrophobic portion of the surfactant monomer is typically a long hydrocarbon chain, referred to as the “tail” of the molecule. The hydrophilic “head” group often includes anions or cations such as sodium, chloride, or bromide. The hydrophilic group of the surfactant monomer provides most surfactants with a high solubility in water. However hydrophobic group of the monomer prefers to reside in a hydrophobic phase as LNAPL (light non-aqueous phase liquid) or DNAPL (dense non-aqueous phase liquid). These competing effects result in the accumulation or assembling of surfactant monomers of NAPL-water interfaces, with the hydrophobic tail group embedded in the NAPL phase and the hydrophilic head group oriented toward the water phase. In addition, surfactant accumulation also occurs at water-air and water-solid interfaces.

4.4 Mechanisms of Surfactant-Enhanced Bioavailability

The biodegradation process consists of several steps (Figure 4.1). A substrate that is initially present in the soil or a porous matrix is inaccessible to microorganisms. The substrate may be adsorbed to the matrix or may be present in the liquid or solid phase. First, this substrate has to be transferred to sites where it can come in direct contact with microorganism. This can occur by desorption, dissolution or mobilization of the contaminant from the soil phase to aqueous phase and eventually by transport, i.e., convection and dispersion. Subsequently, the substrate has to be taken up by the cells and finally converted.

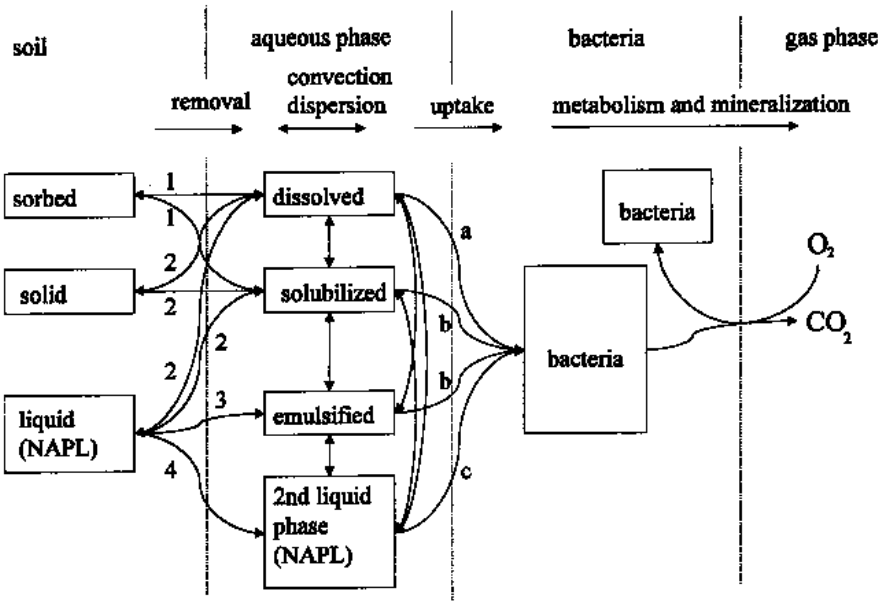


Figure 4.1 Processes involved in the biodegradation of contaminants that are initially present in soil. Processes involved in the transfer of compounds between the soil phase and the bulk aqueous phase: 1: desorption; 2: dissolution; 3: detachment; 4: mobilization. Processes involved in the uptake of contaminants by cells: a) uptake of dissolved substrate; b) uptake of 'pseudo-solubilized' substrate; c) uptake of substrate by direct attachment of the organism to substrate droplets.

Recent studies have showed that surfactant can be used to stimulate the processes that convert the contaminants into more available form for the microorganisms. These studies on the application of surfactant technology for environmental remediation have focused on the coupled solubilization and biodegradation of HOC's (hydrophobic organic compounds). It has been proposed that surfactants may be utilized to enhance the bioavailability of strongly-sorbed compounds. From a mechanistic perspective, the presence of surfactant will increase the apparent solubility of HOC's in the aqueous phase, either through association with dissolved monomers or incorporation within the micelles, and may thereby increase the rate of dissolution/desorption or mass transfer from the solid to liquid phase. The interaction between HOC's, microorganisms, surfactant micelles, monomers, and admicelles, and the solid phase is de depicted conceptually in Figure 4.2 and 4.3 and is described below.

At concentration above the CMC, surfactant monomers aggregate to form micelles (step1). Surfactant monomers may also sorb to solids and form admicelles (single or bilayer coverages) (step 2). Sorbed-phase monomers may cause swelling of the organic and clay fractions of the solid particles and increase the rate of HOC diffusion within the solid matrix. However, adsorbed surfactants provide additional sorptive capacity to the soil, which can enhance sorption of hydrophobic compounds. This effect, known as admicellar sorption or adsolubilization, can negatively influence the amount of contaminants present in the (mobile) aqueous phase and potentially the availability of substrate to microorganisms. On the other hand, at concentrations below the CMC, sorbed surfactants may actually increase the distribution of the HOC towards the solid phase by effectively increasing the organic content of the solid-phase. In addition to sorption to the solid phase, surfactant monomers can also sorb to biomass (step 3). It has been hypothesized that the association of surfactant with cell membranes may facilitate the mass transfer of the HOC across the membrane, thus enhancing its biotransformation. At the same time, an incompatible match between the surfactant and microbial membrane, in terms of surfactant type or concentration, will have the opposite effect of causing inhibition. Lastly, surfactant monomers sorbed to the biomass may also be biodegraded.

Distribution or incorporation of aqueous-phase HOC's into the surfactant micelles leads to enhanced contaminant solubility in the bulk solution (step 4). The exchange of the HOC between the aqueous phase and the micellar pseudophase is often

considered to be very rapid, and thus equilibrium between the two phases is frequently assumed. The increased apparent solubility of the HOC in the bulk liquid-phase leads to greater driving force for the desorption of HOC from the solid-phase (step 5). Finally, sorption and partitioning of the aqueous-phase HOC to the biomass can also take place. HOCs accumulated on the microbial cells will then be transported into the cell and subsequently biotransformed (step 6).

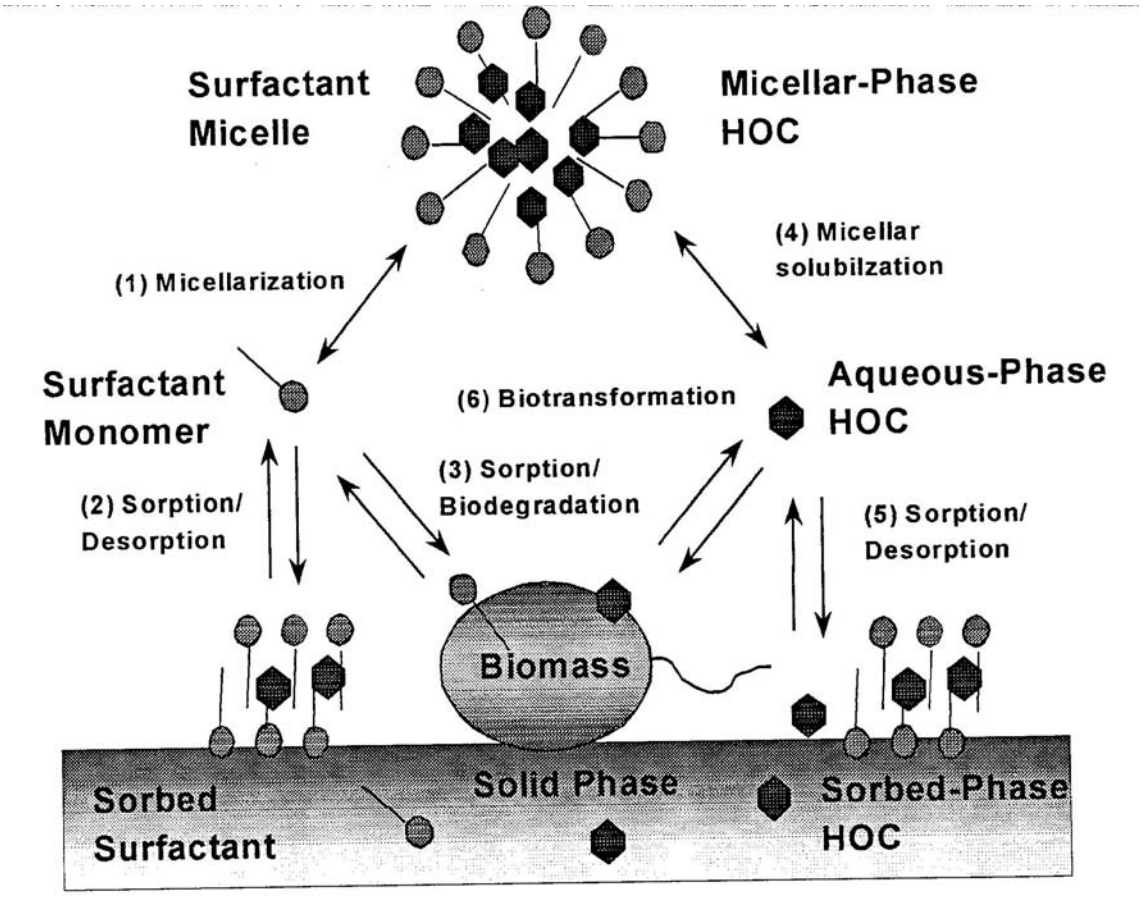


Figure 4.2 Conceptual representation of several processes and process interactions which may affect contaminant bioavailability in the presence of biosurfactant.

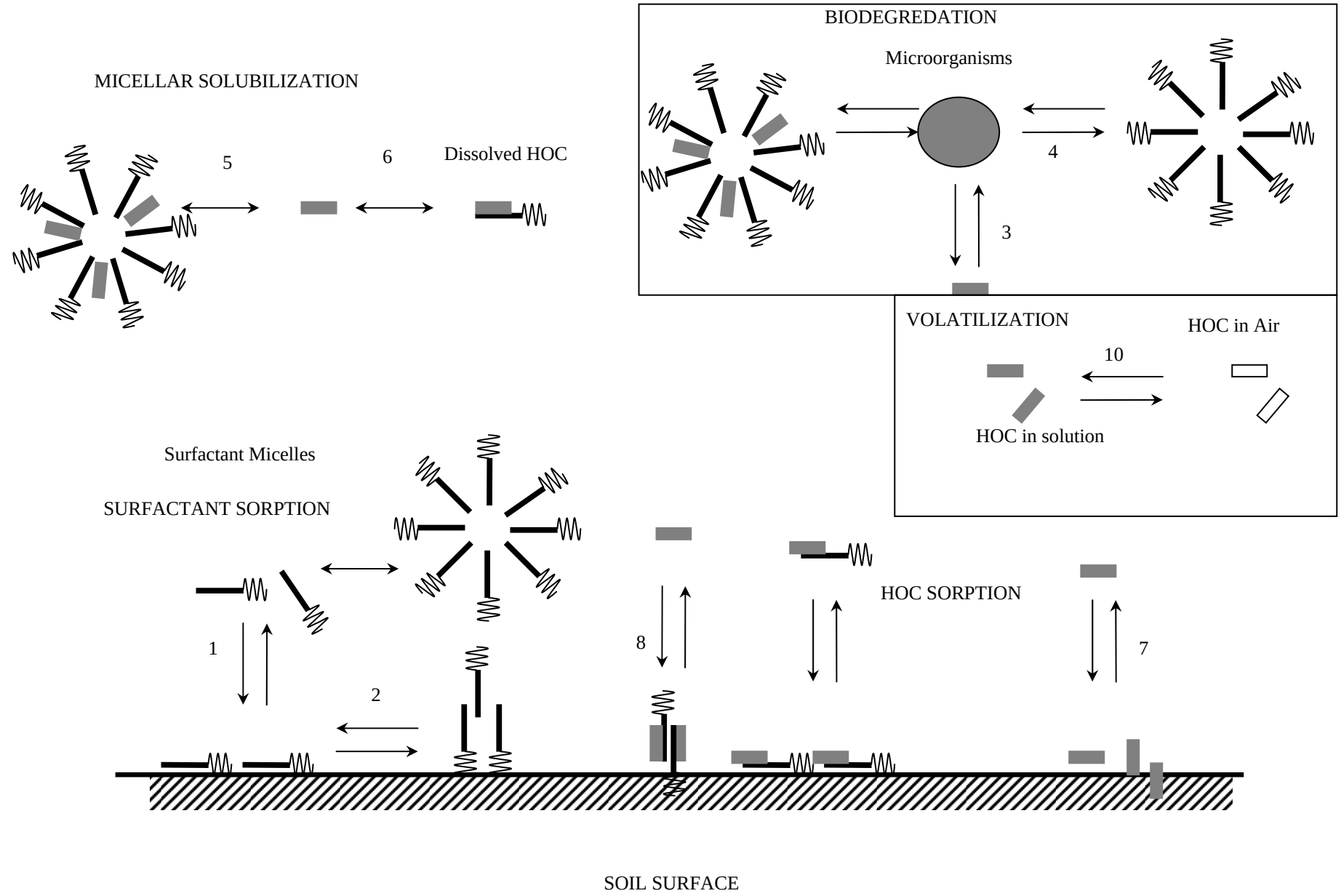


Figure 4.3 Schematics illustrating interactions between surfactant, hydrophobic organic compounds and soil in soil/aqueous systems

Chapter 5
EXPERIMENTAL STUDY

5.1 Materials

5.1.1 Soil

Soil was taken from “Ege University, Faculty of Agriculture Farm” in Menemen where investigations on the soil fertility and land use capability classes of the soils were studied. The soil sample used for thesis belonged to 36. parcel of the Agricultural Faculty Farm. Physical-chemical characteristics of the soil are given in Table 5.1

5.1.2 Pesticides

Two types of pesticides, endosulfan and trifluralin were selected as model contaminants in this study. Endosulfan and trifluralin are widely used in Turkey and they have low bioavailability due to their low solubility and high hydrophobicity. Therefore, they have potential for long term contamination.

5.1.3 Biosurfactants

Biodegradation of endosulfan and trifluralin were investigated by two experimental studies.

In the first experiments, sophorolipid type biosurfactant was utilized in order to understand its effect on the removal of endosulfan in the soil. In the second experiment, rhamnolipid type of biosurfactant called as JBR 425 was examined in terms of the degradation of trifluralin pesticide in the same soil samples.

Table 5.1 Physical and chemical properties of soil used in the experimental study (Parcel No: 36)

Profile	Depth **(cm)**	**(%) Sand**	**(%) Silt**	**(%) Clay**	**(%) Silt+Clay**	**pH[1] (25C)**	**(%) Org.C[1]**	**(%) Org. Matter[1]**	**C/N[1]**	**Cation exchange capacity (C.E.C.) (me/100 g)**
P 18	0-10	26.92	52.00	21.08	73.08	8.00	1.26	2.18	8.87	14.02

Profile	Depth **(cm)**	**(%) Field Capacity**	**(%) Wilting Capacity**	**(%) Useful Water**	**(%) Total-N[1]**	**Useful-P (ppm)[1]**	**Useful-K (ppm)[1]**
P 18	0-10	26.83	7.98	18.85	0.142	3.58	585

Sources : Investigations on the soil fertility and land use capability classes of the soils of the agricultural faculty farm-Menemen Ege University Research Fund, Research Report Proje No: 88 ZRF 05 Bornova, Izmir, 1990

1. Some values defined in the table changed with time; thus, these parameters were measured again in the beginning of the experiments.

a. General Information About Sophorolipid

In the Stuttgart University, The sophorolipid was produced from deproteinized whey, using a two-stage batch cultivation process. In the first stage, the oleaginous yeast Cryptococcus curvatus ATCC 20509 was grown on deproteinized whey concentrates (DWC). While lactose was completely consumed, biomass as well as intracellular triglyceride, a so-called single-cell oil, was produced. After cell disruption and heat sterilization, the resulting crude cell extract was directly used for growth and sophorolipid production by yeast *Candida bombicola* in the second stage.

The composition of sophorolipid can contain up to 14 different compounds [35]. Their physico-chemical and biological properties depend on the carbon sources and cultivation condition applied.

Crude sophorolipid mixtures used in the study, showed moderate to good surface active properties (STPmin 39 mN/m, CMC 130 mg/L), water solubilities (2 to 3 g/L) and low cytotoxicities (LC50 300 to 700 mg/L). Structure of sophorolipid is shown in Figure 5.1

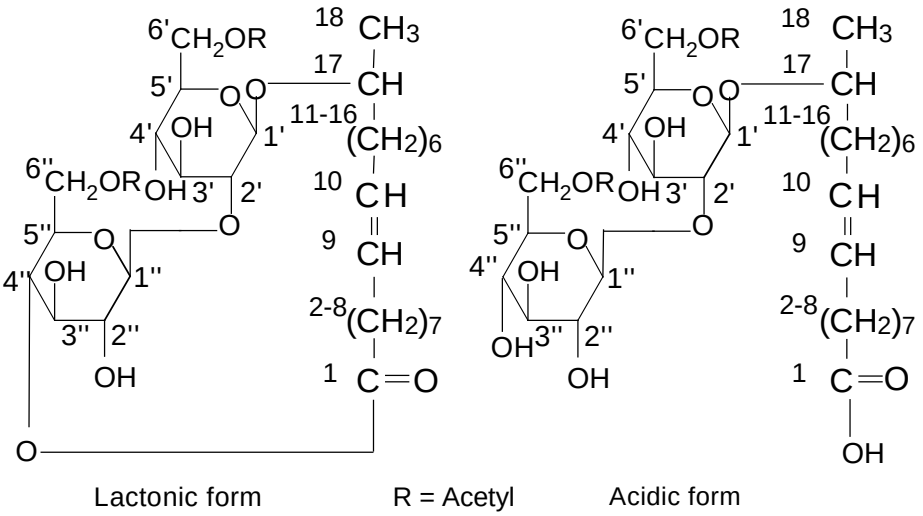


Figure 5.1 Structure classes of sophorolipids: (a) closed 1,4’’lactone form, (b) open acidic form. Main compounds in the present work are derivatives of (17-hydroxyoctadecenoic)-1’,4’’-lactone-6’6’’-diacetate sophorolipid (a).

b. General Information About Rhamnolipid

In the second part of the experiments rhamnolipid type of biosurfactant was used. The biosurfactant was obtained from Jeneil Biosurfactant Company in Saukville, WI. This product was named as JBR 425, which is an aqueous solution of rhamnolipids at 25% concentration. It was produced from sterilized and centrifuged fermentation broth that has had all protein removed and partially decolorized. Two major rhamnolipids, RLL (R1) and RRLL (R2), were present. Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and β-hydroxydecanoic acid. Other properties of JBR 425 were given in Appendix.

Structures, chemical names and molecular formulates of rhamnolipids are shown below in Figure 5.2 and 5.3 and physical and chemical properties are given in Table 5.2.

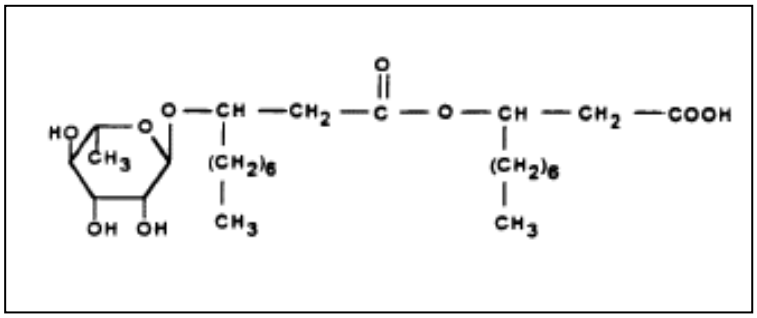


Figure 5.2 Structure of R_1 or RLL

Molecular Formula: $C_{26}H_{48}O_9$

Formal Chemical Names:

Decanoic acid, 3-[(6-deoxy-L—mannopyranosyl)oxy]-,1-(carboxymethyl)octyl ester

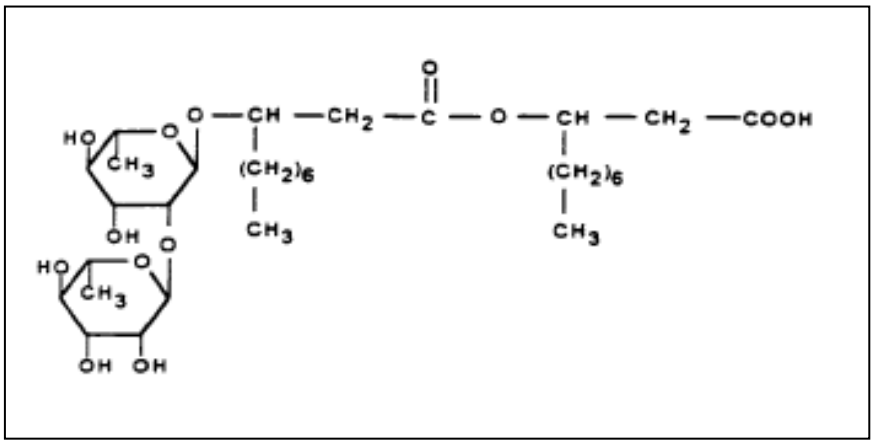


Figure 5.3 Structure of R2 or RRLL

Molecular Formula: $C_{32}H_{58}O_{13}$

Formal Chemical Names:

Decanoic acid, 3-[[6-deoxy-2-O(6-deoxy-L-mannopyranosyl)-

L-mannopyranosyl]oxy]-,1-(carboxymethyl)octyl ester

Table 5.2 Physical and chemical properties of rhamnolipid-JBR 425

TYPICAL PROPERTIES	
Appearance: Odor: Specific Gravity: PH: Solubility in Water: Suitable Diluents: Suggested Starting Concentrations:	Amber solution Soapy 1.05 – 1.06 6.5 – 7.0 Soluble at neutral pH Water, most common alcohols Active rhamnolipid ingredient: 1.0, 0.1, 0.01%

Sources: Jeneil Biosurfactant Co., JBR 425 Product Data Sheet

5.2 Experimental Methods

5.2.1 General

Experimental study was performed in two parts. In both stages, biosurfactant efficiency was investigated in terms of pesticide degradation in soil. Two different types of biosurfactants and the two, namely endosulfan and trifluralin were investigated throughout the studies.

In the first experiment soil was contaminated with endosulfan and the sophorolipid biosurfactant was added into the soil media. The temperature was kept constant by keeping columns in the incubator. In the second experiment, the removal of trifluralin pesticide was investigated in the presence of rhamnolipid type of biosurfactant. The second study was carried out in room temperature since incubator space was unavailable. Five different soil columns were prepared. The column can be seen in the Figure 5.4 and the summary of experimental study are given in Figure 5.5. Columns were made of plexiglass. The dimensions of the columns were 20 cm in diameter, 15 cm in height. Each soil column, which had porous surface at its bottom and its upper part, was open to air. Each column also contained same amount of clay particles at its bottom to supply soil with oxygen by providing porous media.

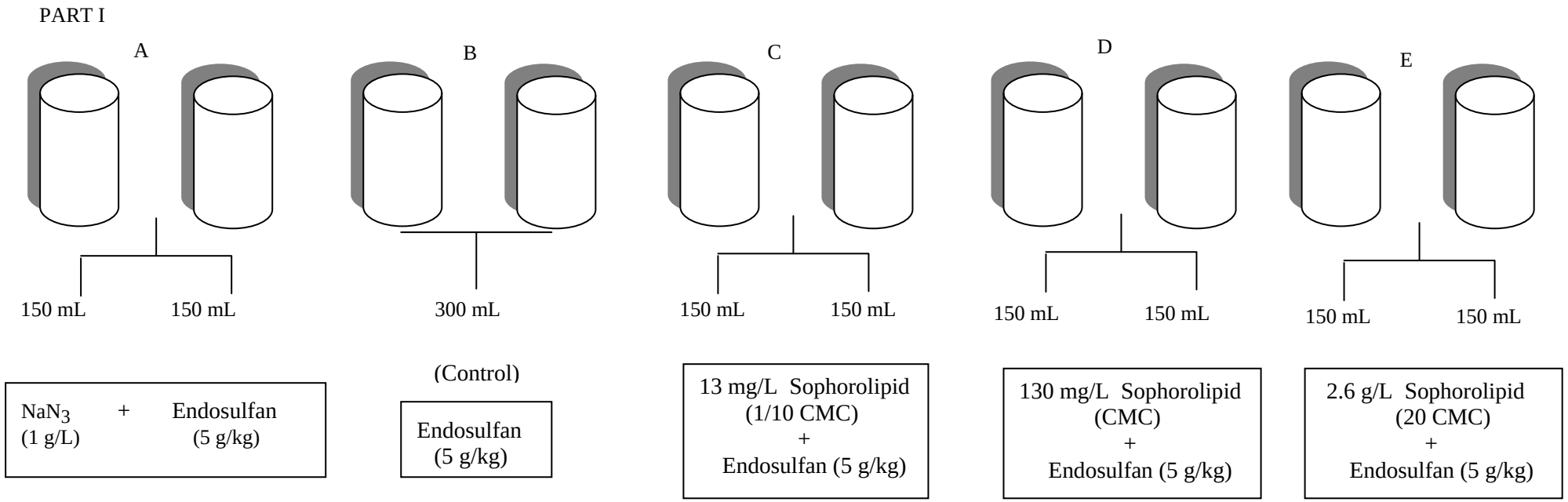
Figure 5.4 Views of soil columns

Each soil column was studied in duplicate. The first pair of the soil column contains NaN_3 (to inhibit the microbial activity) and pesticide mixture. The second pair of the soil column contains only pesticide. These 2 columns are blank samples. Other

three pairs of soil columns contain three different concentrations of biosurfactants and pesticides.

The reason for addition of NaN_3 solution in the first pair of the soil column is to determine the biodegradation rate comparing with the control column. In the columns containing NaN_3 and pesticide, only volatilization occurs, whereas in the control samples containing pesticide, both volatilization and biodegradation take place.

Throughout the study, the effect of biosurfactant on the pesticide degradation is determined by comparing the black samples with the columns containing three different concentrations of biosurfactants and black soil columns.



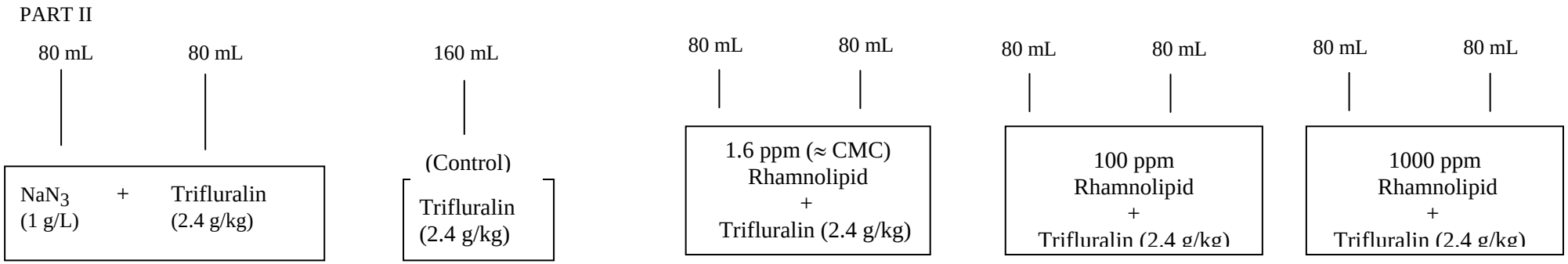


Figure 5.5 Schematic diagrams of experimental study

5.2.2 Effect of Sophorolipid on the Removal of Endosulfan from the Soil (PART I)

As indicated above, two different types of biosurfactants were used throughout the study. In the first part of the study the effect of sophorolipid type of biosurfactant on the removal of endosulfan was investigated. The soil columns contaminated with endosulfan were placed in the incubator and the temperature was set at 28^{o}C.

5.2.2.1 Experimental Procedure in Part I

5.2.2.1.1 Preparation of Soil Samples

Soil sample was obtained from field at different collection points and depths ranging from 0 to 20 cm, and they were mixed thoroughly. Prior to use, the soil was air-dried and sieved through a 2.0 mm screen.

Five different soil columns were prepared for the first part of the experiments. Each soil column contained 2 kg of soil

The prepared soil columns were then kept in the incubator at 28^{o}C throughout the first part of the experiment.

5.2.2.1.2 Preparation of Endosulfan Solution

Commercial endosulfan with purity of 35 % was provided by ASKA Ltd. Sti. Company. Endosulfan standard utilized in the study was obtained from Sigma- Aldrich.

The endosulfan concentration applied to soil was 5 g/kg soil and each soil column contained 2 kg of soil.

Firstly, 10 grams of endosulfan were dissolved in 150 mL deionized water by mixing with magnetic stirrer for a while. In order to achieve a proper homogenization of pesticide with soil, 5g/kg soil concentration was applied to 500 grams of soil at each time instead of applying all 10 grams of endosulfan into 2 kg of soil directly. Thus, this pesticide solution was applied to soil in four stages so that each 500 grams of soil contains 2.5 grams of pesticide. After that, all 500 grams of soil contaminated with endosulfan, were combined to prepare one column of soil.

Apart from the endosulfan contamination, stock endosulfan solutions of 4 ppm, 1 ppm, 0.5 ppm, and 0.1 ppm were prepared for GC calibration curves.

5.2.2.1.3 NaN_3 (Sodium Azide) Solution

1 g/L of NaN_3 solution is prepared for the first pair of soil columns in order to inhibit the bacteria growing in these soil columns.

5.2.2.1.4 Preparation of Sophorolipid Solutions

In the first experiment, the effect of sophorolipid type of biosurfactant on the removal of endosulfan was investigated. sophorolipid was provided by Stuttgart University in Germany.

Three sophorolipid concentrations were applied to soil columns; below the CMC (critical micelle concentration) (1/10 CMC), at the CMC, and above the CMC (20 CMC). These concentrations were13 mg/L, 130 mg/L, and 2600 mg/L. Prior to application of sophorolipid to soil, firstly 2.6 g/L sophorolipid solution was prepared. Then the solution was diluted with deionized water to 130 and 13 mg/L. 37.5 mL of each sophorolipid concentrations was applied to each 500 gr of soils. Then each 500 gr of soil contaminated with pesticide and biosurfactant, was mixed together, so that pesticide and biosurfactant were applied to soil properly. As a result, 2 kg of soil columns, each of them containing 13, 130 and 2600 mg/L of sophorolipid and 10 gr of pesticide were obtained.

5.2.3 Effect of Rhamnolipid on the Removal of Trifluralin from the Soil (PART II)

In this study it was aimed to determine the enhancement effect of rhamnolipid (JBR 425) in the biological treatment of soil contaminated with trifluralin.

5.2.3.1 Experimental Procedure in Part II

In the second part of the study, rhamnolipid type of biosurfactant was used and the soil was contaminated with trifluralin.

The principle of the study is the same as the first experiment. However, the study was carried out in room temperature. Consequently, the temperature values were noted in each day of analysis. The experiment was performed with smaller amount of soils.

5.2.3.1.1 Preparation of Soil Samples

Soil sample was obtained from the same place. Prior to use, the soil was mixed, air-dried and sieved through a 2.0 mm screen.

Five different soil columns were prepared for this experiments and each column was studied in duplicate. The soil columns were designed similarly with the set of columns used in the first experiment except column dimensions. The amount of soil in each column is 500 g. The dimensions of the columns were 10 cm in diameter and 10 cm in height. The prepared soil columns were then kept at the room temperature throughout the experiment.

5.2.3.1.2 Preparation of Trifluralin Solutions

Trifluralin with purity of 48% was provided by ASKA Ltd. Sti. in Bornova. Trifluralin standard was obtained from Sigma-Aldrich.

In this study, application rate of trifluralin was 2.4 g per kg soil and the amount of soil placed into the each column was 500 g. The amount of pesticide and biosurfactant added to soil are calculated for 6 kg of soil instead of the 5 kg of soil in

order to study under excess conditions. After all additions were completed, the soil is separated into the 500 grams and placed into each column.

Firstly, 30 mL (2.4 g) of commercial aqueous trifluralin was dissolved in 380 mL deionized water. Secondly, 5.54 g of Phosphate was weighed and dissolved in 100 mL of deionized water. These two solutions were mixed and applied together to soil. 80 mL of this solution was added to each 500 g of soil so that each 500 g of soil contained 1.6 g of trifluralin and 923 mg Phosphate.

Stock trifluralin solutions of 4 ppm, 1 ppm, 0.5 ppm, and 0.1 ppm were also prepared for GC calibration curves.

5.2.3.1.3 NaN_3 Solution

1 g/L of NaN_3 solution is prepared for the first pair of soil columns as in the first part of the experiment.

5.2.3.1.4 Nutrient Addition

Apart from the first experiment, nutrient addition was performed in order to achieve optimum C: N: P ratio for the bioremediation of pesticide. The C: N: P ratio is between 100:5:1-100:7:1, but optimum ratio is 100:7:1 [50]. Therefore C: N: P ratio was almost kept 100:7:1 by adding potassium di hydrogen phosphate ($K_2H_2PO_4$) (Merck) as phosphorus sources and peat as carbon sources. The amount of phosphorus and carbon necessary to be added to soil was determined from the amount of phosphorus in the soil and peat.

Table 5.3 Addition of nutrient in soil

Total amount of soil (6 kg)		**Phosphorous ($K_2H_2PO_4$)-5.54 g**	**Nitrogen**	**Carbon**
Soil	**Peat**			
5620 g	384 g	1.65 g	11.54 g	170.4 g
Amount of Nutrient per kg of Soil		0.275 g/kg	1.923 g/kg	28.4 g/kg

5.2.3.1.5 Preparation of Rhamnolipid Solutions

In this experiment, the effect of rhamnolipid type of biosurfactant on the removal of endosulfan was investigated. rhamnolipid, namely JBR 425 (Figure 5.6) was provided by Jeneil biosurfactant Company in USA. JBR 425 is a 25% aqueous solution of rhamnolipids.

Figure 5.6 JBR 425-rhamnolipid

Three different rhamnolipid concentrations were applied to soil columns; which were 20 mg/L (1.6 ppm) that was close the CMC (critical micelle concentration), 0.01 % (100 ppm) and 0.1 % (1000 ppm). Firstly, 26.25 g/L rhamnolipid solution was prepared. This solution was then diluted to 20 mg/L with deionized water. 80 mL of this solution was added to each two 500 g of soils which were contaminated with trifluralin. In order to prepare the 0.01 % of JBR 425, 2.625 g/L of rhamnolipid solution was prepared by diluting from the 26.25 g/L of rhamnolipid solution. After that, 38.1 mL (0.1 g) of this solution was taken and diluted to 80 mL with deionized water and added in each of other two soil columns. The last concentration of 0.1 % of JBR 425 was prepared by taking 38.1 mL of the 26.25 g/L of rhamnolipid solution. Then the 38.1 mL of solution was similarly diluted to 80 mL and applied to last two soil columns. The schematic diagram of second experimental study is shown in Figure 5.5

5.3 Analytical Methods

In order to decompose all organic contaminants available microbial growth, conditions must be adjusted. For this reason, some parameters must be controlled. Factors required for the organic matter decomposition that would be ideal include:

a) Soil temperatures near 28^{o}C
b) Moisture of 50 to 70% of the soil's water holding capacity;
c) Aeration must be satisfactory for aerobic decomposition;
d) Providing substrate or organic matter.

Therefore, some parameters were analyzed periodically during the study. These parameters are TOC (total organic carbon), pesticide content (pesticide concentration), soil pH and soil moisture content. Temperature is also recorded in each day of analysis in the second part of the experiment.

5.3.1 Soil Moisture

Moisture content is an important parameter for microorganisms for the suitable degradation of contaminants. Microorganisms are more effective in “field capacity” level and the plants are used water optimum in this value in the soil media. Therefore, soil moisture content was kept constant at the level of 19 %, by adding water twice a week. The amount of water to be added to each soil column was determined from the weight loss of the soil column. The field capacity of the soil is shown in Table 5.1

All applications were performed by considering water content of the soil. In the first experiment; moisture content of the soil was 4%. In order to obtain the soil moisture content at the level of 19 %, 150 mL of water was added to 1 kg of soil. Consequently, each 2 kg of soil column contained 300 mL of water. In the second part of the experiment, soil moisture content was 3 % after the soil was air dried thus, 160 mL of water was added to 1 kg of soil. As a result, each 500 g of soil column contained 80 mL of water. So that each soil column had the same moisture content, 19 %, at the

beginning of the two study. After that, moisture content was maintained by adding deionized water to each column periodically.

5.3.2 Soil pH Measurement

pH plays an important role in bioremediation since it effects microorganisms activities in soil media. The pH of soil is dependent on the parent material, the climate, the native vegetation, the cropping history (for agricultural soils) and the fertilizer or liming practices. The soil pH is also significant for the nutrient availability in the soil. For example; phosphorus and boron are unavailable at both low pH and high pH levels. Presence of Phosphorus is very important in terms of the nutrient balance in the soil for achieving satisfactory bioremediation. Therefore, controlling the pH of the soil is essential for both the microorganisms and the nutrient uptake.

5.3.3 TOC Analysis

In order to determine the microbial degradation in each soil column biologically, total organic carbon analyses were done.

5.3.4 Microwave Assisted Extraction (MAE) Analysis

In the first experiment, 2 g of soil was placed into the extraction vessel. 15 ml of acetone and hexane were added into the vessel as a solvent ratio of 1:1 (v/v). The extraction vessel was then sealed and placed into the microwave system. Extraction was performed at a temperature of 115^{o}C for 10 min and then ventilation was applied for the last 15 minutes. After the extraction, the vessel was allowed to cool down to room temperature before it was opened to avoid loss of analytes. The supernatant was separated from soil by pasteur pipet. The extract was then diluted 100 times by hexane and subjected to GC analysis using appropriate established analytical methods. In the second experiment, extraction was performed with 1 g of soil and 15 mL of solvent. In this process power program was used to extract trifluralin from the soil (Figure 5.8). According to this program, power was not applied to the system for one minute. Then, the power was increased to 600 Watt and kept constant throughout the 5 minutes. After 6th minute the power was decreased to 350 watt for 5 minutes and then ventilation was

applied for the last 15 minutes. After the extraction process, vessels were cooled to room temperature and the solvents in each vessel were separated from the soils. 70 % of extraction yield was obtained at the end of the extraction. After the dilution process, extracts were analyzed by GC.

Figure 5.7 Microwave extraction equipment

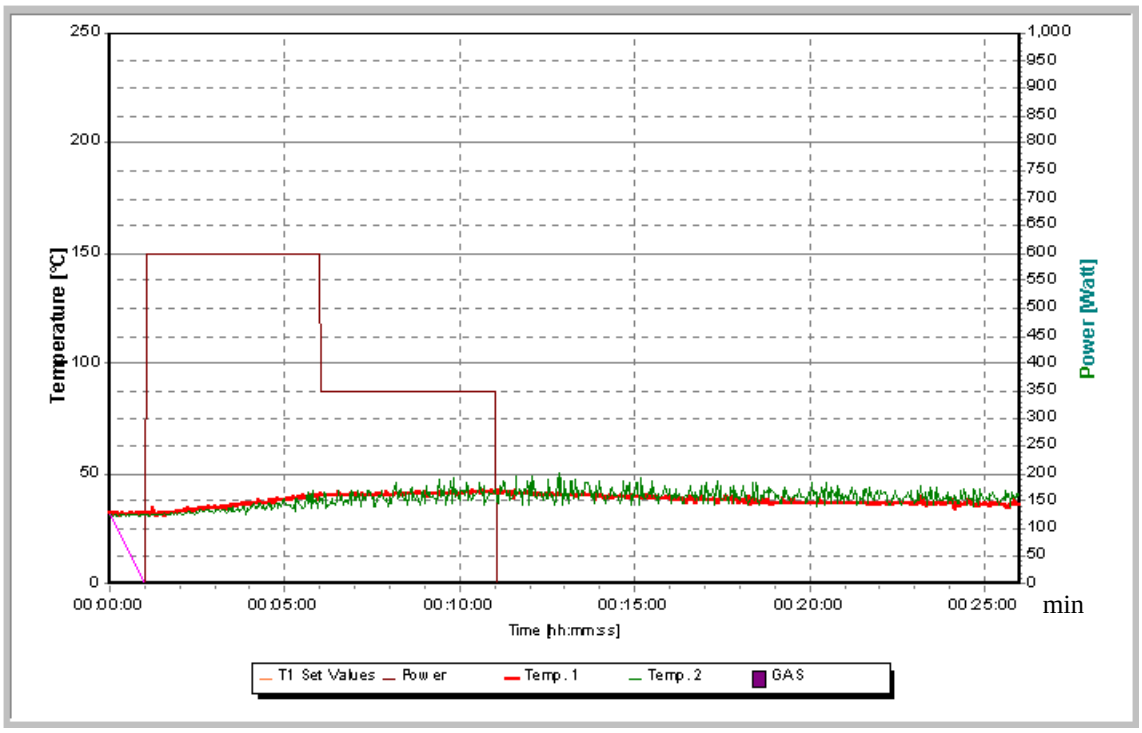


Figure 5.8 Microwave extraction power program used for the extraction of pesticide from soil

5.3.5 GC Analysis

A Shimadzu 17 A Ver. 3 Gas Chromatograph equipped with ECD was used for endosulfan and trifluralin residual analysis. The column used in the study was Optima 5 capillary column, 30 m x 0.25 mm i.d, coated with 95% dimethylpoly siloxane, 5% biphenyl. In order to distinguish the peaks, the temperature program was applied. The temperature program is given below;

60^{o}C (1 min) - (20 ^{o}C/min) - 210 ^{o}C (0 min) - (10 ^{o}C/min)- 280 ^{o}C (3 min)- (30 ^{o}C/min) 300 ^{o}C

Calibration of the instrument response for pesticides was performed by plotting the instrument response (i.e. peak area) against the analytes concentrations. Endosulfan and trifluralin concentrations were calculated from calibration curves. Calibration curves for endosulfan and trifluralin were plotted by preparing 0.1, 0.5, 1 and 4 ppm pesticide solutions (Figure 5.9 and 5.10).

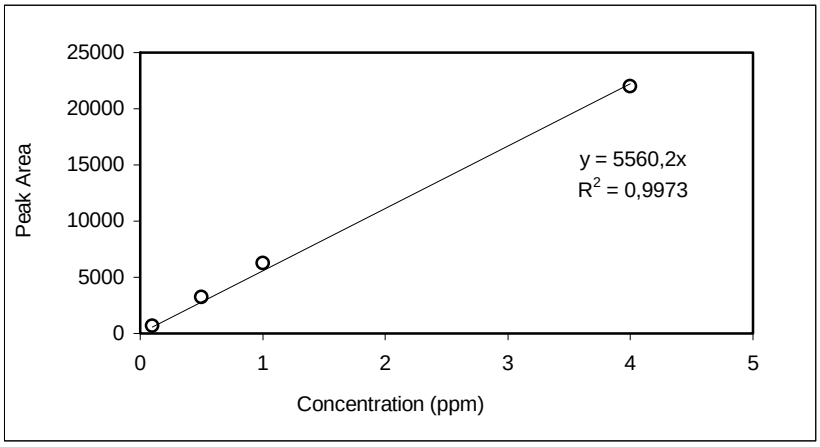


Figure 5.9 Endosulfan calibration curve

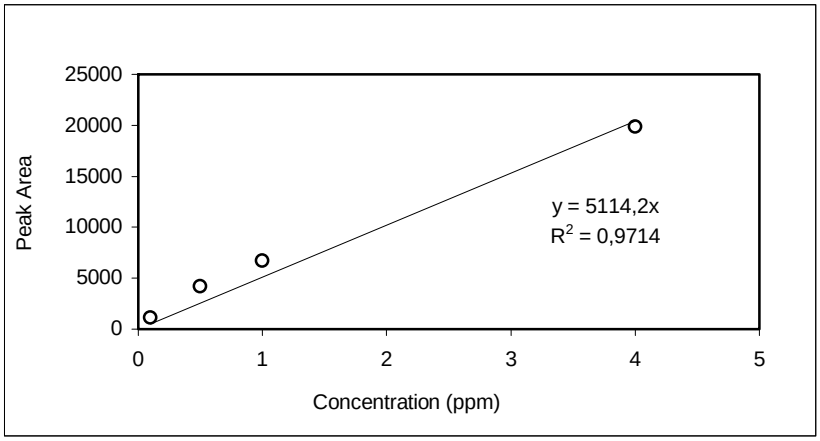


Figure 5.10 Trifluralin calibration curve

According to the temperature program applied, endosulfan and trifluralin peaks appeared in 15th and 10.7th minutes, respectively. GC chromatograms for endosulfan and trifluralin obtained after GC-ECD analysis of soil are shown in Figure 5.11 and 5.12.

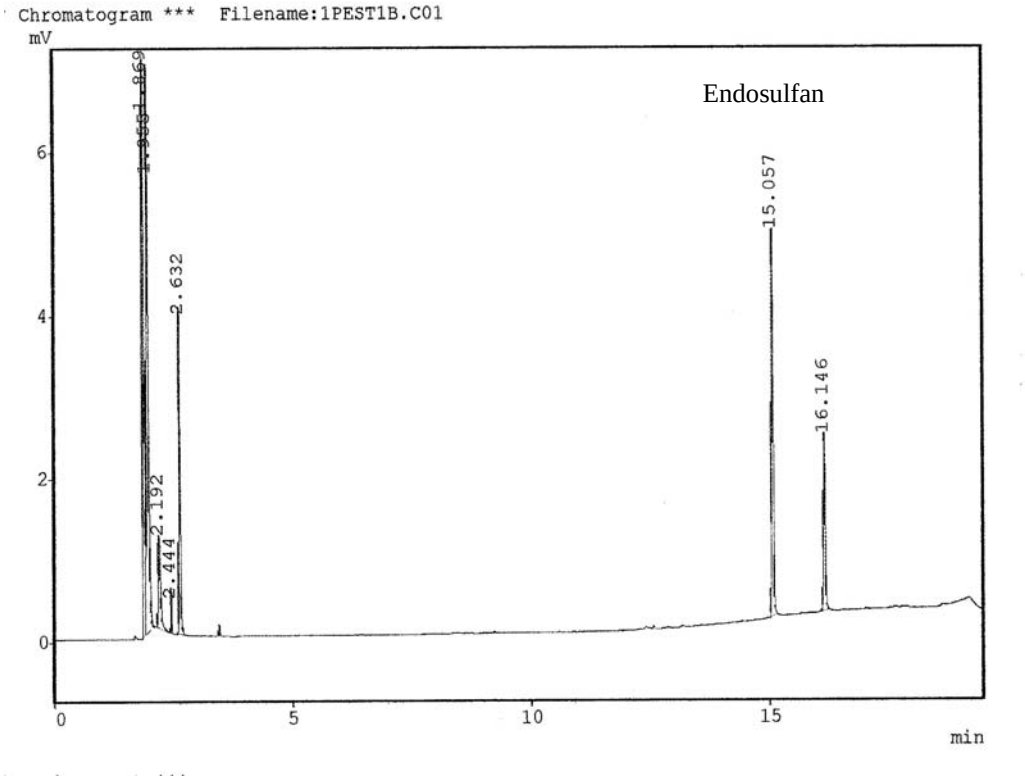


Figure 5.11 GC Chromatogram for endosulfan soil sample in first analysis

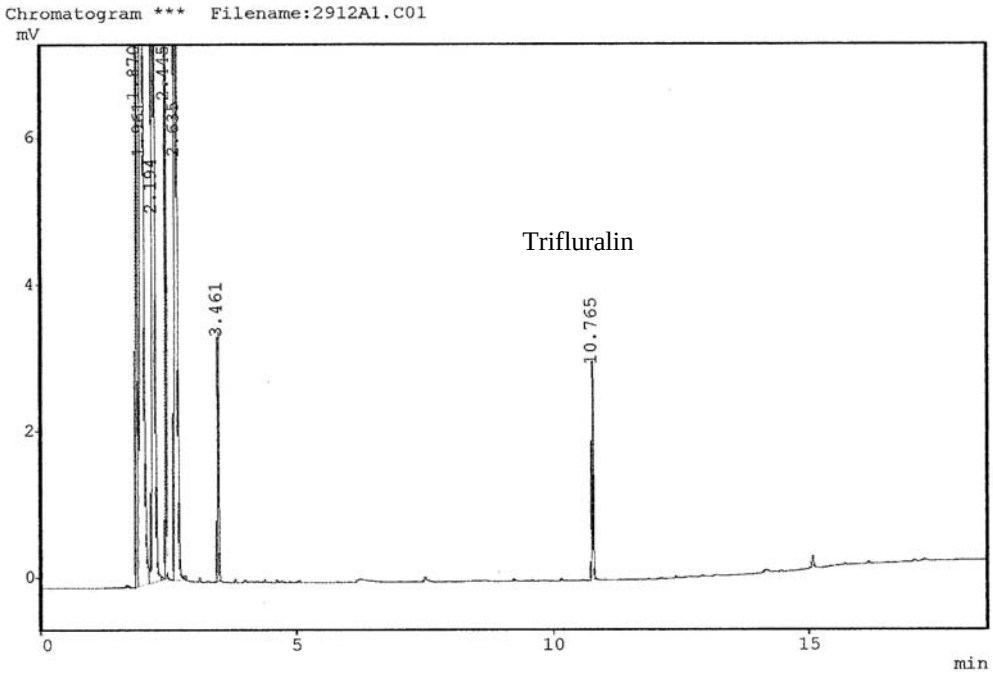


Figure 5.12 GC Chromatogram for trifluralin soil sample in first analysis

In Figure 5.11, the peak appeared in 16th minutes resulted from impurities in the commercial endosulfan product. Because it was not observed any peak in 16th minutes in both soil and standard endosulfan chromatogram.

The other parameters for the GC analysis are given below:

Carrier Gas : N_2

Make up Gas : N_2

Column Pressure : 100 kPa

Column Flow : 1.55911mL/min

Total Flow : 22 mL/min

Injection Port : 250^{o}C

Detector : 300^{o}C

Split Ratio : 10

Chapter 6

RESULTS AND DISCUSSIONS

6.1 Enhanced Biodegradation of Endosulfan-contaminated Soil by Sophorolipid (PART I)

In the first part of the study, three different concentrations of sophorolipid were applied to soil columns to determine the effects of sophorolipid on the removal of endosulfan from the soil. The results of these experiments are given in Table 6.1. The rate of degradation calculated based on the pesticide initial concentration of soil.

Table 6.1. Endosulfan concentrations (ppm) in soil columns during the incubation time

Columns	Column No	1st Day	9th Day	16th Day	42nd Day
NAN_3-added Soil	A	3312	2196	1837	1305
Blank-Endosulfan	B	2812	2123	1763	1266
0.98 ppm Sp.	C	3040	3354	1858	1389
9.75 ppm Sp.	D	1630	3006	1755	1441
195 ppm Sp.	E	2232	3303	1746	1732

In order to determine the biodegradation rate of pesticide, the blank soil column and NaN_3-added soil column were compared in Figure 6.1. Endosulfan concentrations determined in each analysis were found less in blank soil column than that in the NaN_3-added soil column. Because of the toxicity effect of NaN_3 on the soil microorganisms, only volatilization and chemical degradation played significant role in the degradation of pesticide while all processes including biodegradation took place in the blank soil column. Therefore, the rate of degradation was higher in the blank column.

Addition of sophorolipid in the soil columns resulted in the increase of the endosulfan concentration at the beginning of the experiment (Figure 6.1). The increases in the pesticide concentration in the biosurfactant-added soil columns was probably a result of the ability of biosurfactants to desorp the pesticides. In addition, it was clarified that biosurfactant can enhance dissolution rates of liquid and solid

contaminants. After a few days, the degradation of endosulfan was observed in all biosurfactant-added soil columns but degradation was slow in comparison to blank soil column.

The rate of endosulfan degradation was plotted against time of incubation. On comparing the three sophorolipid concentrations, the degradation of endosulfan was maximum in soil containing 13 mg/L of sophorolipid (Table 6.1). However, endosulfan was found to be more degradable in the blank soil column than the other soil columns containing sophorolipid. Throughout the 42 days of incubation, sophorolipid did not show positive effect on the removal of endosulfan from the soil as compared to the control soil column. After the 42 days of incubation, 75% of removal was observed in control soil column whereas the biosurfactant removed only 72, 71 and 65% of endosulfan in soil columns C, D and E, respectively (Figure 6.5). On comparing the three biosurfactant-added soil columns (C, D and E), 0.98 ppm sophorolipid showed the highest removal as compared to the other 9.75 and 195 ppm of sophorolipid. However, more incubation time was required in order to see whether the sophorolipid was effective on the removal of endosulfan from the soil. Since, the degradation rates of endosulfan were very close to each other after 42 days. It is probable that, the sophorolipid can be effective on the degradation of endosulfan after a long period of time.

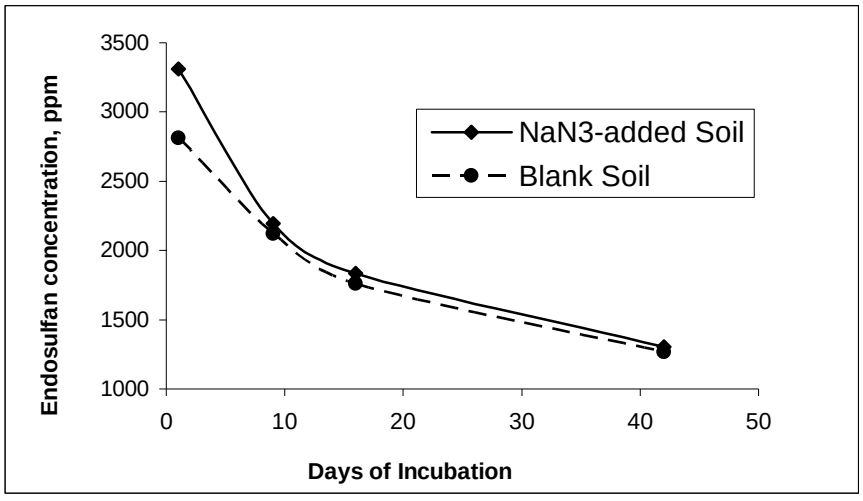


Figure 6.1 Comparison of endosulfan concentration in blank and NaN_3-added soil columns

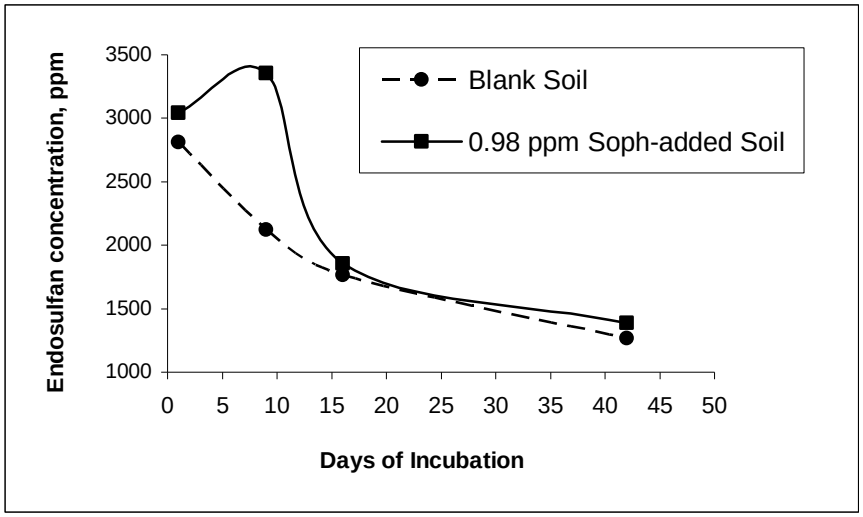


Figure 6.2 Comparison of endosulfan concentration in blank and 0.98 ppm sophorolipid-added soil

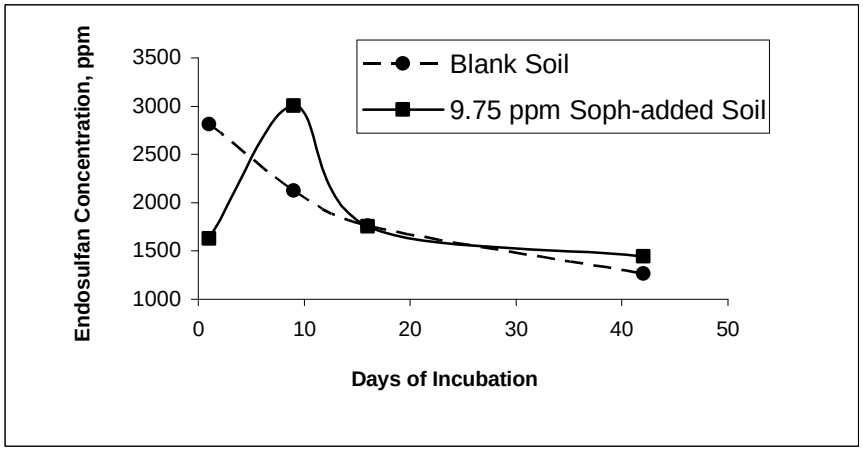


Figure 6.3 Comparison of Endosulfan concentration in Blank and 9.75 ppm sophorolipid-added Soil

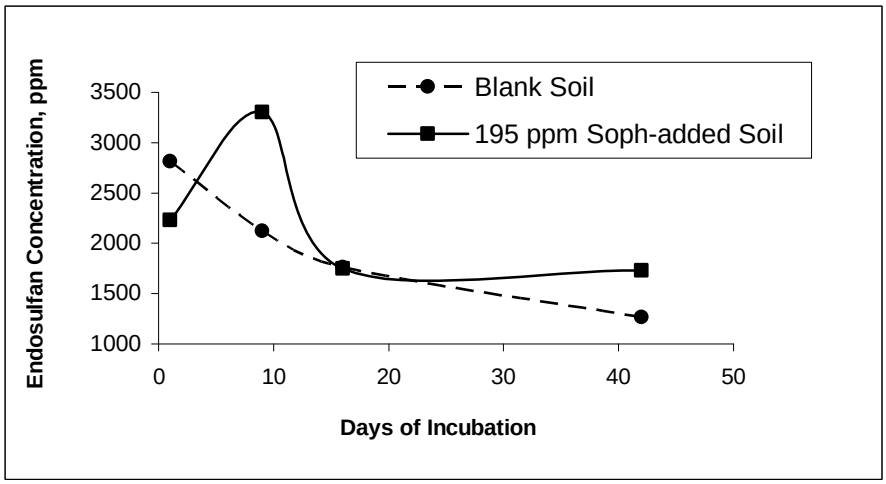


Figure 6.4 Comparison of endosulfan concentration in blank and 195 ppm sophorolipid-added soil

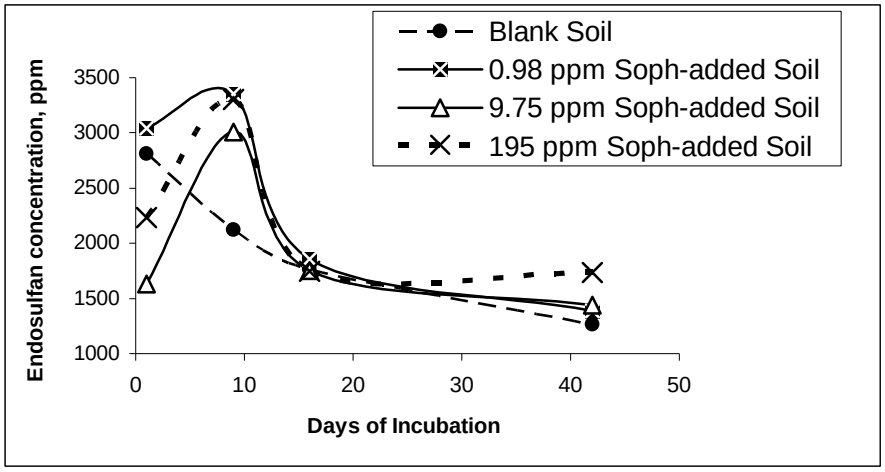


Figure 6.5 Comparison of Endosulfan concentration in Blank and 0.98, 9.75 and 195 ppm Sophorolipid-added Soil

6.1.1 Soil pH

Table 6.2 PH measurements in each soil columns

Columns	pH		
	7th Day	20th Day	40th Day
NAN_3-added Soil	8.10	8.22	8.05
Blank-Endosulfan	8.01	7.87	7.84
13 mg/L Sp. (0.98 ppm)	7.98	7.96	7.85
130 mg/L Sp. (9.75 ppm)	8.02	7.95	7.90
2,6 g/L Sp. (195 ppm)	8.04	7.95	7.88

At the beginning of the experiment, the pH of the soil was 7.89. In order to understand the changes in soil pH, three pH measurements were performed during the experiment. According to pH measurements, it was observed that there are no significant changes in soil pH. The pH values ranged between 7.84-8.22

6.1.2 Total Organic Carbon of the Soil

In order to determine the microorganism activity in pesticide-contaminated soil Total Organic Carbon (TOC) analysis were done. TOC % was found 1.26 % in uncontaminated soil. After 42 days of incubation, variations in the TOC % of the soil samples were shown in Table 6.3. According to these results, maximum degradation was seen in the blank soil column with regard to others.

Table 6.3 TOC values in each soil column

Columns	Total Organic Matter (%)	Total Organic Carbon (%)
	Initial TOC 1.26%	
NAN_3-added Soil	2.16	1.25
Blank-Endosulfan	2.07	1.20
13 mg/L Sp. (0.98 ppm)	2.09	1.21
130 mg/L Sp. (9.75 ppm)	2.16	1.25
2.6 g/L Sp. (195 ppm)	2.21	1.28

6.2 Enhanced Biodegradation of Trifluralin-contaminated soil by Rhamnolipid (PART II)

In the second part of the study, the ability of rhamnolipid to remove the trifluralin from the soil was studied. Similar to the first experiment, three concentrations of rhamnolipid solutions were applied into the soil columns.

Table 6.4 Trifluralin concentrations (ppm) in soil columns during the degradation process

Columns	Columns	0^{th} Day	3rt Day	7^{th} Day	12^{th} Day	24^{th} Day
NAN_3-added Soil	A	2276	2254	2169	1970	1893
Blank-Trifluralin	B	2249	2251	2097	1962	1876
1.6 ppm Rh.	C	2148	2155	2010	1929	1830
100 ppm Rh.	D	2042	2363	2017	1978	1760
1000 ppm Rh.	E	1903	2319	2168	1904	1568

NaN_3-added soil and only trifluralin-containing soil are compared in Figure 6.6. The decreases in the trifluralin concentrations in two columns were almost the same during the 24 days. However, blank soil columns showed little more degradation at the end of the experiment.

As in the first part of the study, biosurfactant increased the trifluralin concentration after adding to the soil column at the beginning of the study. This was probably because of the desorption of the pesticide from the soil solution and increase in the solubility of trifluralin in the soil media. Therefore, the pesticide concentration was increased only in the biosurfactant-added soil columns. After a few days, a rapid decrease in the trifluralin concentration was observed in the rhamnolipid-containing soil columns.

After 24 days, more degradation rate of trifluralin was detected in rhamnolipid-added soil columns as compared to the blank soil column. Adding of biosurfactant into the soil enhanced the bioremediation, resulting in (24-35%) removal of trifluralin in the soil. Increasing the rhamnolipid concentration further to ten times (100-1000 ppm), improved the removal of trifluralin by 8 %. However, 100 ppm of rhamnolipid only increased the removal of trifluralin further to 3 % in comparison to soil containing 1.6 ppm rhamnolipid.

These results showed that, rhamnolipid was more effective at concentration of 100-1000 ppm for the removal of trifluralin. When 1.6, 100 and 1000 ppm rhamnolipid additions are compared, it can be seen that the best results were obtained for 1000 ppm rhamnolipid addition. This indicates the importance of biosurfactant concentration in the decay process of trifluralin. More degradation time was necessary in order to comment on the effect of JBR 425-rhamnolipid for the trifluralin degradation.

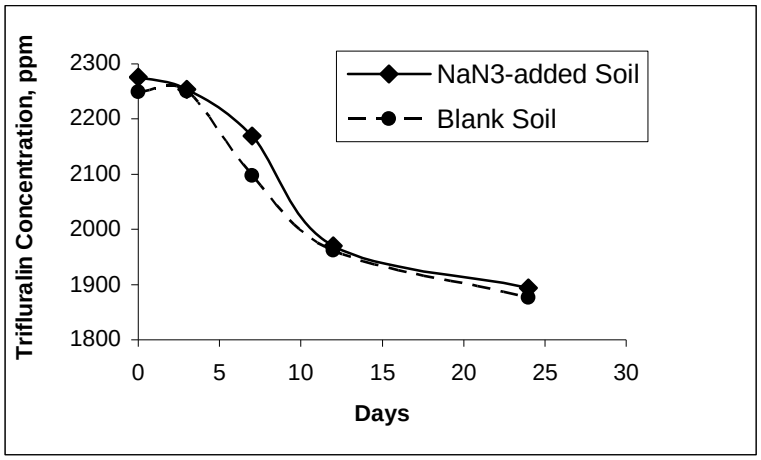


Figure 6.6 Comparison of trifluralin concentrations in blank and NaN_3-added soil columns

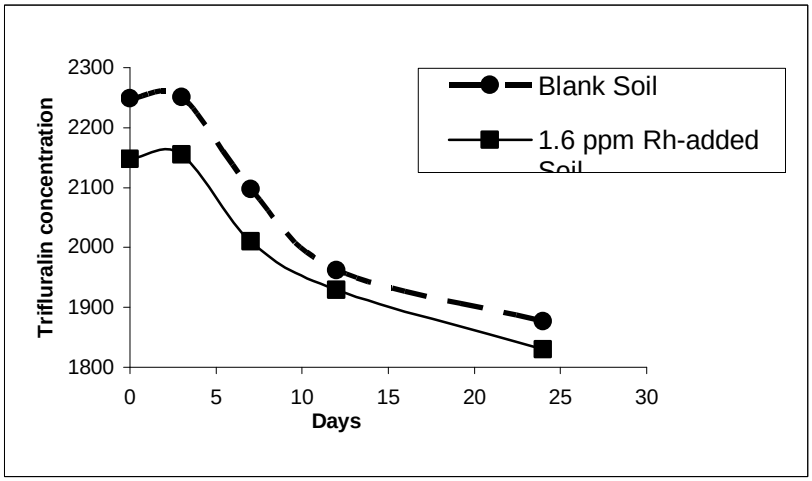


Figure 6.7 Comparison of trifluralin concentrations in blank and 1.6 ppm rhamnolipid-added soil

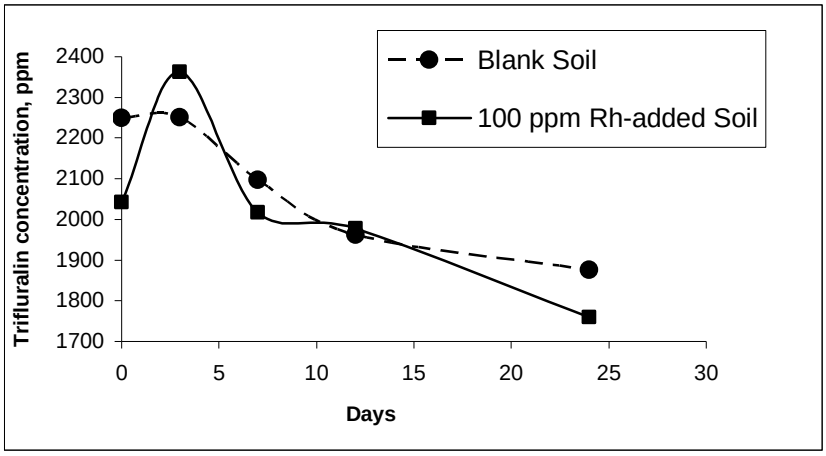


Figure 6.8 Comparison of trifluralin concentrations in blank and 100 ppm rhamnolipid-added soil

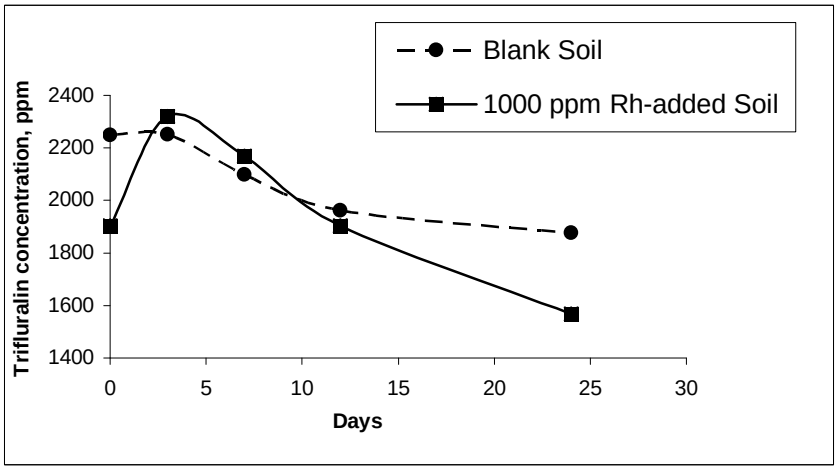


Figure 6.9 Comparison of trifluralin concentrations in blank and 1000 ppm rhamnolipid-added soil

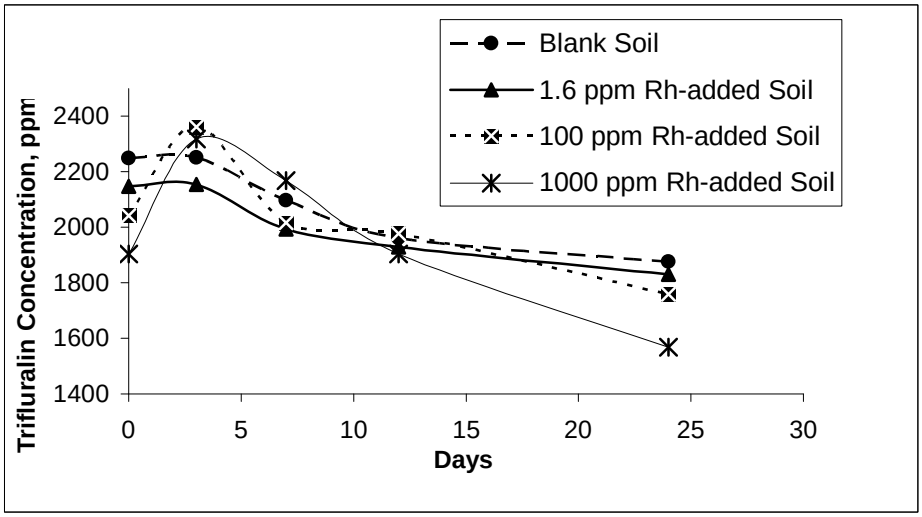


Figure 6.10 Comparison of trifluralin concentrations in blank and 1.6, 100 and 1000 ppm rhamnolipid-added soil

6.2.1 Soil pH

The pH values for second experiment are given in table below. According to Table 6.4, pH values ranged between 7.5 and 7.9 during the experiment. Previous work showed that rhamnolipid solubilization of organics was optimal at pH 7 or slightly less [49]. Additionally, microorganisms maintain the activities in pH range of 6-8. Since the pH values of the soil were not so high than these values for the bioremediation process, there was no need to adjust pH of the soil.

Table 6.5 PH measurements in each soil columns

Columns	pH	
	2nd Day	24th Day
NAN_3-added Soil	7.64	7.91
Blank-Trifluralin	7.55	7.52
1.6 ppm	7.51	7.63
100 ppm Rh.	7.60	7.68
1000 ppm Rh	7.63	7.72

6.2.2 Total Organic Carbon of the Soil

The bacteria and fungi in the soil digest or "oxidize" carbon as an energy source and ingest nitrogen for protein synthesis. Carbon can be considered the "food" and nitrogen the digestive enzymes. For organic matter with just enough nitrogen to aid the decomposition the process will proceed smoothly. Therefore, carbon and nitrogen are the two fundamental elements in organic matter decomposition and their ratio (C: N) is significant for achievement suitable microbial activity. When more organic matter is added, the populations of organisms also increase. In order to achieve suitable C: N ratio, “peat” which is a kind of humus was added to soil at the beginning of the experiments.

After addition of peat in to the soil, TOC (%) background concentration of soil was determined as 2.88 %. The changes in the TOC (%) values in each soil samples were determined and shown in Table 6.6. According to these results, TOC (%) levels in each soil column were shown to decrease. However, it was found that the decrease in the TOC (%) was maximum in soil containing 1000 ppm of rhamnolipid.

Table 6.6 TOC values in each soil column

Columns	Total Organic Matter (%)	Total Organic Carbon (%)
	Initial TOC 2.88 %	
NAN_3-added Soil	4.90	2.84
Blank-Trifluralin	4.79	2.78
1.6 ppm Rh.	4.67	2.71
100 ppm Rh.	4.60	2.67
1000 ppm Rh	4.28	2.48

6.2.3 Temperature

In second part of the experiment, trifluralin degradation in the presence of rhamnolipid was studied in room temperature. The effect of temperature in trifluralin degradation was not studied since there were no significant differences between recorded temperatures. Recorded temperatures in each analysis are given below;

0^{th} Day : 30.0^oC

3^{rt} Day : 29.0^oC

7^{th} Day : 28.8^oC

12^{th} Day : 26.0^oC

24^{th} Day : 27.2^oC

CONCLUSIONS AND RECOMMENDATIONS

The first part of the experiment in the study showed that the sophorolipid-biosurfactant did not show enhancement effect on the endosulfan degradation. The second experiment showed the potential of rhamnolipid type of biosurfactant in remediating trifluralin-contaminated soil. The concentrations of trifluralin in rhamnolipid-applied columns were less than blank column in 24 days. The maximum degradation of trifluralin was found to be only 35 % in column containing 1000 ppm of JBR 425 whereas the total removal of trifluralin was 22 % in blank column. Addition of JBR 425 into the soil can only increase the degradation by 13 % as compared to the blank column. The total trifluralin removed by rhamnolipid ranged from 24-35 %, with the increase in removal being a function of higher biosurfactant concentrations. Increase in the JBR 425 application concentration from 1.6 ppm to 100 ppm, increased the trifluralin removal by 3 %. Similarly, 1000 ppm JBR 425-applied soil column has shown only 8 % more removal than 100 ppm JBR 425 containing soil. From the economic point of view, using JBR 425-rhamnolipid would be more expensive in field applications. Additionally, degradation of pesticides in soil required long period of time ranging from a few weeks to many years depending on the physiological and ecological factors. Therefore, longer period of time is required in order to see the effects of this biosurfactant on trifluralin degradation.

Adding of Sophorolipid in to the endosulfan-contaminated soil only removed 65-72% of endosulfan while the 75% of removal was obtained in blank soil column. This probably resulted from the sophorolipid which was not appropriate for the removal of endosulfan type of pesticide or more incubation time is required in order to understand whether sophorolipid was effective in endosulfan removal.

On comparing the blank and NaN_3-containing soil columns, it was observed that the decrease in the concentration of pesticides were almost the same. This was probably due to the microorganism's inability to adapt to the pesticide-contaminated soil media in these incubation times.

Several factors such as type, structural characteristics and concentration of biosurfactant, and the type of contaminant play a significant role on the biosurfactant-aided degradation of hydrophobic organic contaminants in soil. Because biosurfactants may have different properties, these parameters should be investigated with other type of biosurfactants. Since the processes involved in the biodegradation of a contaminant

are dependent on the physical state of the contaminant (i.e. dissolved, sorbed, solid, liquid) it might be expected that the effect of biosurfactants depends on the physical state. Thus, beside the type of contaminant and the amount of biosurfactant, other parameters such as, adsorption and desorption kinetic and also types of cultures which are effective in degrading the specific contaminant should be examined to achieve optimum conditions and better removal efficiencies.

The other parameters such as pH and temperature effects on the biodegradation process should be examined. For example; optimum pH for the production of rhamnolipid is 6.5-7 according to the JBR 425 Product Data Sheets. The soil pH range may be kept 6.5-7 in other studies. Additionally, the study can be performed at least two different temperatures in order to examine the temperature effects.

In addition to positive effect, negative effects of biosurfactant and factors limiting the bioremediation process should be investigated. Because, it is still difficult to determine how the effects of biosurfactants on biodegradation come about, since often the effects of the biosurfactants on the separate processes have not been investigated.

Although the specific interaction between biosurfactant and the certain contaminants in soil is unclear, it is possible to say that biosurfactants are found to be effective on the removal of some certain contaminants. Thus, additional investigations for the effectiveness of different types of biosurfactant on other contaminants will provide further information about the processes.

Before biosurfactants can be applied on a wide scale for soil remediation, it must be established whether the positive effects of biosurfactants on the soil quality outweigh the negative effects. Generally, negative effects of biosurfactants include the increased leaching of contaminants and the toxicity of biosurfactants to soil fauna and flora. In order to prevent the negative effects of biosurfactants caused by leaching the toxic substances in soil, the amount of toxic impurities contained in the biosurfactant should be detected and required purification levels for the different biosurfactants should be determined in the later studies.

The applications of biosurfactants in the environmental applications have potentially increased in a few years. However, the cost of process is limiting factor for the application of biosurfactants in these areas. The overall production cost of the biosurfactants involves the biosynthesis and purification cost and these are depending on the type and purity of the biosurfactant. As the purity of biosurfactant increases, the

cost of biosurfactant also increases. One advantage of biosurfactants on environmental applications is that, it does not require high purity of biosurfactant (99 % or more). However, the toxicity is important factor for the remedial applications. Therefore, cheaper production alternatives should be investigated in order to extent the applications of biosurfactants for the environmental areas.

The affectivity of biosurfactant for stimulating biodegradation of contaminants is uncertain given the specificity observed between biosurfactant and organism. Addition of biosurfactants will stimulate some organisms but will inhibit others. Therefore, further experiments under field conditions must be revealed whether the balance is positive or negative. Because of the specific interactions between biosurfactant and organisms, it might be beneficial to use biosurfactants produced by the indigenous population. It can be also argued that due to the natural selection a population that can profit from biosurfactant addition will automatically adapt. However, the adaptation of microbial community might be too slow for stimulating biodegradation. In addition, specific organisms which are to be more effective for the specific contaminants should be investigated and biosurfactant-producing these microorganisms should be applied to soil. In-situ production of biosurfactant will also decrease the cost of biosurfactant production.

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