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**MODELING THE POTENTIAL OF MICROORGANISMS
ON THE REMOVAL OF 2,4-DICHLOROPHENOXYACETIC
ACID FROM AQUEOUS SOLUTIONS BY ADSORPTION**

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Duygu OVA tarafından Yüksek Lisans tezi olarak sunulan “Sulu Çözeltilerden 2,4-Diklorofenoksiasetik Asit’in Adsorpsiyon ile Uzaklaştırılmasında Mikroorganizmaların Etkisinin Modellenmesi / Modeling The Potential of Microorganisms On The Removal of 2,4-Dichlorophenoxyacetic Acid From Aqueous Solutions By Adsorption” başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi’nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 27/07/2010 tarihinde yapılan tez savunma sınavında aday oybirliği ile başarılı bulunmuştur.

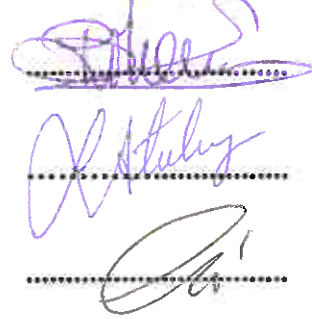
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ÖZET

SULU ÇÖZELTİLERDEN 2,4-DİKLOROFENOKSİASETİK ASİT'İN ADSORPSİYON İLE UZAKLAŞTIRILMASINDA MİKROORGANİZMALARIN ETKİSİNİN MODELLENMESİ

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Bu çalışmada, 2,4-D'nin sulu çözeltilerden uzaklaştırılması ile ilgili bilgi elde edebilmek için adsorpsiyon çalışmaları kesikli ve sürekli olmak üzere iki farklı yöntemle gerçekleştirilmiştir ve adsorbent olarak granüler aktif karbon kullanılmıştır. Bu deneyler akvaryum suyu, *Gracilaria verrucosa*, *Acidovorax Avenae Subsp. Avenae LMG 17238*, *Chlorella sp.*, *Spirulina platensis* olmak üzere beş farklı mikroorganizma varlığında ve herhangi bir mikroorganizma kullanılmadan karşılaştırmalı olarak yürütülmüştür.

Kesikli deneylerde, kinetik/zaman, izoterm/konsantrasyon, termodinamik ve biyolojik kirliliğin adsorpsiyona etkisi çalışmaları yapılmıştır. Mikroorganizma olmadığı durumda 25°C'deki deneysel veriler ikinci mertebeden kinetik modele uyumluluk göstermiş ve hesaplanan adsorpsiyon kapasitesi 256.41 mg/g iken, mikroorganizma varlığında veriler birinci mertebe kinetik modele uyumluluk göstermiş ve hesaplanan adsorpsiyon kapasitesi 164.17 mg/g olarak bulunmuştur. Görünen şudur ki mikroorganizmaların varlığı aktif karbonun adsorpsiyon kapasitesini olumsuz etkilemiştir.

Deneysel veriler Langmuir, Freundlich, Toth, Redlich-Peterson, Sips ve BET modelleri ile tanımlanmış, MATLAB 7.7.0 yardımıyla modellenmiştir. Korelasyon katsayılarına bakıldığında, mikroorganizma olmadığı durumda deneysel verilerin sırasıyla Toth, Sips ve Redlich-Peterson; mikroorganizma olduğu durumda sırasıyla Redlich-Peterson, Toth ve Langmuir modellerine uyum gösterdiği anlaşılmıştır.

Adsorpsiyonun termodinamiği 25°C, 35°C ve 45°C olmak üzere üç farklı sıcaklıkta incelenmiştir. Her iki durumda da proses endotermik olup, mikroorganizma olduğu durumda entalpi 1.370 kJ/mol'den 14.858 kJ/mol'e yükselmiştir. Öte yandan, entropi çok değişmeyip (mikroorganizma yokken 0.017 kJ/molK, varken 0.059 kJ/molK) sıfıra yakın değerlerde kalmıştır.

Sürekli deneylerde ise, 2,4-D'nin aktif karbon ile giderimine 2,4-D'nin başlangıç konsantrasyonunun, akış hızının, adsorbent miktarının ve biyolojik kirlilik yüklenmesinin etkileri sabit yataklı kolonlarda incelenmiştir. Sürekli veriler, Thomas, Yoon-Nelson, Adams-Bohart olmak üzere üç farklı kinetik modellerle incelenmiş olup, en uygun modellerin Thomas ve Yoon-Nelson olduğuna karar verilmiştir.

Maksimum adsorpsiyon kapasitesi (mg/g) değerlerine bakıldığında 2,4-D'nin kesikli sistemde adsorplanma sıralaması:

Acidovorax Avenae Subsp. Avenae LMG 17238 < *Gracilaria verrucosa* < Aquarium water < *Chlorella sp.* < *Spirulina platensis* < Mikroorganizmasız

şeklinde dir. Bu sıralama sürekli sistemin sonuçlarına bakıldığında tam terstir.

Sonuç olarak, kesikli ve sürekli deneylerle granüler aktif karbon ile etkin bir giderim sağlandığı görülmüştür. Biyolojik kirlilik olarak adlandırılan bakteri, alg gibi mikroorganizmaların varlığının adsorpsiyon verimini düşürdüğü gözlenmiştir. Dolayısıyla içme suyunu aktif karbondan geçirmeden önce bir şekilde mikroorganizmaların elimine edilmesi gerektiği sonucuna varılmıştır.

Anahtar sözcükler: 2,4-Diklorofenoksiasetik asit (2,4-D), pestisit, aktif karbon, içme suyu, adsorpsiyon, mikroorganizmalar, biyolojik kirlilik, MATLAB.

ABSTRACT**MODELING THE POTENTIAL OF MICROORGANISMS ON THE
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In this study, experiments were done with two different methods for the removal of 2,4-D in a batch and continuous mode and granular activated carbon was used as the adsorbent. These experiments were performed in the presence of microorganisms, which are aquarium water, *Gracilaria verrucosa*, *Acidovorax Avenae Subsp. Avenae LMG 17238*, *Chlorella sp.*, *Spirulina platensis*, and without any microorganism in comparison.

In the batch experiments, kinetics/time, isotherm/concentration, thermodynamic, and the effect of biological contamination on adsorption were studied. Blank experimental data fit to the pseudo-second order kinetic model with a calculated adsorption capacity of 256.41 mg/g at 25°C where in the case of microorganism, the process turns out to obey the pseudo-first order kinetic model with a calculated adsorption capacity of 164.17 mg/g at 25°C. It is obvious that the presence of microorganisms affect the adsorption capacity of granular activated carbon negatively.

The batch studies data were described using the Langmuir, Freundlich, Toth, Redlich-Peterson, Sips and BET model isotherms with the help of MATLAB 7.7.0 Curve Fitting Tool Box. Regarding the correlation coefficients, the experimental data best fit in the order of Toth, Sips and then Redlich-Peterson for the blank case and in the order of Redlich-Peterson, Toth and then Langmuir for microorganism case.

The thermodynamics of adsorption were investigated at 25°C, 35°C and 45°C. The enthalpy of adsorption increased from 1.370 kJ/mol to 14.858 kJ/mol in the presence of microorganisms both being endothermic processes. On the other hand, the entropy did not change very much (0.017 kJ/molK for blank and 0.059 kJ/molK for microorganism conditions) and stayed nearly at zero.

In the continuous experiments, the experiments were carried out as a function of the initial concentration of 2,4-D, flow rate, adsorbent mass, and the effect of biological contamination load in fixed-bed columns. Three kinetic models; Thomas, Yoon-Nelson and Adams-Bohart models were applied to the experimental data and the Thomas and Yoon-Nelson kinetic models showed better fit for granular activated carbon.

The order of batch adsorption of 2,4-D in terms of maximum adsorption capacity (mg/g) from the least to the most is:

Acidovorax Avenae Subsp. Avenae LMG 17238 < *Gracilaria verrucosa* < Aquarium water < *Chlorella sp.* < *Spirulina platensis* < Blank

where the order is just the opposite for the continuous mode studies.

As a consequence, by the help of all the batch and continuous experiments done, it was found that although granular activated carbon is a very effective adsorbent in the removal of 2,4-D, the efficiency of the adsorption decreases in the presence of biological contamination namely microorganisms like bacteria and algae. So it was concluded that before treating the drinking water with activated carbon, it is necessary to get rid of such microorganisms somehow.

Keywords: 2,4-Dichlorophenoxyacetic acid, pesticide, activated carbon, drinking water, adsorption, microorganisms, biological contamination, MATLAB.

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NOMENCLATURE

Symbol		Unit
b	: Langmuir adsorption constant	L/mg
Q_0	: Monolayer adsorption capacity	mg/g
$Q_{\max}$	: Amount adsorbed	mg/g
K_F	: Freundlich adsorption constant	(mg/g)(mg/L) ⁿ
n	: Freundlich adsorption constant	
A	: Toth Isotherm Constant	
B	: Toth Isotherm Constant	
D	: Toth Isotherm Constant	
k_{rp}	: Redlich-Peterson Isotherm Constant	L/g
a_{rx}	: Redlich-Peterson Isotherm Constant	(L/mg) ^b
β	: Redlich-Peterson Isotherm Constant	
k_s	: Sips Isotherm Constant	
a_s	: Sips Isotherm Constant	
b_s	: Sips Isotherm Constant	
B	: BET Isotherm Constant	
Q_0	: BET Isotherm Constant	
λ	: The wave length	nm
T	: Transmission	
I_0	: Initial light intensity	
I	: Final light intensity	
Abs	: Absorbance	
C_b	: bulk concentration in the solution in the column	(mg/L)
C_0	: inlet (feed) or initial concentration	(mg/L)
C_{ad}	: (= $C_0 - C$) adsorbed concentration in the column	(mg/L)

NOMENCLATURE (Continued)

Symbol		Unit
C	: break outlet concentration at breakthrough (or limit effluent concentration)	(mg /L)
C _{eq}	: nonadsorbed concentration at equilibrium in the column	(mg/L)
C _s	: concentration at the solid/liquid interface in the column	(mg/L)
D _p	: diffusion coefficient	(cm ² /s)
k _{AB}	: kinetic constant in the Adams-Bohart model	(L/mg x min)
k _{Th}	: kinetic constant in the Thomas model	(mL/mg x min)
k _{YN}	: kinetic constant in the Yoon and Nelson model	(L/min)
N _o	: saturation concentration in the Adams-Bohart model	(mg/L)
m _{total}	: total amount of adsorbate sent to column	(mg)
q	: concentration in the solid phase in the column at any time	(mg/L)
q _{eq}	: equilibrium uptake per g of adsorbent in the column or in the batch system	(mg/g)
q _{total}	: total adsorbed quantity of adsorbate in the column	(mg)
q _o	: (=q _{eq}) constant in the Thomas model	(mg/g)
Q	: flow rate	(ml/min)
r	: adsorption rate	(mg/L x min)
t	: flow time	(min)
t _{break}	: time at breakthrough	(min)
t _{total}	: total flow time	(min)

NOMENCLATURE (Continued)

Symbol		Unit
U_0	: superficial velocity	(cm/min)
V_{eff}	: effluent volume	(mL)
X	: amount of sorbent in the column (g) or sorbent concentration in the batch system	(g /L)
Z	: height of the column	(cm)
ε	: void fraction in the bed	
γ	: migration rate	(cm/min)
τ	: time required for 50% adsorbate breakthrough	(min)

1. INTRODUCTION

Water is a basic need of life and is used in many ways to meet the needs of daily life. Supplied by a combination of surface water and underground water sources, drinking water can become contaminated at the original water source, during treatment, or during distribution to the home.

Indiscriminate use of pesticides in the modern agricultural practices leads to the contamination in of soils, surface, and ground waters. These contaminants arise predominantly from agricultural and industrial sources (Vroumsia et al., 1999).

Pesticides are among the major organic compounds encountered in wastewater effluents of the pesticide industry and domestic activities. These compounds are commonly used in agriculture to protect crops from pest organisms. Since most pesticides are used in the open environment such as ploughed fields, their residues have become one of the major diffuse pollution risks to the world drinking water supplies. Numerous cases of pesticide residue have been reported in literature (Moore and Ramamoorthy, 1984). Their residual concentrations in food products are alarming especially in developing countries. Leafy vegetables, cereals, fruits, rice, meat, milk, fish intestine, and edible portions and even human milk have been reported to be contaminated by various pesticides in a concentration range of 0.1 mg/L - 25.7 mg/L.

The contamination of water in ponds, rivers, and ground water sources with various pesticides and herbicides is posing a direct threat to human health. They are considered dangerous and harmful to living systems because of their toxicity and carcinogenicity even at low concentrations (IARC, 1987).

Environmental regulations in developed countries have become very strict for drinking water treatment over the last few years, especially regarding pesticide compounds.

To protect consumers from the adverse effects associated with high contamination intake, the United States, Canada, the European Union (EU), and

the World Health Organization (WHO) have set standards to regulate the contamination in water (Sayre, 1988; Deininger et al., 1998; Nalcaci et al., 2006).

The subject of pesticide contamination in water has reached a particularly problematic level as a consequence of the European Commission directive on the Quality of Water Intended for Human consumption (1980), which defines a maximum admissible concentration of 0.1 $\mu\text{g/L}$ for individual pesticides and of 0.5 $\mu\text{g/L}$ for the total of all pesticides present (Nalcaci et al., 2006).

The removal of pesticides from water is an extremely complex problem due to the wide range of the pesticides chemical structures and properties, so various treatment techniques have been employed to treat water with pesticides for example ion exchange, reverse osmosis, and adsorption etc. Nevertheless, adsorption processes using suitable adsorbent have shown high removal efficiency and many economical, ecological, and technological advantages.

In water treatment, activated carbon is a powerful adsorbent because it has a large surface area and pore volume, which remove liquid-phase contaminants, including organic compounds (like pesticides), heavy metal ions, and colors. In order to design effective activated carbon adsorption units and to develop mathematical models that can accurately describe their operation, sufficient information on both the adsorption and desorption of individual pollutants under different operating conditions is required (Khan et al., 1997).

Granular activated-carbon (GAC) filters have been used for 35 years to remove dissolved organic matter by adsorption. More recently, it has been shown that, in addition to adsorption of organic matter, GAC is also a good material for the development of attached bacteria. The colonization of GAC filters by bacteria has been observed in some industrial treatment plants by various techniques: scanning electron microscopy (Weber et al. 1978). Studies have shown that GAC columns continue to yield effective organic matter removal far beyond the point where the adsorption capacity would normally be exhausted (Rittmann and Snoeyink 1984; McFeeters et al., 1987). Also in addition to the advantage of the

microbiological removal of organic matter, biological GAC filters need no regeneration of the GAC.

Biological contamination is a situation which occurs when microorganisms end up in a location where they are not supposed to be. It is often used to refer to the contamination of food by microorganisms which can cause disease, but biological contamination can also occur in other settings. In the case of drinking water, the analysis of the microbiological flora of the water supplied for drinking purposes may show that the water is biologically contaminated. This situation is not desirable, because it can pose a health threat and cause other problems. As a result, steps are taken to avoid biological contamination in settings where it can become an issue.

The aim of the study is to eliminate the 2,4-D pesticide from drinking water by the adsorption process both in batch and continuous systems, to study this adsorption process in the presence of biological contamination/microorganisms (like bacteria and algae) and to model this process.

2. THEORETICAL BACKGROUND

2.1 Drinking Water

Drinking water is usually supplied by a combination of surface water and groundwater sources. Surface water includes rivers, lakes, and reservoirs. Groundwater lies under the surface of the land, where it travels through and fills openings in rocks.

Drinking water can become contaminated at the original water source, during treatment, or during distribution to the home. Surface water (river or lake) sources can be exposed to acid rain, storm water runoff, pesticide runoff, and industrial waste. This water is cleansed somewhat by exposure to sunlight, aeration, and microorganisms in the water. For groundwater (private wells and some public water supplies), it generally takes longer to become contaminated, but the natural treatment process also may take much longer. Groundwater moves slowly and is not exposed to sunlight, aeration, or aerobic (requiring oxygen) micro-organisms. Groundwater can be contaminated by disease-producing pathogens, leachate from landfills and septic systems, careless disposal of hazardous household products, agricultural chemicals, and leaking underground storage tanks.

2.2 Drinking Water Standards

The WHO, set up the latest guidelines for drinking-water quality on 1993 which are the international reference point for standards setting and drinking-water safety.

The EU drew up the Council Directive 98/83/EC on the quality of water intended for human consumption by reviewing the parametric values of the old Drinking Water Directive of 1980. The EU standards are more recent (1998), complete and strict than the WHO standards (1993).

The United States (U.S.) Environmental Protection Agency (EPA) is required by Safe Drinking Water Act (1974) to determine safe levels of chemicals

in drinking water which do or may cause health problems. The comparison of standards of some contaminants in drinking water respect to WHO (1993) and EU (1998) are tabulated in Table 2.1.

Table 2.1 Comparison of standards of some contaminants in drinking water respect to WHO and EU

Contaminants	WHO standards (1993)	EU standards (1998)
Aluminium (Al)	0.2 mg/L	0.2 mg/L
Ammonia (NH₄)	No guideline	0.50 mg/L
Antimony (Sb)	0.005 mg/L	0.005 mg/L
Arsenic (As)	0.01 mg/L	0.01 mg/L
Barium (Ba)	0.3 mg/L	Not mentioned
Boron (B)	0.3 mg/L	1.00 mg/L
Bromate (Br)	Not mentioned	0.01 mg/L
Cadmium (Cd)	0.003 mg/L	0.005 mg/L
Chromium (Cr)	0.05 mg/L	0.05 mg/L
Copper (Cu)	2 mg/L	2.0 mg/L
Iron (Fe)	No guideline	0.2 mg/L
Lead (Pb)	0.01 mg/L	0.01 mg/L
Manganese (Mn)	0.5 mg/L	0.05 mg/L
Mercury (Hg)	0.001 mg/L	0.001 mg/L
Molibdenum (Mo)	0.07 mg/L	Not mentioned
Nickel (Ni)	0.02 mg/L	0.02 mg/L
Nitrogen (total N)	50 mg/L	Not mentioned
Selenium (Se)	0.01 mg/L	0.01 mg/L
Silver (Ag)	No guideline	Not mentioned
Sodium (Na)	200 mg/L	200 mg/L
Uranium (U)	1.4 mg/L	Not mentioned
Zinc (Zn)	3 mg/L	Not mentioned
Chloride (Cl)	250 mg/L	250 mg/L
Cyanide (CN)	0.07 mg/L	0.05 mg/L
Fluoride (F)	1.5 mg/L	1.5 mg/L
Sulfate (SO₄)	500 mg/L	250 mg/L
Nitrate (NO₃)	(See Nitrogen)	50 mg/L
Nitrite (NO₂)	(See Nitrogen)	0.50 mg/L
2,4-D	30 µg/L	0.1 µg/L for individual pesticides and of 0.5 µg/L for total of all pesticides present
MCPA	2 µg/L	
Simazine	2 µg/L	
2,4-DB	90 µg/L	
Dichlorprop	100 µg/L	
Fenoprop	9 µg/L	
Mecoprop	10 µg/L	
2,4,5-T	9 µg/L	

2.3 Pesticides

A pesticide may be a chemical substance or biological agent (such as a virus or bacteria) used against pests including insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes and microbes that compete with humans for food, destroy property, spread disease or are a nuisance. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other animals (WHO, 1985; Tomlin, 2000).

By the way, pesticides are chemicals used to control unwanted organisms such as weeds and pesticides can be derived from plants (e.g., pyrethrin, neem) or minerals, or they can be chemically manufactured (e.g., DDT, 2,4-D).

There are basically two ways properly applied pesticides may reach surface and ground waters through runoff and leaching (Figure 2.1). Runoff is the physical transport of pollutants over the ground surface by rainwater that does not penetrate the soil. Leaching is a process whereby pollutants are flushed through the soil by rain or irrigation water as it moves downward (Ka et al., 1994).

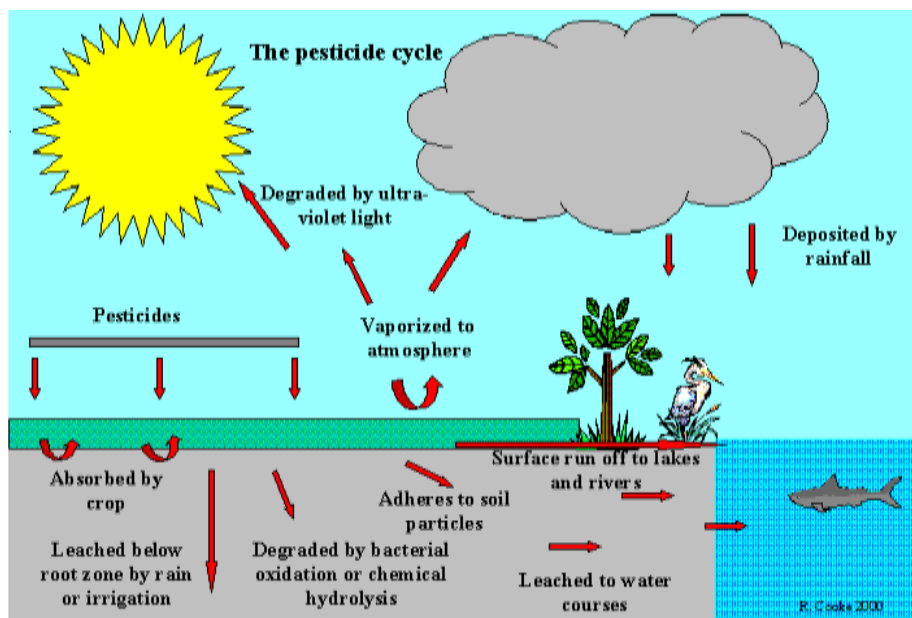


Figure 2.1 The pesticide cycle

2.3.1 Types of Pesticides

Pesticides are classified according to the type of pest they control. Types of pesticides and the target affect of those pesticide types are tabulated in Table 2.2. Pesticides can also be referred as synthetic pesticides or biological pesticides (biopesticides), although the distinction can sometimes blur.

Table 2.2 Types of pesticides

Algaecides	For the control of algae
Avicides	For the control of birds
Bactericides	For the control of bacteria
Herbicides	For the control of weeds
Fungicides	For the control of fungi
Insecticides	For the control of insects
Miticides	For the control of mites
Molluscicides	For the control of slugs and snails
Nematicides	For the control of worms
Rodenticides	For the control of rodents
Virucides	For the control of viruses

Herbicides:

Herbicide is a chemical compound that kills plants or inhibits their normal growth. A herbicide in a particular formulation and application can be described as selective or nonselective.

In agriculture, selective herbicides are often used instead of tillage, or in combination with tillage and other agronomic practices, to control weeds without damaging crops. For these no-till or low-till systems, scientists are using biotechnology to develop crop varieties with increased tolerance for herbicides. Nonselective herbicides (e.g., paraquat) toxic to all plants are used where complete control of plant growth is required.

Contact herbicides kill only the parts of the plant they touch; systemic herbicides are absorbed by foliage or roots and translocated to other parts of the plant. Preemergence herbicides, mixed into the soil, will kill germinating seeds and small seedlings. Postemergence herbicides either hinder photosynthesis or inhibit growth.

For a translocated herbicide to have a potentially lethal effect on a plant:

- (1) the herbicide must penetrate or be absorbed into the plant,
 - (2) it must move through the plant, generally in the phloem,
 - (3) it must have a toxic reaction with the cells and tissues of the plant
- (Leonard and Crafts, 1956; Butts and Fang, 1956).

Herbicides vary widely. Some are water soluble enough to enter lakes or streams with rainfall or runoff irrigation water, but the hazard they represent depends on their persistence and interaction with the soil. They can also leach into ground water or move with eroding soil.

Many herbicides designed to be applied to emerge plants are inactivated once they reach the soil surface. Soil-applied herbicides, however, must be soluble in soil water in order to move into the root zones of target weeds. Some move deeply into the ground to kill deep-rooted perennials. Others don't move as deeply in order to kill shallow-rooted weeds and spare a deeper rooted crop.

Early chemical herbicides were inorganic compounds. Organic herbicides began to be produced in earnest with dinitrophenol compounds in 1932. A breakthrough occurred in the 1940s with 2,4-D (2,4-dichlorophenoxyacetic acid), a compound similar to plant hormones, which is a highly selective systemic herbicide when used in very small quantities. The phenoxyaliphatic acids and their derivatives, another major group of organic herbicides, succeeded because of their selectivity and ease of translocation.

Phenoxyalkanoic Acid Herbicides:

Phenoxyalkanoic acid herbicides are widely used in agriculture to control the growth of broad-leaf weeds in crops (Figure 2.2). They are also used in lakes, ponds, and ditches for aquatic weed control (Smith, 1989; Smith et al., 1994). These compounds are considered potential pollutants owing to their high soil mobility, long half-lives and large scale of application. Due to their persistence, polar nature and water solubility, they are present in several environmental compartments including water, soil, cereals and vegetables. Hence, there is increasing interest in methods for the determination of phenoxyalkanoic acid herbicide residues as well as their phenolic metabolites in natural samples (Biesaga et al., 2005).

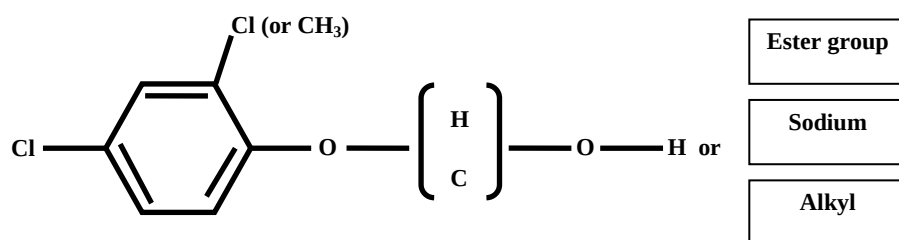


Figure 2.2 The general chemical structure of phenoxyalkanoic acid herbicides

One of the classes of phenoxy herbicides includes:

(1) the phenoxyacetic acids:

- a. 2-methyl-4-chlorophenoxyacetic acid (**MCPA**),
- b. 2,4-dichlorophenoxyacetic acid (**2,4-D**),
- c. 2,4,5 trichlorophenoxyacetic acid (**2,4,5-T**)

(2) the phenoxypropionic acids:

- a. 2-(4-chloro-2 methylphenoxy)- propionic acid (**MCP**),
- b. 2-(2,4-dichlorophenoxy)propionic acid (**2,4-DP**),
- c. 2-(2,4,5-trichlorophenoxy) propionic acid (**2,4,5-TP**)

(3) the phenoxybutyric acids:

- a. 4-(4- chloro-2-methylphenoxy)butanoic acid (MCPB)
- b. 4-(2,4-dichlorophenoxy)butyric acid (**2,4-DB**) as in Figure 2.3 (Argese et al., 2005).

These herbicides are known as synthetic auxins and bring to cellular proliferation of components of the plants, which is rapid and out of control and causes their death. The phenoxy herbicides have low to moderate toxicity toward humans and animals. Some of them, such as 2,4-D and 2,4,5-T, have been extensively studied; however, their mechanism of action towards non-target organisms is not fully elucidated yet (Zychlinski and Zolnierowicz, 1990; Suwalsky et al., 1996; Palmeira et al., 1994).

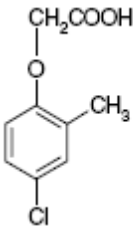
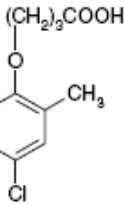
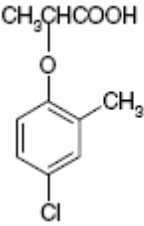
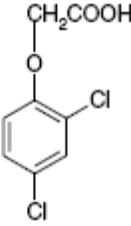
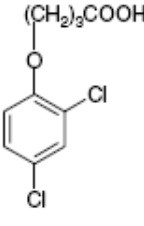
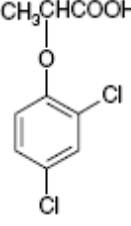
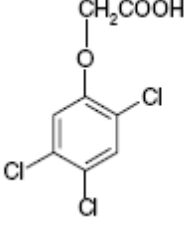
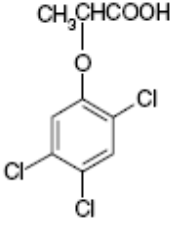
MCPA		MCPB	
MCPP		2,4-D	
2,4-DB		2,4-DP	
2,4,5-T		2,4,5-TP	

Figure 2.3 Names and structural formulas of the some phenoxyalkanoic acid herbicides

2.3.2 Pesticide Consumption in EU and Turkey

The agricultural area in the current 15 member states of the EU comprises over 130 million hectare. Almost 7 million farm holdings exist. By the sales data taken from Eurostat (the statistical agency of the European Union) and Oskam et al. (1997), the highest consumption of pesticides was in Italy, followed by the United Kingdom and the lowest consumption of pesticides was in Finland between 1996 and 2000 as seen in Table 2.3 (Eurostat; Oskam et al., (1997)).

Table 2.3 Pesticide consumption in EU

Country	1996 (tons)	1997 (tons)	1998 (tons)	1999 (tons)	2000 (tons)
Belgium	4,787	4,715	4,718	4,724	4,124
Denmark	2,632	2,685	3,283	2,915	-
Germany	15,941	17,790	15,394	12,696	14,834
Greece	-	2,252	2,442	2,579	-
France	-	-	27,419	36,052	-
Italy	24,468	25,848	25,921	24,913	24,943
Austria	1,873	1,549	1,607	1,536	-
UK	20,885	22,733	22,753	24,131	24,118
Sweden	1,097	1,507	975	1,235	-
Finland	841	915	815	674	734

Comparing the statistical values in Table 2.3 and Table 2.4, it is seen that the pesticide consumption in Turkey in 1996 (tons) is less than some of the EU countries like Germany, Italy, UK; but more than Belgium, Denmark, Austria, Sweden and Finland. On the other hand, as known, pesticide consumption in Turkey is quite heterogeneous (Delen et al.,1995). Table 2.4 is an evidence for heterogeneity of the pesticide consumption in Turkey. Between years 1979 and 2002, 45.29 % increase was observed in the consumption of pesticide, although the increase of world pesticide consumption seemed to be standing still (Delen et al., 2004).

Table 2.4 Pesticide consumption in Turkey according to years (tons)

Pesticide Groups	1979	1987	1994	1996	2002
Insecticides	2,287	3,303	2,064	3,027	2,250
Acaricide	203	240	192	223	296
Lipids	1,594	2,147	2,147	2,871	2,428
Fumigant & Nematicides	315	322	530	1,076	1,559
Rodenticides & Molluscicides	5.6	2.1	2.5	3.2	1.7
Fungicides	1,537	2,611	2,201	2,951	1,964
Herbicides	2,451	3,495	3,902	3,643	3,697
TOTAL	8,395	12,112	10,871	13,797	12,198

Not only the pesticide consumption is quite heterogeneous in Turkey, and also consumption of regions is heterogeneous too. While consumption in Aegean and Mediterranean regions had more than 33.3%, consumption in Eastern Anatolia and South Eastern Anatolia regions was 10% of country consumption (Delen et al., 2004).

Amount of widely used pesticides in Izmir between 2000 and 2004, is given in Table 2.5 (Izmir Agriculture Management, Plant Protection Department 2004). It is seen that 2,4-dichlorophenoxyacetic acid (2,4-D) which involved 17.41% of total consumption between 2000-2004 is the second more common used pesticide after Trifluralin, an aniline group herbicide.

Table 2.5 Most used herbicides (kg) in Izmir according to a yearly change between the years 2000 and 2004

Herbicide	Herbicide Group	2000	2001	2002	2003	2004	% in total
2,4-D	Phenoxy Herbicide	25,340	17,801	13,281	14,752	24,632	17.41
Trifluralin	Aniline	26,280	32,875	30,382	113,073	122,740	59.12
Glyphosate isopropylamin	Amino phosphonates	12,852	15,814	28,942	27,656	34,163	21.70
Metalochlor	Amides and Anilides	3,100	1,543	515	500	4,090	1.77
TOTAL		67,572	68,033	73,120	155,981	185,625	100

Pesticides are chemicals use of which has increased worldwide since the Second World War in order to improve the quality and the quantity of agricultural products. Their harmful effects on the environment and the human health have also been seen as usage increases. Although there have been studies about the harmful effects of pesticides on human health in the developing countries, there exists limited data about the use of pesticides in Turkey. About 131 greenhouse workers were interviewed with within the province of Izmir: 63.8% graduated from primary school; 49.2% were living in region where the greenhouses were located; 46.2% were working in their own greenhouses; 42.3% had health insurance; 86.9% used pesticides and 59.3% did not take protective cautions during use pesticide. The application technique, location, amount, time or any other required information of the pesticides used have not been recorded by the greenhouse workers (Ergonen, et. al, 2003).

2.3.3 Pesticide Contamination in Drinking Water

Pesticides not taken up by plants, adsorbed by soils or broken down by sunlight, soil organisms or chemical reactions may ultimately reach groundwater sources of drinking water. This will depend upon the nature of the soil, depth to groundwater, chemical properties of the pesticide, and the amount and timing of precipitation or irrigation in an area. Usually the faster a pesticide moves through the ground, as with sandy soils and heavy rainfall or irrigation, the less filtration or breakdown. Heavier soils, combined with lower moisture levels and warmer temperatures, provide a greater opportunity for pesticides to break down before reaching groundwater.

The amount of a pesticide detected in well samples also relates to the kind of pesticide and the amount originally applied. Contamination problems can result from using high concentrations of water-soluble pesticides for a specific crop in a vulnerable area.

Pesticides that are applied correctly may wash away from the application site. Rain falling on a treated area before the pesticide binds or degrades may carry the pesticide to surface water sources. Pesticides can seep into and through the soil during recharge of ground water and get into aquifers. Aquifers are underground areas that can be tapped to provide fresh water. Water slowly flows

through an aquifer like underground water. Pesticides are sometimes applied directly to lakes or wetlands for control of aquatic weeds, insects, or fish. However, these products are labeled to avoid use near drinking water systems. Some pesticides can move in air from the application site to surface waters used in a drinking water system. When a pesticide is spilled, dumped, or misused, the chance of it reaching drinking water is greater than with labeled uses. Pesticides can get into drinking water when homeowners illegally dump unused pesticides down the drain.

Pesticides used in the recharge zone might be found in drinking water. For example, drinking water in an agricultural area may contain pesticides that were sprayed on agricultural crops. Pesticides with certain chemical characteristics, such as high water solubility, are more likely to be found in drinking water. If pesticides are spilled or misused near a well, they can get into drinking water.

Pesticides found in drinking water may be harmful to human health. The toxicity of pesticides and the amount detected determine if any health effects are likely. Pesticides can affect human health and the environment depending on how much chemical is present, the length and frequency of exposure, and the toxicity of the pesticide. Effects also depend on the health of a person and the condition of the environment when exposure occurs. Laboratories can detect extremely low levels of pesticide in drinking water that are unlikely to be harmful to humans. Individual pesticides have different effects on humans. Variation exists in the toxicity of pesticides and the sensitivity of people to chemicals. In large amounts, some pesticides can cause long-term health effects, such as cancer or organ damage, in laboratory animals. Some pesticides at high doses can cause reproductive effects in laboratory animals. Most pesticides will have toxic effects on animals at high levels. Some pesticides are toxic at medium or low levels. Pesticides in drinking water are usually found at very low levels.

Drinking water contaminated with pesticides can be treated by a local water treatment facility to remove most of the pesticides. Activated carbon or reverse osmosis filters can be effective at removing pesticides from water. Scientists are conducting research to better understand the long term effects of certain pesticides in drinking water (Hamilton, et. al, 2004).

2.3.4 Pesticide Regulations

The WHO guidelines for drinking-water quality on 1993 set the standard pesticide range between 0.03 µg/L and 100 µg/L. These levels are detected according to the consequence of any potential health problems. For example, the level for 2,4-D is set to be 30 µg/L.

In the EU Drinking Water Directive (1998), the values for individual pesticides and for total pesticides were retained 0.1µg/L and 0.5µg/L respectively, and more stringent values were introduced for certain pesticides 0.03µg/L.

EPA National Primary Drinking Water Regulations set some non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals (MCLG). The directory of MCLG values of contaminants are tabulated in EPA Drinking Water Regulation sheets on web pages. For example, the MCLG for 2,4-D has been set at 70 parts per billion (ppb) because this level of protection would not cause any of the potential health problems. Based on MCLG, EPA has set an enforceable standard called a Maximum Contaminant Level (MCL). MCLs are set as close to the MCLGs as possible, considering the ability of public water systems to detect and remove contaminants using suitable treatment technologies. The MCL has been set at 70 ppb for 2,4-D because by means of the present technology and resources, this is the lowest level to which water systems can reasonably be required to remove this contaminant that occurs in drinking water.

2.4 2,4-Dichlorophenoxyacetic Acid

2,4-D is a common synthetic auxin, which is a class of plant growth regulators. It is absorbed through leaves and is translocated to the meristems of the plant. Uncontrolled, unsustainable growth ensures causing stem curl-over, leaf withering, and eventual plant death.

2,4-D was developed during World War II by a British team, aiming to increase crop yields for a nation at war. When it was commercially released in

1946, it became the first successful selective herbicide and allowed for greatly enhanced weed control in wheat, corn, rice and similar cereal grass crop, because it only kills dicots, leaving behind monocots.

Nowadays, 2,4-D is the third-most widely used herbicide in North America and the most widely used herbicide in the world. Commercial names for products containing 2,4-D include Aqua-Kleen, Barrage, Lawn-Keep, Malerbane, Planotox, Plantgard, Savage, Salvo, Weedone and Weedtrine-II.

2,4-D is mostly used for:

- Weed control in lawns and other turf
- No-till burndown
- Control weeds and brush along fences and highway
- Conifer release
- Grass hayfields and pastures
- Cereal grains
- Corn and sorghum
- As a synthetic auxin analogue

2,4-D continues to be used, where legal, for its low cost. However, where municipal lawn pesticide bylaws exist, such as Canada, alternatives such as corn gluten meal and vinegar based products are increasingly being used to combat weeds.

2.4.1 Chemical Characteristics of 2,4-Dichlorophenoxyacetic Acid

The basic form of 2, 4-D is 2,4-Dichlorophenoxyacetic acid (CAS 94-75-7) but is often formulated as an inorganic salt, amine or ester. The chemical characteristics of 2,4-D are given in Table 2.6 (WHO, 1984).

Table 2.6 Chemical characteristics of 2,4-D

Characteristics	Values
Molecular weight (g/mol)	221.04
Melting point (°C)	135-142
Boiling point (°C) (at 4 mmHg)	160
Water solubility (ppm) (average of 2 values near 25°C at pH=7)	3.39×10^4
Vapor pressure (mmHg) (at 25°C)	1.4×10^{-7}
Hydrolysis half-life (days) (average of two values at 25°C, pH=7)	39
Aqueous photolysis half-life (days) (at 25°C)	13
Anaerobic aquatic half-life (days)	312
Aqueous aerobic half –life (days)	15
Aerobic half-life (days)	66
Soil photolysis half-life (days)	393
Field dissipation half-life (days)	59.3
Henry’s constant	1.76×10^{-12}
Octonal-water coefficient (Kow)	$(9.15- 6.74) \times 10^2$
Soil adsorption coefficient (Koc) (cm ³ /g) (data for four soil types)	0.067-1.1

2.4.2 Behavior of 2,4-Dichlorophenoxyacetic Acid in the Environment

The WHO (1984) concluded that 2,4-D does not accumulate or persist in the environment. The fate of 2,4-D in the environment is largely dependent on the ambient pH (Aly & Faust, 1964). At pH levels above 7, 2,4-D is converted rapidly to the anion (negatively charged) form, which is more susceptible to photo degradation and microbial metabolism, and less likely to adsorb to soil particles. At pH levels < 4, microbial degradation is inhibited and 2,4-D retains its molecular form and resists degradation (Johnson et al., 1995a). The primary degradation mechanism is microbial metabolism, but mineralization and possibly photolysis may also play a role. 2,4-D degrades rapidly in sunlight under

laboratory conditions, but photo degradation has not been demonstrated in the field. The average half-life (the time it takes for the herbicide concentration to decline by 50%) is 10 days, but rates of degradation can vary from several hours to several months or longer. Degradation rates are determined by the microbial population, environmental pH, soil moisture and temperature (Sandmann et al., 1988). The type of 2,4-D formulation applied does not significantly affect the rate of degradation (Wilson et al., 1997). 2,4-D does not readily undergo chemical degradation, relative to other herbicides (Que Hee and Sutherland, 1981).

Behavior in Soil

2,4-D may be applied in acid, salt, or ester formulations, but in most cases, each of these formulations are apparently converted rapidly to the acid form once it contacts soil. Consequently, the rate of dissipation from soils is often the same regardless of the formulation of 2,4-D that is applied .

Half-lives are short, ranging from a few days to several months but detectable residues can persist for up to a year.

Degradation is almost entirely through microbial metabolism. Soil conditions that maximize microbial populations (i.e. warm and moist with a high organic content) maximize degradation rates. Wilson et al. (1997) found that adequate soil moisture was the most influential parameter affecting degradation rates. Johnson et al. (1995b) found that plants do not play a significant role in eliminating 2,4-D from soils.

Most formulations of 2,4-D do not bind tightly with soils and therefore, have the potential to leach down into the soil column and to move off-site in surface or subsurface water flows. In many cases, extensive leaching does not occur, most likely because of the rapid degradation of the herbicide (Que Hee and Sutherland, 1981). Where 2,4-D does leach, however, it will be more persistent because populations of microbes responsible for the degradation of 2,4-D tends to decrease with soil depth (Wilson et al., 1997).

2,4-D can also be lost from soils through volatilization. Volatilization rates are determined by the temperature and molecular form of the herbicide at the surface of the soil, which, in turn, is determined primarily by the soil's pH.

Johnson et al. (1995a) found that soil organic content and soil pH are the main determinants of 2,4-D adsorption in soils. As the herbicide concentration increases, the percentage of herbicide adsorbed decreases, possibly because the number of binding sites on soil particles are finite and become filled. Inorganic clays can also bind 2,4-D particles. A relatively high concentration of clay particles however, is required to bind small concentrations of 2,4-D (Aly and Faust, 1964).

Behavior in Water

2,4-D will change form and function with changes in water pH (Que Hee & Sutherland, 1981). In alkaline (high pH; $\text{pH} > 7$) waters, 2,4-D takes an ionized (negatively charged) form that is water-soluble and remains in the water column. Theoretically, in water of a lower pH, 2,4-D will remain in a neutral molecular form, increasing its potential for adsorption to organic particles in water and increasing its persistence (Wang et al., 1994a). 2,4-D is most likely to adsorb to suspended particles in muddy waters with a fine silt load, but little adsorption has been observed in the field

Degradation mechanisms are difficult to isolate in the field. Aly & Faust (1964) concluded that, in sediments with sufficient microbial populations, 2,4-D can be degraded in a matter of hours and it was unlikely that sufficient amount of ultraviolet radiation would reach the surface of natural waters to degrade 2,4-D.

Que Hee and Sutherland (1981) reported that concentrations of most 2,4-D residues found in lakes and streams are < 1 ppm, although concentrations of up to 61 ppm have been reported immediately following direct application to water bodies. These concentrations are well above the 0.1 ppm established as "permissible" levels for potable water by the E.P.A. (EPA, 1998).

2.4.3 Environmental Effects of Excess 2,4-Dichlorophenoxyacetic Acid

The lethal potential of a chemical is usually measured as the dose (mg/kg body weight), or as the concentration in the air, diet or water (mg/kg, mg/m³, mg/liter, respectively). These amounts are referred to as the LD₅₀ or LC₅₀.

The LC₅₀ value indicates the lethal concentration of an active ingredient which is expected to cause death to 50% of the test animals treated. The higher the LC₅₀ is the lower the acute toxicity of the compound.

Toxicity on Birds and Mammals

2,4-D is considered of moderate toxicity to animals, although LD₅₀ levels vary significantly between formulations and animal species. The majority of LD₅₀ values range between 300-1,000 mg/kg. For example, the LD₅₀ for 2,4-D acid in rats and bobwhite quail is 764 mg/kg and 500 mg/kg, respectively. Some animals such as dogs, however, are significantly more sensitive to 2,4-D organic acids than are rats and humans (Ibrahim et al., 1991).

2,4-D can bio-accumulate in animals. In Russia, residues of more than ten times the allowable level were found in eggs, milk, and meat products served by public caterers and one study reported residues in 46% of tested cattle (Que Hee & Sutherland, 1981). Risk to browsing wildlife, however, is low, it is analyzed 2,4-D residues in forest browse following aerial application to forests in Oregon and found them to be below the concentrations known to cause effects in mammals.

Toxicity on Aquatic Species

LC₅₀ levels for bluegill sunfish and rainbow trout are 263 and 377 mg/L, respectively. Wang et al. (1994b) studied bioaccumulation of 2,4-D in carp and tilapia and found that accumulation of up to 18 times the ambient concentration occurred within two days of exposure. 2,4-D was found in oysters and clams in concentrations up to 3.8 ppm, and it persisted for up to two months (Thomas &

Duffy, 1968). The highest concentrations of 2,4-D were generally reached shortly after application, and dissipated within three weeks following exposure.

2,4-D can accumulate in fish exposed to concentrations as low as 0.05 ppm (Wang et al 1994b) and concentrations of 1.5 ppm can kill the eggs of fathead minnows in 48 hours (Thomas & Duffy, 1968). After animals are removed from contaminated waters, they tend to excrete residues.

Toxicity on Other Non-Target Organisms

It is found that honey-bees directly sprayed with 2,4-D showed no injury and no residues were found in the bees or their honey (cited in Que Hee & Sutherland, 1981). These results, however, are questionable as the LD₅₀ for honey-bees is only 1 µg/bee (WSSA, 1994).

2.4.4 Human Toxicology of Excess 2,4-Dichlorophenoxyacetic Acid

2,4-D can be absorbed through the skin or through the lungs if inhaled. It is reported airborne residues of 1-35 micrograms/cubic meter of air when 2,4-D was applied using hand-held spray guns along power line right-of-ways. These workers later excreted <0.01-30 mg/kg of body weight in their urine. Absorption through the skin accounts for 90% of the 2,4-D absorbed by applicators.

Once in the body, 2,4-D is distributed rapidly with the greatest concentrations appearing in the kidneys and liver. The majority of the compound is excreted unmetabolized. Due to its solubility in water, 2,4-D is not believed to accumulate in tissues, but is excreted in the urine in less than a week (Ibrahim et al., 1991). Nevertheless, some agricultural workers and other applicators have experienced long term complications including pain, paresthesias (tingling or numbness), and paralysis following exposure to 2,4-D.

Accidental inhalation resulted in one reported case of acute poisoning. Symptoms included brief loss of consciousness, urinary incontinence, vomiting, muscular hypertonia (an abnormal increase in skeletal or smooth muscle tone), fever, headache and constipation. Workers that entered an area shortly after

treatment with 2,4-D experienced weakness, headache, dizziness, stomach pains, nausea, brief loss of consciousness, and moderate leukopenia (an abnormal reduction in the number of white blood cells, often reducing immune system function) (Stevens & Sumner, 1991).

In 1991, the case-control studies showed evidence of a relationship between 2,4-D exposure and non-Hodgkins lymphoma in humans, with some studies showing an increased risk with increased exposure level.

In another study of human exposure, female applicators were found to have a significant increase in cervical cancer associated with 2,4-D application. Due to the many confounding factors that make identification of cause and effect mechanisms difficult, other expert review panels including the EPA, Agriculture and Agri-food Canada, and the World Health Organization concluded that 2,4-D alone is not carcinogenic (Mullison and Bond, 1991).

2.5 Water Treatment Methods

It is well known fact that clean water is absolutely essential for healthy living. Exactly why a clean and reliable water supply was needed was not known until the second half of the nineteenth century, when the nature of infectious disease was first recognized and the ability of water supplies to transmit diseases such as cholera and typhoid was first demonstrated. After this, concerns about the quality of drinking water focused on disease-causing microorganisms (pathogens) in public water supplies.

Scientists discovered that contaminations in the water that could also indicate a health risk. As a result, drinking water treatment systems were designed to reduce these contaminations, for example removing pathogens that were causing typhoid, dysentery and cholera, or pesticides that were causing cancerogenic effects.

The choice of which treatment methods to use from the great variety of available processes depends on the characteristics of the water, the types of water quality problems likely to be present and the costs of different treatments.

Precipitation, coagulation, sedimentation and flocculation are very effective at removing fine suspended particles. They can remove up to 99.9 percent of the bacteria and 99 percent of the viruses from water supplies. They also remove some of the organic matter that gathers as water travels across the landscape, from raindrop to river.

Ion exchange units can be used to remove any charged (ionic) substance from water, in other word; ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. Water is pretreated to reduce the suspended solids and total dissolved solids (TDS) load to the ion-exchange unit.

Reverse osmosis is one option for drinking water treatment problems which is an effective method to reduce certain ions and metals, such as nitrate and arsenic. It also can remove certain pesticides, organic and inorganic compounds, though it is not effective for others.

Adsorption occupies an important position among the unit operations in water and wastewater treatment. Adsorption operations exploit the ability of certain solids preferentially to concentrate specific substances from solution onto their surfaces.

The treatment techniques that are used in the removal of 2,4-D from water is tabulated with working principles, advantages and disadvantages in Table 2.7.

The comparison of treatment of water by adsorption (potential adsorbents to remove various contaminants such as color, heavy metals, COD, pesticides etc.) is tabulated in Table 2.8.

Table 2.7 The comparison of water treatment techniques that are used in the removal of 2,4-D from water

Water Treatment Techniques	Advantages & Disadvantages	References
Precipitation	Advantage: *Greater removal efficiency, the feasibility of using higher overflow rates, and more consistent performance. Disadvantages: *Coagulation results in a larger mass of primary sludge that is often more difficult to thicken and dewater. *Entails higher operational costs.	Porras and Talens, 1999 Porras and Talens, 2000
Ion Exchange	Advantages: * In process, like reverse osmosis, can be used with fluctuating flow rates. *Effluent contamination is virtually impossible. *Large variety of specific resins are available from suppliers. Each resin is effective in removing specific contaminants. Disadvantages: *Ion exchange waste is highly concentrated and requires careful disposal. *Potential for unacceptable levels (peaks) of contamination in effluent. *Usually not feasible with high levels of TDS. *Pretreatment required for most surface waters. * Ion exchange units also are sensitive to the presence of competing ions	Kwan and Chu, 2006 Legrouiri et al., 2005 Qui et al., 2006
Reverse Osmosis	Advantages: *Removes nearly all contaminant ions and most dissolved non-ions. *Relatively insensitive to flow and TDS level, and thus suitable for small systems with a high degree of seasonal fluctuation in water demand. *RO operates immediately, without any minimum break-in period. * Low effluent concentration possible. * Bacteria and particles are also removed. *Operational simplicity and automation allow for less operator attention and make RO suitable for small system applications. Disadvantages: *High capital and operating costs. (but less expensive than ion exchange) *Managing the wastewater (brine solution) is a potential problem. *High level of pretreatment is required in some cases. *Membranes are prone to fouling.	Edwards and Schubert, 1974 Hofman et al., 1997
Adsorption	Advantages: *Low cost (in most biosorbents) *High efficiency Disadvantage: *Possibility of regeneration of adsorbent	Aksu and Kabasakal, 2004 Kim et al., 2002 Goyne et al., 2004

Table 2.8 Comparison of treatment of water by adsorption

Adsorbent	Adsorbate	Parameter Investigated	Conclusion	References
PAC	Distillery Spent wash	*Initial conc. *Adsorbent dosage	Removal of 18% is noted and when spent was pretreated with polyelectrolyte as a flocculating agent, the color removal was increased up to 99%.	Sekar and Murthy, 1998
PAC	Synthetic dye wastewater of Disperse-red-60	*Effect of contact time *Concentration of PAC.	95-98% of COD removal for 25-200 ppm & 88-98% for various particle sizes. Freundlich, Langmuir, Dziubek & Kowals adsorption isotherms fit well. Film-pore double resistance diffusion model describes the mass transfer. External film mass transfer coefficient increases with decrease in particle size.	Yeh and Thomas, 1995
GAC [Industrial grade; Laboratory grade]	Catechol	*Initial Concentration *pH *Adsorbent dosage *Particle Size	Equilibrium studies showed that IGGAC has the maximum adsorption capacity. Diffusion studies showed that initial part of the adsorption is attributed to external mass transfer effects followed by intraparticle diffusion.	Mahesh et al., 1998
Fly ash	Copper (Synthetic)	*Initial conc. *pH *Adsorbent dosage	Maximum efficiency was at the pH of 6.0. The fly ash treatment raises the pH of effluent.	Kapadia et al., 2000
GAC	Nitro phenol	*Bed depths *Flow rates	The experimental half lives are very close to predicted by self-sharpening wave approach and proportional to bed depth-flow rate ratio. Experimental and predicted breakthrough curves are in good agreement.	Chern and Chien, 2002
Lignite	Cr (IV)	*Adsorbent dosage	Kinetic showed the applicability of Lagergren model. The data tend to follow first order rate kinetics.	Balasubramanian et al., 1998
Blast furnace dust Slag	Raw Paper mill effluent	*Initial COD conc. *pH *Temperature *Adsorbent dosage.	COD removal efficiency of BFD is significantly better than Basic slag.	Das and Patnaik, 2001
PAC	2,4-D	*pH *Temperature *Initial concentration *Capacity vs Kinetics	The max. adsorption capacity occurs at pH=2; adsorption increased with increasing conc., and decreased with increasing temp. up to 45 °C.	Aksu and Kabasakal, 2005
GAC	2,4-D CPA 2,4-DNP	*pH *Kinetics	Adsorption equilibrium capacities of herbicides on GAC increased with decreasing of pH.	Kim et al., 2005
GAC	2,4-D 2,4 DNP	*Type of adsorbent *Capacity vs Kinetics *pH *Temperature	Adsorption capacities of adsorbents; decreased with increasing pH and temperature.	Kim et al., 2002

2.6 Adsorption from Theory to Practice

Innumerable physical, chemical and biological processes take place at the boundary between two phases, while others are initiated at that interface. The change in concentration of a given substance at the interface as compared with the neighbouring phases is referred to as “**adsorption**” and the material in the adsorbed state is defined as “adsorbate.” Depending on the type of phases in contact, this process can be considered in the following systems: liquid-gas, liquid-liquid, solid-liquid and solid-gas (Dabrowski, 2001).

The term “adsorption” deals with the process in which molecules accumulate in the interfacial layer whereas the penetration by the adsorbate molecules into the bulk solid phase is determined as “absorption”. The term ‘sorption’ - together with the terms ‘sorbent’, ‘sorbate’, and ‘sorptive’ – is also used to denote both adsorption and absorption, when both occur simultaneously or cannot be distinguished (Dabrowski, 2001).

2.6.1 Types of Adsorption

Adsorption can result either from the universal van der Waals interactions (physical adsorption, physisorption) or it can have the character of a chemical process (chemical adsorption or chemisorption). Contrary to physisorption, chemisorption occurs only as a monolayer. Physical adsorption can be compared to the condensation process of the adsorptive. As a rule, it is a reversible process that occurs at a temperature lower or close to the critical temperature of an adsorbed substance.

Physical adsorption is very effective particularly at a temperature close to the critical temperature of a given gas. Chemisorption occurs usually at temperatures much higher than the critical temperature and – by contrast to physisorption – is a specific process which can only take place on some solid surfaces for a given gas. Under favorable conditions, both processes can occur simultaneously or alternately. Physical adsorption is accompanied by a decrease

in free energy and entropy of the adsorption system and, thereby, this process is exothermic (Dabrowski, 2001).

Kumar et al. (2004) were summarized the physical and chemical adsorption characteristics (Table 2.9). Although this distinction is conceptually useful, many cases are intermediate and it is not always possible to categorize a particular system unequivocally (Aly and Faust, 1987).

Table 2.9 Typical characteristics of adsorption processes

Characteristics	Physical Adsorption (Van der Waal's Adsorption)	Chemical Adsorption (Activated Adsorption)
Adsorption Temperature	Near or below boiling point temperature of adsorbate	Unlimited range
Adsorption Enthalpy	5-40 kJ/mol	40-800 kJ/mol
Crystallographic Specificity	Independent of surface geometry	Marked difference for between crystal planes
Nature of Adsorption	Non-dissociative & Reversible	Dissociative & Irreversible
Saturation	Monolayer/Multilayer	Monolayer
Adsorption Kinetics	Fast, non-activated process	Activated process

2.6.1.1 The Physical Adsorption Theory

It is well known that in the literature there are more than 100 isotherm equations derived based on various physical, mathematical, and experimental considerations. The different types of adsorption, solid/gas (S/G), solid/liquid (S/L), and liquid/gas (L/G), have, apparently, various properties and, therefore, these different phenomena should be explained with different physical pictures and mathematical treatments. These models are very important for the calculation of the energy distribution functions (Gaussian, multi-Gaussian, quasi-Gaussian, exponential) and so it is possible to characterize the solid adsorbents. Evidently, for these calculations, one must apply different isotherm equations based on various theoretical and mathematical treatments. Thus, all of these different isotherm equations have a common thermodynamical base which makes possible a common mathematical treatment of physical adsorption (Toth, J., 2001).

2.6.1.1.1 The Forces Involved in Physical Adsorption

The forces involved in physical adsorption include both van der Waals forces (dispersion-repulsion) and electrostatic interactions comprising polarization, dipole and quadrupole interactions. The van der Waals contribution is always present while the electrostatic contributions are significant only in the case of adsorbents such as zeolites which have an ionic structure (Ruthven, 1984).

Neglecting the higher-order contributions to the dispersion energy and combining the inverse sixth-power attraction with the inverse twelfth-power repulsion leads to the Lennard-Jones potential function which is also sketched in the Figure 2.4 (Ruthven, 1984):

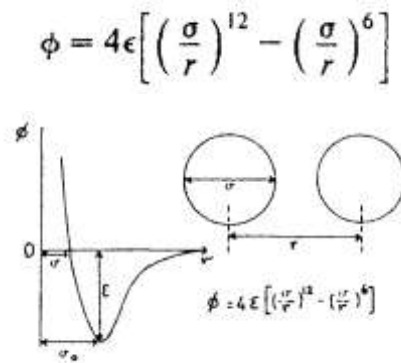


Figure 2.4 Lennard-Jones Potential

The bonding energy ϵ and the tension force σ are characteristics of the particular molecular species and tabulated values for many common molecules are available. The values may be determined either from transport properties (viscosity, thermal conductivity) or from detailed measurements of the deviations from the ideal gas law (second virial coefficients).

The interaction between two different molecules is generally represented by the arithmetic mean of σ and the geometric mean of ϵ :

$$\sigma_{12} = \frac{1}{2}(\sigma_1 + \sigma_2), \quad \epsilon_{12} = \sqrt{\epsilon_1 \epsilon_2}$$

Setting $d\phi/dr = 0$ in the equation above gives, for the equilibrium separation between centers of an isolated pair of molecules:

$$\sigma_0 = 2^{1/6}\sigma$$

2.6.1.1.2 The General Classification of Physical Adsorption Isotherms

Brunauer et.al. have divided the isotherms for physical adsorption into five classes as illustrated in the Figure 2.5 (Oscik, 1982; Ruthven, 1984).

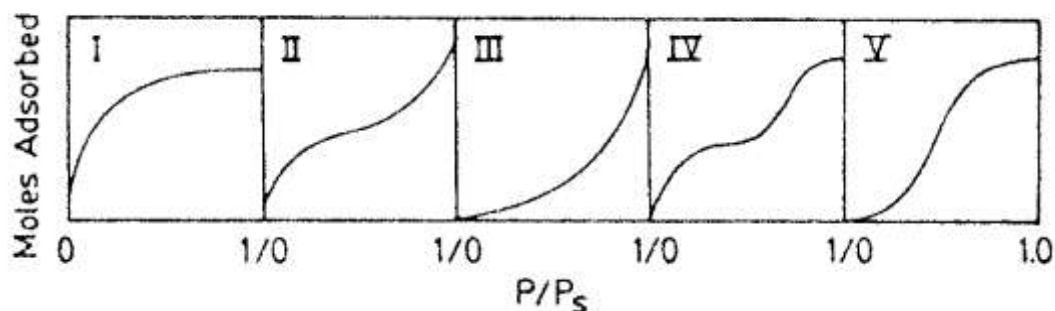


Figure 2.5 Types of Physical Adsorption Isotherms

Type I: It is valid for monolayer adsorption. This is a typical Langmuir type of isotherm. The pore size of the microporous adsorbents is not very much greater than the molecular diameter of the adsorbate molecule. The equilibrium concentration approaches monotonically to the limiting adsorption value.

Type II: It is valid for multilayer adsorption. An example to this type may be the adsorption of benzene vapors onto the graphitized carbon black.

Type III: Like Type II, it is valid for multilayer adsorption, but is very uncommon. Type II and III are generally observed only in adsorbents in which there is a wide range of pore sizes. In such systems, there is a continuous progression with increasing loading from monolayer to multilayer adsorption and then to capillary condensation. An example to this type may be the adsorption of bromine onto the silica gel.

Type IV: It is valid for multilayer adsorption. It suggests the formation of two surface layers either on a plane surface or on the wall of a pore very much wider than the molecular diameter of adsorbate.

Type V: If intermolecular attraction effects are large, this type of isotherm is observed as for example in the sorption of phosphorous vapor on NaX.

2.6.1.2 The Chemical Adsorption Theory

Chemisorption is a sub-class of adsorption, driven by a chemical reaction occurring at the exposed surface. A new chemical species is generated at the adsorbant surface (e.g. corrosion, metallic oxidation). The strong interaction between the adsorbate and the substrate surface creates new types of electronic bonds - ionic or covalent, depending on the reactive chemical species involved (Oura et. al, 2003).

It is characterised by:

- a high activation energy for the chemical reaction that takes place only in a monolayer
- a high enthalpy change/ temperature gain, indicating an exothermic chemical reaction: $-50 \text{ kJ/mol} > \Delta H > -800 \text{ kJ/mol}$

Due to specificity, the nature of chemisorption can greatly differ from system to system, depending on the chemical identity and the surface structure.

The main way in which most chemists utilize the effect of chemisorption is in catalyzed reactions. The process of chemisorption is actually pivotal to the role of heterogeneous catalysis where the catalyst is in a solid phase—particularly transition metal catalysts. In many cases the chemical reagents will both bind to the catalytic surface. The chemical bonds then form and draw electrons away from the chemisorption bonds. The molecule then desorbs and is free to leave the surface.

As a substance of adsorption, chemisorption follows the adsorption process. The first stage is for the adsorbate particle to come into contact with the surface. The particle needs to be trapped onto the surface by not possessing enough energy to leave the gas-surface potential well. If it elastically collides with the surface, then it would return to the bulk gas. If it loses enough momentum through an inelastic collision, then it “sticks” onto the surface, forming a precursor state bonded to the surface by weak forces, similar to physisorption. The particle diffuses on the surface until it finds a deep chemisorption potential well. Then it reacts with the surface or simply desorbs after enough energy and time (Rettner et. al., 1996).

The reaction with the surface is dependent on the chemical species involved. Applying Gibbs free energy equation for reactions: $\Delta G = \Delta H - T\Delta S$. General thermodynamics states that for reactions, the change in free energy should be negative. Since a free particle is restrained to a surface, and unless the surface atom is highly mobile, entropy is lowered. This means that the enthalpy term must be negative, implying an exothermic reaction (Gasser, 1985).

Physisorption is given as a Lennard-Jones potential and chemisorption is given as a Morse potential. There exists a point of crossover between the physisorption and chemisorption, meaning a point of transfer. It can occur above or below the zero-energy line (with a difference in the Morse potential) representing an activation energy requirement or lack of. Most simple gases on clean metal surfaces lack the activation energy requirement.

A particular brand of gas-surface chemisorption is the dissociation of diatomic gas molecules, such as hydrogen, oxygen, and nitrogen. One model used to describe the process is precursor-mediation. The absorbed molecule is adsorbed onto a surface into a precursor state. The molecule then diffuses across the surface to the chemisorption sites. They break the molecular bond in favor of new bonds to the surface. The energy to overcome the activation potential of dissociation usually comes from the translational energy and vibrational energy (Rettner et. al., 1996).

The most striking example is the hydrogen and copper system, one that has been studied many times over. It has a large activation energy of 0.35 - 0.85 eV. The vibrational excitation of the hydrogen molecule promotes dissociation on low index surfaces of copper (Rettner et. al., 1996).

2.6.2 Adsorbent Materials

Some adsorbents that are used in the adsorption process of 2,4-D are tabulated in Table 2.10.

Table 2.10 Some adsorbents that are used in the adsorption process of 2,4-D

Adsorbate	Material Type	Adsorbent Type	Adsorbent	References
2,4-D	Inorganic	Alumina	α -Alumina	Clausen et al., 2001
			Alumina	Goyne et al., 2004
			Zinc-aluminium-chloride layered double hydroxides	Legrouri et al., 2005
		Silica	Silica-supported β -cyclodextrin derivatives	Phan et al., 2002
			Chemically modified silica gel surface	Prado and Airolidi, 2001
			Silica	Goyne et al., 2004
		Zeolites	Zeolite-supported TiO ₂	Shankar et al., 2006
		Clay	Organo-clays	Akçay and Yurdakoç, 2000
		Hydrotalcite	Calcinated Hydrotalcite	Pavlovic, 2005
			Sepiolite and DAS	Akçay et al., 2005
	Organic	Polymer	Modified hypercrosslinked polystyrene (NDA-99)	Qui et al., 2003
			Crosslinked Poly(N-vinyl pyrrolidone)	Saraydin and Karadag, 1996
			Waste tyre rubber granules	Alam et al., 2006
			Rubber granules,	Alam et al., 2000
		Carbon	Gac SLS103, WWL	Cho et al., 2003
			H- type Gac 6-16 mesh size	Aksu and Kabasakal, 2004
			PAC	Aksu and Kabasakal, 2005
			Gac F400	Chingombe et al., 2006
				Cho et al., 2003
				Kim et al., 2002
			Viz.wood charcoal, bottom ash	Alam et al., 2000
			CH supplied PAC & (CL)(grad 20070D)	Belmouden et al., 2000
	Biological		Macro fungi sajor caju and florida	Alam et al., 2000
			Fertilizer and Steel Industry Wastes	Gupta et al., 2006

2.7 Operational Modes

2.7.1 Batch Mode

In batch contact unit systems, a predetermined amount of adsorbent is mixed with the water for a given contact period and subsequently is separated by sedimentation or filtration. The optimum of application should allow adequate dispersion of the carbon and sufficient contact time to ensure maximum adsorption.

The first step in the evaluation of carbon dosage is determining the contact period. This is determined by adding a known quantity of carbon to a given volume of the water to be treated and placing the mixture in a stirring apparatus. A sample is removed at various time intervals and the carbon is immediately removed by filtration or centrifugation (for powdered forms). The residual concentration of the contaminant subsequently is determined in each sample. The data are plotted to show the change in concentration with time. Adsorption equilibrium is achieved at the contact period where no significant change in concentration is observed with increased time (Faust and Aly, 1987).

The amount of adsorbate adsorbed onto activated carbon at equilibrium is calculated from the following mass balance equation.

$$q = (C_i - C) \frac{V}{W} \quad (1.1)$$

Here q is the equilibrium amount adsorbed on the adsorbent (mg/ g), C_i is the initial concentration of bulk fluid (mg/L), C is the equilibrium concentration of the solution (mg/L), V is the volume of solution (L), and W is the weight of adsorbent (g) (Cho et al., 2003).

Comparison of batch mode studies is tabulated in Table 2.11.

Table 2.11 Comparison of batch mode studies with activated carbon

Adsorbent Form	Adsorbent Size	Adsorbate	Parameter Investigated	Parameter Range	Conclusion	References
GAC	0,055-0,065cm in diameter	2,4-D 2,4 DNP	*Type of adsorbate *Capacity vs Kinetics *pH *Temperature	pH=3,5; 7,0; 10,0 T=288,15K; 298,15K; 313,5K	Adsorption capacities of adsorbents; decreased with increasing pH and temperature.	Kim et al., 2002
AC Polymeric resins	-	Beta-lactam antibiotics	*Type of adsorbent *Capacity *pH	pH=3,5; 5,0; 7,0	AC provides higher adsorption capacity than studied polymeric resins.	Dutta et al., 1999
GAC Biologic adsorbents& rubber granules	0,15-0,30mm	2,4-D Atrazine	*Type of adsorbent *Capacity vs Kinetics	Adsorption capacity	Adsorption capacity of the Activated charcoal is the max compared to other adsorbents.	Alam et al., 2000
GAC AC&AC-S	8-20 mesh size	Lead(II)	*Type of adsorbent *Capacity	Adsorption capacity	Pb(II) adsorption onto AC-S shows about 35% increase respect to adsorption on AC.	Goel et al., 2005
PAC Afsin-elbistan fly ash	-	C.I Reactive Black	*Type of adsorbent *Capacity vs Kinetics *Initial dye concentration *Adsorbent dosage	Adsorption capacity	Afsin-elbistan fly ash shows lower adsorption capacity.	Eren and Acar, 2006
GAC AC-C	*0,6-1.7mm *Cloth	Zn(II)	*Type of adsorbent *pH *Capacity	pH=3-6	GAC shows the min adsorption capacity. Increasing the pH causes decreases Zn (II) ions removal.	Alvarez-Merino et al., 2005
PAC	<0,15mm	2,4-D	*pH *Temperature *Initial concentration *Capacity vs Kinetics	pH=2-8 T=25; 35; 45 °C C=106; 204,1; 416,8; 628,6 mg/L	The max. adsorption capacity occurs at pH=2; adsorption increased with increasing conc., and decreased with increasing temp.up to 45 °C.	Aksu Kabasakal, 2005
PAC	CH&(CL) (grad 20070D)	2,4-D	*Type of adsorbent *Contact time *Adsorbent concentration *pH	Time=0-180 min pH=1-10	CH shows the max adsorption capacity. The adsorption decreases with the increase in adsorbent conc. Because of aggregation of AC particles.	Belmouden et al., 2000
GAC	F400	2,4-D CPA 2,4-DNP	*pH *Kinetics	pH=3,5; 7,0; 10,0	Adsorption equilibrium capacities of herbicides on GAC increased with decreasing of pH.	Kim et al., 2005

2.7.1.1 Kinetic Modeling of Adsorption in a Batch System

If the movement of organic pollutant molecule from the bulk liquid to the liquid film or boundary layer surrounding the adsorbent is ignored, the following sequence of steps can take place in the adsorption process of porous adsorbent transport of solute molecules from the boundary film to the external surface of the adsorbent (film diffusion), transfer of molecules from the surface to the intraparticle active sites and uptake of molecules by the active sites of sorbent. In the removal of organics from wastewater, it is important for design purposes to investigate the mechanisms of adsorption and potential rate controlling steps which control the adsorption rate. In order to find the contribution of rate controlling steps such as external mass transfer, intraparticle diffusion, and adsorption process and also, mass transfer and kinetic models have been used to test the experimental data.

In the first step of adsorption, the film diffusion is an important rate-controlling step and external mass transfer or boundary layer diffusion can be characterized by the initial rate of solute sorption. In this case, adsorption rate is expected to be proportional to the first power of concentration; it means that this step is a first-order process and can be defined as:

$$\frac{dC}{dt} = k_{1,ad} C \quad (1.2)$$

where C is the pollutant concentration in the wastewater remaining at each contact time and $k_{1,ad}$ is the adsorption rate constant. After integration and applying boundary conditions, $t = 0$ to $t = t$ and $C = C_0$ to $C = C$; the integrated form of Eq. (1.2) becomes;

$$\log \frac{C_0}{C} = \frac{1}{2.303} k_{1,ad} t \quad (1.3)$$

where C_0 is the initial pollutant concentration (Chu and Chen, 2002).

If intraparticle diffusion is involved in the sorption process, the model developed by Weber and Morris, (1963) can be used to find the region where intraparticle diffusion is rate-limited and to determine intraparticle diffusion rate.

An empirically found functional relationship, common to the most adsorption processes, is that the uptake varies almost proportionally with $t^{1/2}$, the Weber–Morris plot, rather than with the contact time t (Weber and Morris, 1963).

$$q_t = k_{id}t^{1/2} + C \quad (1.4)$$

where k_{id} is the intra-particle diffusion rate constant. According to Eq. (1.4), a plot of q_t versus $t^{1/2}$ should be a straight line with a slope k_{id} and intercept C when adsorption mechanism follows the intra-particle diffusion process. Values of intercept give an idea about the thickness of boundary layer, i.e., larger the intercept with greater is the boundary layer effect (Kannan and Sundaram, 2001).

Assuming spherical geometry for the adsorbent, the time for half adsorption can be correlated to the pore diffusion coefficient (Michelson et al., 1975).

$$t_{1/2} = 0.03r_0^2 / D_p \quad (1.5)$$

where $t_{1/2}$ is the time for half adsorption (s), r_0 is the radius of the adsorbent particle (cm) and D_p is the diffusion coefficient (cm^2/s). Values of D_p can be calculated for different temperatures etc.

Kinetic data can further be used to check whether pore diffusion is the only rate-controlling step or not in the adsorption system using Bangham's equation (Aharoni et al., 1979).

$$\log \log (C_o / (C_o - q_t m)) = \log (k_o m / 2.303V) + \log (t) \quad (1.6)$$

where C_0 is the initial concentration of adsorbate in solution (mg/L), V is the volume of solution (mL), m is the weight of adsorbent per liter of solution (g/L), q_t (mg/g) is the amount of adsorbate retained at time t and k_0 is constant.

In many cases, the kinetics of adsorption based on overall adsorption rate by adsorbents are described by the first-order Lagergren (Lagergren, 1898) and pseudo second-order (McKay and Ho, 1999) kinetic models.

The sorption kinetics may be described by a pseudo-first-order according to Lagergren (Lagergren, 1898), Ho and McKay (Ho and McKay, 1998), Namasivayam and Kardivelu (Namasivayam and Kardivelu, 1999), the first-order rate expression of Lagergren based on the sorption capacity of adsorbent is generally expressed as follows:

$$\frac{dq}{dt} = k_{1,ad}(q_{eq} - q) \quad (1.7)$$

where q_{eq} is the amount of solute adsorbed at equilibrium per unit weight of adsorbent (mg/g), q the amount of solute adsorbed at any time (mg/g) and $k_{1,ad}$ (L/min) is the rate constant of Lagergren first-order adsorption.

After integration and applying boundary conditions, $t = 0$ to $t = t$ and $q = 0$ to $q = q$; the integrated form of Eq. (1.7) becomes

$$\log(q_{eq} - q) = \log q_{eq} - \frac{k_{1,ad}}{2.303} t \quad (1.8)$$

A straight line of $\log(q_{eq} - q)$ versus t suggests the applicability of this kinetic model. In order to fit Eq. (1.8) to experimental data, the equilibrium sorption capacity, q_{eq} , must be known. In many cases q_{eq} is unknown and as adsorption tends to become immeasurably slow, the amount sorbed is still significantly smaller than the equilibrium amount. For this reason it is necessary to obtain the real equilibrium sorption capacity, q_{eq} , by extrapolating the experimental data to $t = \infty$ or by using a trial and error method. Furthermore, in most cases the first-order equation of Lagergren does not fit well for the whole

range of contact time and is generally applicable over the initial 20–30 min of the sorption process.

The pseudo second-order equation is also based on the sorption capacity of the solid phase. Contrary to the other model it predicts the behavior over the whole range of adsorption. The sorption kinetics may also be described by a pseudo-second-order according to Ho and McKay (Ho and McKay, 1998-1999) and the pseudo second-order kinetic rate equation is expressed as

$$\frac{dq}{dt} = k_{2,ad} (q_{eq} - q)^2 \quad (1.9)$$

where q_{eq} is the amount of solute adsorbed at equilibrium per unit weight of adsorbent (mg/g) (the equilibrium adsorption capacity), q is the amount of solute adsorbed at any time (mg/g) and $k_{2,ad}$ (g/mg min) is the rate constant of second-order adsorption. For the boundary conditions $t = 0 \rightarrow t = t$ and $q = 0 \rightarrow q = q$; the integrated and linear form of Eq. (1.9) becomes

$$\frac{t}{q} = \frac{1}{k_{2,ad} q_{eq}^2} + \frac{1}{q_{eq}} t \quad (1.10)$$

If second-order kinetics are applicable, the plot of t/q against t of Eq. (1.10) should give a linear relationship, from which q_{eq} and $k_{2,ad}$ can be determined from the slope and intercept of the plot and there is no need to know any parameter beforehand.

2.7.1.2 Equilibrium Modeling of Adsorption Isotherms in a Batch System

A common way to represent the equilibrium in adsorption systems is the equilibrium isotherm. The equilibrium isotherm represents the distribution of the adsorbed material between the adsorbed phase and the solution phase at equilibrium. This isotherm is characteristic for a specific system at a particular temperature (Poulopoulos et. al., 2006).

The Langmuir Isotherm Model

The Langmuir isotherm was developed by Irving Langmuir in 1916 to describe the dependence of the surface coverage of an adsorbed gas on the pressure of the gas above the surface at a fixed temperature. There are many other types of isotherm (Temkin, Freundlich...) which differ in one or more of the assumptions made in deriving the expression for the surface coverage; in particular, on how they treat the surface coverage dependence of the enthalpy of adsorption. While the Langmuir isotherm is one of the simplest, it still provides a useful insight into the pressure dependence of the extent of surface adsorption.

The Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites is given by Eq.(1.11).

$$q_{eq} = \frac{Q_o b C_{eq}}{1 + b C_{eq}} \quad (1.11)$$

where q_{eq} is the amount adsorbed (mg/g), C_{eq} is the equilibrium concentration of the adsorbate (mg/L), and parameters Q_o and b are Langmuir constants related to maximum adsorption capacity (mg/g) (monolayer capacity) and bonding energy of adsorption (L/mg), respectively, which are functions of the characteristics of the system as well as time (Langmuir, 1918).

Plots were obtained from $\left[\frac{1}{q_{eq}} = \frac{1}{Q_o} + \frac{1}{b Q_o C_{eq}} \right]$ which was the linearized form of the Langmuir equation, for $1/q_{eq}$ versus $1/C_{eq}$, for the adsorption of pesticides showing the applicability of the equation. The values of monolayer capacity (Q_o) and Langmuir constant (b) have been evaluated from the intercept and the slope of these plots.

The adsorption capacity can be correlated with the variation of surface area and porosity of the adsorbent. Higher surface area and pore volume will result in higher adsorption capacity. The Langmuir equation is used for homogeneous surfaces.

The essential characteristics of Langmuir model can be expressed by a dimensionless constant called equilibrium parameter R_L , defined by Weber and Chakkravorti (1974); as $R_L = 1/(1+bC_0)$ (where C_0 is the initial concentration of adsorbate in (mg/L), and b is the Langmuir constant). The Langmuir parameters can be used to predict the affinity between the sorbate and sorbent using the dimensionless separation factor (R_L).

This indicates the nature of adsorption as:

Seperation Factor, R_L	Characteristiscis of Langmuir Models
$R_L > 1$	Unfavorable
$R_L = 1$	Linear
$0 < R_L < 1$	Favorable
$R_L = 0$	Irreversible

The Freundlich Isotherm Model

The Freundlich model assumes neither homogeneous site energies nor limited levels of sorption. The Freundlich equation has the general form:

$$q_{eq} = K_F C_{eq}^{1/n} \quad (1.12)$$

where q_{eq} is the amount adsorbed (mg/g), C_{eq} is the equilibrium concentration of the adsorbate (mg/L), K_F ((mg/g)*(mg/L)ⁿ) and n are the Freundlich constants related to adsorption capacity and adsorption intensity, respectively (Freundlich, 1906). Eq. (1.12) can be linearized in logarithmic form.

Plots were obtained from $\left[\ln q_{eq} = \ln K_F + \frac{1}{n} \ln C_{eq} \right]$ which was the linearized form of Freundlich equation. $\ln q_{eq}$ versus $\ln C_{eq}$ linear plots would give the value of $\frac{1}{n}$ as slope and K_F as intercept. The value of K_F can be used as alternative measure of adsorption capacity, while $1/n$ determines the adsorption intensity.

The Toth Model

This is a Langmuir-based isotherm belonging to the left-handed widened Gaussian energy distribution and assuming a continuous distribution of site affinities. The equation may be written as:

$$q_e = \frac{A * C_e}{(B + C_e^D)^{1/D}} \quad (1.13)$$

where the terms q_e , A , C_e , B , and $1/D$ are the same as those in the Langmuir and Freundlich isotherms.

The Redlich-Peterson Model

The Redlich–Peterson isotherm model (Redlich and Peterson, 1959; Ho and McKay, 1999) combines both Langmuir isotherm and Freundlich isotherm equation. The mechanism of adsorption is a hybrid and does not follow ideal monolayer adsorption. The equation is:

$$q_e = \frac{K_R * C_e}{1 + a_R * C_e^\beta} \quad (1.14)$$

where K_R (L/g), a_R (L^β/mg^β) and β are Redlich–Peterson isotherm constants and β lies between 0 and 1. For $\beta = 1$ the equation converts to the Langmuir form. For $\beta=0$, it simplifies to Henry's law equation; and for $1 \ll (a_R C_e^\beta)$, it is identical with the Freundlich isotherm (Aksu and Kabasakal, 2004).

The Sips Model

The Sips model, which always describes the adsorption of the adsorbent with heterogeneous surface, is a three-parameter equation and expressed as:

$$q_e = \frac{q_{\max} * K_{eq} * C_e^n}{1 + K_{eq} * C_e^n} \quad (1.15)$$

where K_{eq} (L^n/mg^n) is a constant related to adsorption energy, $q_{\max}$ (mg/g) is the maximum adsorption capacity and n is the Sips exponent. The Sips isotherm model is characterized by the heterogeneity factor, n , and specifically when $n = 1$, the Sips isotherm equation reduces to the Langmuir equation and it implies a homogeneous adsorption process. Sips equation similar in form to the Freundlich

equation, but it has a finite limit when the concentration is sufficiently high (Sips R., 1948).

The BET Model

Langmuir fails to account for multilayer adsorption and dependency of ΔH_{ads} on surface coverage i.e more difficult to adsorb gas in between adsorbed molecules ΔH_{ads} decreases with coverage V/V_m . Hence Brunauer, Emmett and Teller BET model (1938) derived the first isotherm for multilayer adsorption. It assumes a random distribution of sites that are empty or that are covered with by one monolayer, two layers and so on. The main equation of this model is:

$$q_e = \frac{B * Q_0 * C_s * C_e}{(C_s - C_e) * [C_s + (B - 1) * C_e]} \quad (1.16)$$

All batch isotherm models together with their model equations and the meanings of constants in the equations are tabulated in Table 2.12.

2.7.1.3 Thermodynamic Studies in a Batch System

The thermodynamic parameters of adsorption, i. e., free energy change, ΔG° , enthalpy change, ΔH° , and change in entropy, ΔS° , are evaluated by using Eq. (1.17):

$$\Delta G^\circ = -RT \ln K_o \quad (1.17)$$

where R is the universal gas constant (8.314 J/molK) and T is the temperature in K (Ovez et. al., 2008). When the concentration of the solute in the solution approaches zero, the activity coefficient will approach unity and, as a consequence, K_o can be evaluated by plotting $\ln (q_e/C_e)$, i.e., the adsorbed solute/the solute in the solution at equilibrium vs. q_e and extrapolating to zero. Subsequently, the intercept with the ordinate axis gives the value of K_o .

The enthalpy change, ΔH° , and entropy change, ΔS° , are calculated by Eq. (1.18):

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \quad (1.18)$$

Hence, the numerical values of ΔS° and ΔH° were obtained from the slope and intercept of the plot. The thermodynamic parameters evaluated in literature for different adsorbents and adsorbates are tabulated in Table 2.13.

Table 2.12 The batch isotherm model equations and constants

Batch Isotherm Models	Model Equation	Constants
Langmuir Isotherm	$q_e = \frac{b * Q_0 * C_e}{1 + b * C_e}$	b : Heat of adsorption (l mg ⁻¹) Q₀ : Adsorption capacity (mg/g)
Freudlich Isotherm	$q_e = K_F * C_e^{1/n}$	K_F : Sorption capacity of the adsorbent (mg/g (L/mg) ^{1/n}) n : Sorption intensity of the adsorbent (-)
Toth Isotherm	$q_e = \frac{A * C_e}{(B + C_e^D)^{1/D}}$	A : Toth isotherm constant B : Toth isotherm constant D : Toth isotherm exponent (-)
Redlich-Peterson Isotherm	$q_e = \frac{k_{rp} * C_e}{1 + a_{rx} * C_e^\beta}$	k_{rp} : Constant (L/g) a_{rp} : Constant (L/mmol) ^β β is the exponent lies 1 to 0
Sips Isotherm	$q_e = \frac{K_s * C_e^{1/bs}}{1 + a_s * C_e^{1/bs}}$	K_s : Sips model isotherm constant a_s : Sips model constant as → 0 isotherm is Freundlich b_s : Sips model exponent β → 1 the isotherm obeys Langmuir model
BET Isotherm	$q_e = \frac{B * Q_0 * C_s * C_e}{(C_s - C_e) * [C_s + (B - 1) * C_e]}$	B : Amount of adsorbate sorbate on adsorbent (mg/g) Q₀ : Energy constant

Table 2.13 Thermodynamic parameters evaluated in literature for different adsorbents and adsorbates

Adsorbent	Adsorbed Material	Thermodynamic Parameters					Reference	
		ΔG° (kJ/mol)			ΔH° (kJ/mol)	ΔS° (J/molK)		
		25°C	35°C	45°C				
F400-AC F400AN-Mod.AC	2,4-D	-16.825	-17.231	-18.466		7.764	82.000	Chingombe et. al., 2006
	Benazolin	-16.540	-17.217	-19.337		25.376	139.900	
	2,4-D	-17.223	-17.832	-18.805		6.410	79.100	
	Benazolin	-17.445	-18.319	-19.414		11.930	98.500	
Organophilic sepiolite	2,4-D	25°C		40°C		−23.084	−0.074	Akçay et. al., 2004
		−2.831		−1.863				
Capsicum annum	Cu (II)	20°C	30°C	40°C	50°C	−7.630	28.350	Ozcan et. al., 2005
		−15.930	−16.230	-16.520	−16.770			
Fertilizer and steel industry wastes	2,4-D	25°C	35°C	45°C		−2.030	−61.220	Gupta et. al., 2006
		-21.080	-20.890	-20.020				
	Carbofuran	-21.040	-20.970	-20.610		−0.810	−65.440	
Activated carbon derived from olive bagasse	Cr (VI)	25°C	35°C	45°C		39.450	0.145	Demiral et. al., 2008
		−4.070	−5.050	−7.020				
Fly ash	Boron	25°C	35°C	45°C		−8.950	−0.029	Ozturk et. al., 2005
		−0.310	−0.020	0.270				

2.7.2 Continuous Mode

When an organic pollutant containing solution passes through a packed bed column, at the beginning, most of organic pollutant get sorbed on adsorbent so organic pollutant concentration in the effluent remains either very low or in some cases is not detectable. As adsorption continues, organic pollutant concentration in the effluent rises, slowly at first, and then abruptly. When this abrupt rise or breakthrough occurs the flow is stopped.

The performance of continuous packed bed is described through the concept of the breakthrough curve. The time for breakthrough appearance (breakthrough time) and the shape of the breakthrough curve are very important characteristics for determining the operation and the dynamic response of an adsorption column. The general position of the breakthrough curve along the time or volume axis depends on the capacity of the column with respect to the feed concentration and flow rate. The breakthrough curves show the loading behaviour of pollutant to be removed from solution in a fixed bed and is usually expressed in terms of normalized concentration defined as the ratio of effluent pollutant concentration to inlet pollutant concentration (C/C_0) as a function of time or volume of effluent (V_{eff}) for a given bed height (Figure 2.6).

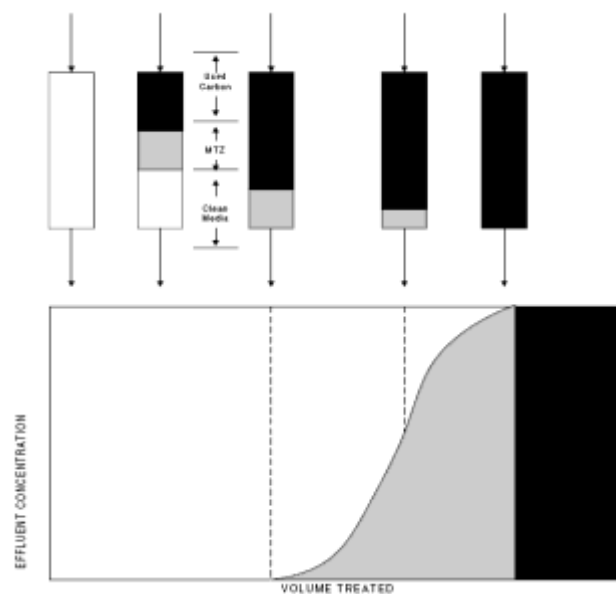


Figure 2.6 Adsorption column mass transfer zone and idealized breakthrough zone

Effluent volume (V_{eff}) can be calculated from Eq. (1.19).

$$V_{eff} = Qt_{total} \quad (1.19)$$

where t_{total} and Q are the total flow time (min) and volumetric flow rate (mL min^{-1}). The area under the breakthrough curve (A) obtained by integrating the adsorbed concentration (C_{ad} ; mg L^{-1}) versus t (min) plot can be used to find the total adsorbed adsorbate quantity (maximum column capacity). Total adsorbed adsorbate quantity (q_{total} ; mg) in the column for a given feed concentration and flow rate is calculated from Eq. (1.20):

$$q_{total} = \frac{QA}{1000} = \frac{Q}{1000} \int_{t=0}^{t=t_{total}} C_{ad} dt \quad (1.20)$$

Total amount of adsorbate sent to column (m_{total}) is calculated from Eq. (1.21).

$$m_{total} = \frac{C_o Qt_{total}}{1000} \quad (1.21)$$

Total removal percent of adsorbate (column performance) with respect to flow volume can be also found from the ratio of total adsorbed quantity of adsorbate (q_{total}) to the total amount of adsorbate sent to column (m_{total}) Eq. (1.22).

$$\text{Total removal \%} = \frac{q_{total}}{m_{total}} \times 100 \quad (1.22)$$

Adsorption is also a well-known equilibrium separation process for wastewater treatment. Equilibrium studies on adsorption give information about the capacity of the sorbent or the amount required to remove a unit mass of pollutant under the system conditions. As the adsorption isotherms obtained from sorption data in a batch system are not able to represent the column equilibrium, the experimental data from the column experiments should be used to fit isotherm models. Equilibrium adsorbate uptake (q_{eq}) (or maximum capacity of the column)

in the column is defined by Eq. (1.23) as the total amount of adsorbate sorbed (q_{total}) per g of sorbent (X) at the end of total flow time.

$$q_{eq} = \frac{q_{total}}{X} \quad (1.23)$$

Unadsorbed adsorbate concentration at equilibrium in the column (C_{eq} ; mg L⁻¹) can be defined by Eq. (1.24):

$$C_{eq} = \frac{m_{total} - q_{total}}{V_{eff}} \times 1000 \quad (1.24)$$

Successful design of a column adsorption process requires prediction of the concentration-time profile or breakthrough curve for the effluent under given specific operating conditions. Developing a model to accurately describe the dynamic behavior of adsorption in a fixed bed system is inherently difficult. Since the concentration of the adsorbate as the feed moves through the bed, the process does not operate at steady state. The fundamental transport equations derived to model the fixed bed with theoretical rigor are differential in nature and usually require complex numerical methods to solve. Such a numerical solution is not usually difficult, but often does not fit experimental results especially well. Some solutions for very limiting cases have been reported, but in general, complete time-dependent analytical solutions to differential equation based models of the proposed rate mechanisms are not available. Because of this, various simple mathematical models have been developed to predict the dynamic behavior of the column and the models used to characterize the fixed bed performance for the removal of organics are presented in the literature (Rao and Viraraghavan, 2002; Volesky, 2001; Aksu 1998; Aksu and Gönen, 2004; Guibal et al., 1995).

The sorption capacity parameter obtained from a batch experiment is useful in providing information about effectiveness of adsorbate- adsorbent system. But, batch reactors were easy to use in the laboratory study, less convenient for industrial applications. The data obtained under batch conditions are generally not applicable to most treatment system (such as column operations) where contact

time is not sufficient long for the attainment of equilibrium. In batch mode adsorption studies, the same solution remains in contact with a given quantity of adsorbent. The adsorption process continues, however, till equilibrium between the solute concentration in solution, and the solute adsorbed per unit weight of adsorbent is reached. This equilibrium established is static in nature, as it does not change further with time. In continuous column adsorption, solution continuously enters and leaves the column, so that the complete equilibrium is never established, as each time, it meets the fresh concentrations, hence, equilibrium in column mode is termed as dynamic equilibrium. Fixed-bed columns were widely used in various chemical industries because of their operation. Hence, there is a need to perform equilibrium studies using columns.

Comparison of column mode studies is tabulated in Table 2.14.

Table 2.14 Comparison of column mode studies with activated carbon

Adsorbent Form	Adsorbent Size	Adsorbate	Parameter Investigated	Parameter Range	Conclusion	References
GAC	0.9mm mean diameter	TNT	*Concentration *Temperature *Flowrate *Bed height	C:32,76 to 171 mg/dm ³ T=10-60°C v= 1, 2, 4 dm ³ /h h= 70 mm&135 mm	Adsorption capacity increased with increasing temperature, but decreased with increasing inlet concentration and flow rate.	Kim et al., 2002
GAC	8-20 mesh size	Lead(II)	*Bed height *Hydraulic Loading Rate *Initial feed concentration	h: 0.3-0.5m HLR:3-10.5 m ³ /(h*m ²) C:2-6 mg/L	0,4m is chosen as optimized bed height. Increase in HLR, resulting decrease in the time to achieve breakthrough. Increase in Concentration causes increase in adsorption capacity.	Goel et al., 2005
GAC	*0.6-1.7mm *Cloth	Zn(II)	*Type of adsorbent	Adsorbents: ACC&ACC-ox	Oxidizing carbon ions causes decrease in the time to achieve breakthrough...	Alvarez-Merino et al., 2005
GAC ACF	100µm	Atrazine	*Type of adsorbent	3 types of ACF and GAC	Pitch based ACF are more effective than GAC if they are highly activated.	Gullon and Font, 2001
GAC	F400	Acid Dyes	*Bed height *Flow rate *Inlet dye concentration *Adsorbent particle size	µ=0.025; 0.1; 0.5 dm ³ min ⁻¹ c=100; 200; 300 mg/dm ³ Particle size=0.06; 0.0425; 0.030 cm	By using low linear flow rates and use fine adsorbent particle size ; the optimum bed results are obtained.	Walker and Weatherley, 1998
GAC	F400 SLS103 WWL	2,4-D 2,4-DNP	*Capacity vs Kinetics	Types of Adsorbents	A simple dynamic model (LDFA) successfully simulated the experimental adsorption breakthrough.	Kim et al., 2003
GAC	24-32 mesh	nitro phenol	*Bed depths *Flow rates	h=3-6 cm v=21.6-86.4 cm ³ /h	The experimental half lifes are very close to predicted by self-sharpening wave approach and proportional to bed depth-flow rate ratio. Experimental and predicted breakthrough curves are in good agreement.	Chern and Chien, 2002

2.7.2.1 Kinetic Modeling of Adsorption in a Continuous Packed Bed Column

The Thomas Model

The data obtained in column in continuous mode studies was used to calculate maximum solid phase concentration of adsorbate on adsorbent and the adsorption rate constant using the kinetic model developed by Thomas (Thomas, 1944). The Thomas solution is one of the most general and widely used methods in column performance theory. The expression by Thomas for an adsorption column is given as follows:

$$\frac{C_t}{C_o} = \frac{1}{1 + \exp[k_{Th}(q_o x - C_o V_{eff})/v]} \quad (1.25)$$

where k_{Th} is the Thomas rate constant (mL/min x mg), q_o the equilibrium adsorbate uptake per gram of the adsorbent (the maximum solid-phase concentration of the solute) (mg/g), x the amount of adsorbent in the column (g), V_{eff} the effluent volume (mL), C_o the influent concentration (mg /L), C_t the effluent concentration at time t (mg/L) and v the flow rate (mL/min). The value of C_t/C_o is the ratio of effluent and influent concentrations. The value of t is time (min, $t = V_{eff}/v$).

The linearized form of the Thomas model is as follows:

$$\ln\left(\frac{C_o}{C_t} - 1\right) = \frac{k_{Th} q_o x}{v} - \frac{k_{Th} C_o V_{eff}}{v} \quad (1.26)$$

The kinetic coefficient k_{Th} and the adsorption capacity of the column q_o can be determined from a plot of $\ln [(C_o/C_t)-1]$ against V_{eff} (or t) at a given flow rate (Aksu and Gönen, 2004).

The Thomas or reaction model, which assumes Langmuir kinetics of adsorption–desorption and no axial dispersion is derived with the adsorption that the rate driving force obeys second-order reversible reaction kinetics (Fu and Viraraghavan, 2003; Vijayaraghavan et al., 2005).

Thomas' solution also assumes a constant separation factor but it is applicable to either favorable or unfavorable isotherms. The primary weakness of the Thomas solution is that its derivation is based on second order reaction kinetics. Adsorption is usually not limited by chemical reaction kinetics but is often controlled by interphase mass transfer. This discrepancy can lead some error when this method is used to model adsorption process (Thomas, 1944).

The Yoon and Nelson Model

Yoon and Nelson model is based on the assumption that the rate of decrease in the probability of adsorption for each adsorbate molecule is proportional to the probability of adsorbate adsorption and the probability of adsorbate breakthrough on the adsorbent. The Yoon and Nelson equation regarding to a single component system is expressed as;

$$\ln \frac{C_t}{C_o - C_t} = k_{YN}t - \tau k_{YN} \quad (1.27)$$

where k_{YN} is the rate constant (L/min); τ the time required for 50% adsorbate breakthrough (min); t the breakthrough time (min).

The kinetic coefficient k_{YN} and τ can be determined from a plot of $\ln [C_t/(C_o - C_t)]$ against t at a given adsorption conditions. If the theoretical model accurately characterizes the experimental data, this plot will result in a straight line with slope of k_{YN} and intercept $\tau \times k_{YN}$ (Aksu and Gönen, 2004).

The Adams–Bohart Model

The fundamental equations describing the relationship between C/C_o and t in a flowing system were established by Adams and Bohart (1920) for the adsorption of chlorine on charcoal. Although the original work by Adams–Bohart was done for the gas–charcoal adsorption system, its overall approach can be applied successfully in quantitative description of other systems. This model assumes that the adsorption rate is proportional to both the residual capacity of the activated carbon and the concentration of the sorbing species.

Adams–Bohart model based on the surface reaction theory and it assumes that equilibrium is not instantaneous; therefore, the rate of the sorption is proportional to the fraction of sorption capacity still remains on the sorbent (Goel et al., 2005).

The Adams–Bohart model is used for the description of the initial part of the breakthrough curve (Aksu and Gönen, 2004; Liao et al., 2004).

The mass transfer rates obey the following equations:

$$\frac{\partial q}{\partial t} = -k_{AB} q C_b \quad (1.28)$$

$$\frac{\partial C_b}{\partial Z} = -\frac{k_{AB}}{U_o} q C_b \quad (1.29)$$

where k_{AB} is the kinetic constant (L /mg x min). Some assumptions are made for the solution of these differential equation systems: (i) the concentration field is considered to be low, e.g. effluent concentration $C < 0.15C_o$; (ii) for $t \rightarrow \infty$, $q \rightarrow N_o$, (where N_o is the saturation concentration (mg/L). When the differential equation systems solved, the following equation is obtained with parameters k_{AB} and N_o :

$$\ln \frac{C_t}{C_o} = k_{AB} C_o t - k_{AB} N_o \frac{Z}{U_o} \quad (1.30)$$

where C_o and C_t are the inlet and effluent adsorbate concentrations (mg/L), respectively. Z is the height of the column (cm), U_o is the superficial velocity (cm/min), N_o is saturation concentration in the Adams–Bohart model (mg/ L) and k_{AB} is the Adams–Bohart kinetic constant (the mass-transfer coefficient) (L/mg x min). The range of t should be considered from the beginning to the end of breakthrough. A straight line was attained for this system by plotting $\ln (C_t/C_o)$ against t , which gives the value of k_{AB} from the slope of the line (Texier et al., 2002; Bohart and Adams, 1920; Wolborska, 1989; Aksu and Gönen 2004).

The studies done with different adsorbents and adsorbates in a continuous system are tabulated and the parameters of the Thomas, Yoon-Nelson and Adams-Bohart kinetic models are compared in Tables 2.15, 2.16, and 2.17 respectively.

Table 2.15 Thomas model parameters evaluated in comparison with the studies in literature

Adsorbent	Adsorbate	Thomas Model				Reference
		Variables	k_{Th} (mL/min x mg)	q_0 (mg/g)	R^2	
NaOH-modified oil palm frond	Copper	Hydraulic retention time = 12 min	1.241	0.192	0.980	Salamatinia et al., 2008
Olive Stone	Cr (III)	$C_0 = 50$ mg/L	0.179	0.330	0.800	Calero et al., 2009

Table 2.16 Yoon-Nelson model parameters evaluated in comparison with the studies in literature

Adsorbent	Adsorbate	Yoon-Nelson Model				Reference
		Variables	k_{YN} (L/min)	τ (min)	R^2	
NaOH-modified oil palm frond	Copper	Hydraulic retention time = 12 min	0.039	257	0.980	Salamatinia et al., 2008
Olive Stone	Cr (III)	$C_0 = 50$ (mg/l)	7.510	56.9	0.800	Calero et al., 2009

Table 2.17 Adams-Bohart model parameters evaluated in comparison with the studies in literature

Adsorbent	Adsorbate	Adams-Bohart Model				Reference
		Variables	k_{AB} (mL/min x mg)	N_0 (mg/L)	R^2	
Vinly Alcohol	Cadmium	PVA/Hap Ratio= 1:1	0.06	370.50	0.959	Wang et al., 2008
NaOH-modified oil palm frond	Copper	Hydraulic retention time = 12 min	1.39	2652.00	0.940	Salamatinia et al., 2008
Olive Stone	Cr (III)	C_0 = 50 mg/l	2.11	95.90	0.930	Calero et al., 2009
Phanerochaete chrysosporium	Lead	Bed Depth=10.5 cm	77.43	6.08	0.970	Pakshirajan et al., 2006

2.8 Biological Contamination in the Systems

Besides pesticides as chemical contaminants, the water can be contaminated biologically. So it is decided to add biological contaminants such as bacteria and algae to the systems.

2.8.1 The Classification of Living Things

Throughout the history, many scientists have made the classification of living things, but the latest one accepted is the classification done by Carl Woese in 1990. According to that classification, from comparative rRNA sequencing, three phylogenetically distinct lineages of cells have been identified. The lineages, called domains, are: *Bacteria*, *Archaea* (both consisting prokaryotes) and *Eukarya* including protists (slime molds, protozoa, and algae), fungi, plants and animals which are all eukaryotes (Madigan, 2009). The domains are thought to have diverged from a common ancestral organism or community of organisms early in the history of life on Earth.

The phylogenetic tree of life reveals two very important evolutionary facts:

1. All prokaryotes are not phylogenetically closely related
2. *Archaea* are more closely related to *Eukarya* than to *Bacteria* (Figure 2.7). *Archaea* are special types are bacteria which can resist extreme conditions such as high temperature, pH or salinity.

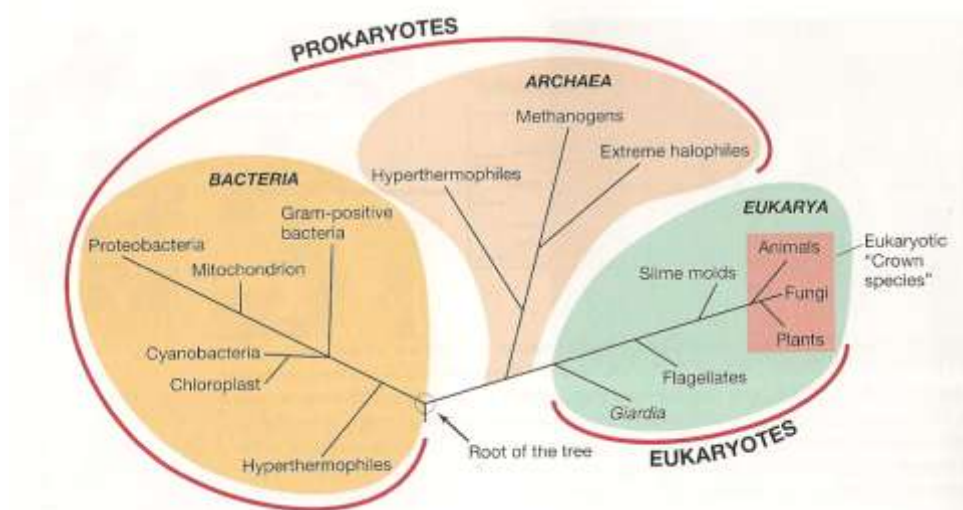


Figure 2.7 The three domains of life as defined by comparative rRNA gene sequencing

Thus from the last universal common ancestor of all life, evolutionary diversification initially went in two directions: *Bacteria* and a second main lineage. This second lineage eventually diverged to yield the *Archaea*, which retained a prokaryotic cell structure, and the *Eukarya*, which did not as seen in Figure 2.8 (Madigan, 2009).

When the word “bacteria” (lower case b and no italics) is used, the reference can either be some species of the domain *Bacteria* or *Archaea*. Otherwise, if written with upper case b and italics, then it is specifically indicated as a member of the domain *Bacteria*.

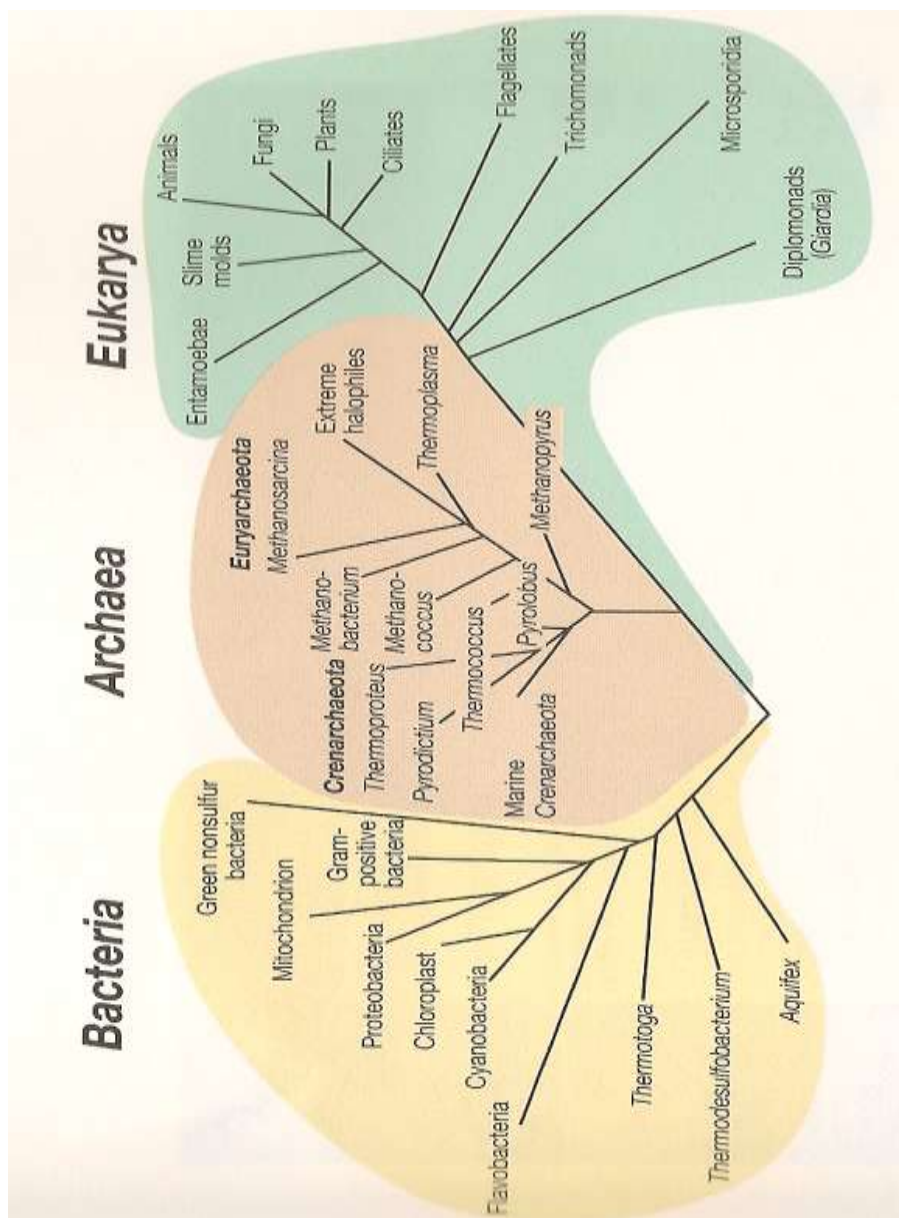


Figure 2.8 The phylogenetic tree of life

The examination of the internal structure and other features of cells reveal two patterns: prokaryote and eukaryote seen in Figure 2.9 (Madigan, 2009). (a) *Heliobacterium modesticaldum*, the cell measures $1 \times 3 \mu\text{m}$. (b) *Methanopyrus kandleri*, the cell measures $0.5 \times 4 \mu\text{m}$. (c) *Saccharomyces cerevisiae*, the model organism of eukaryotes, the cell measures $8 \mu\text{m}$ in diameter. Eukaryotes have their DNA in a membrane-enclosed nucleus and are typically larger and structurally more complex than prokaryotic cells.

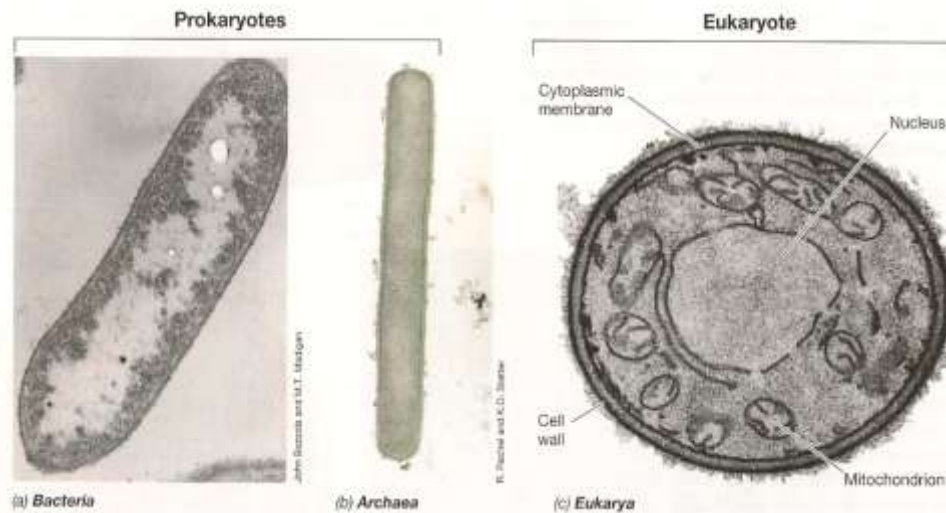


Figure 2.9 Electron micrographs of cells from each of the domains of living organisms

2.8.2 Bacteria and Biofilm

Bacteria domain has at least 18 phylums as seen in Figure 2.10, but three of them are the most important ones which are *Cyanobacteria*, *Gram-positive bacteria* and *Proteobacteria*.

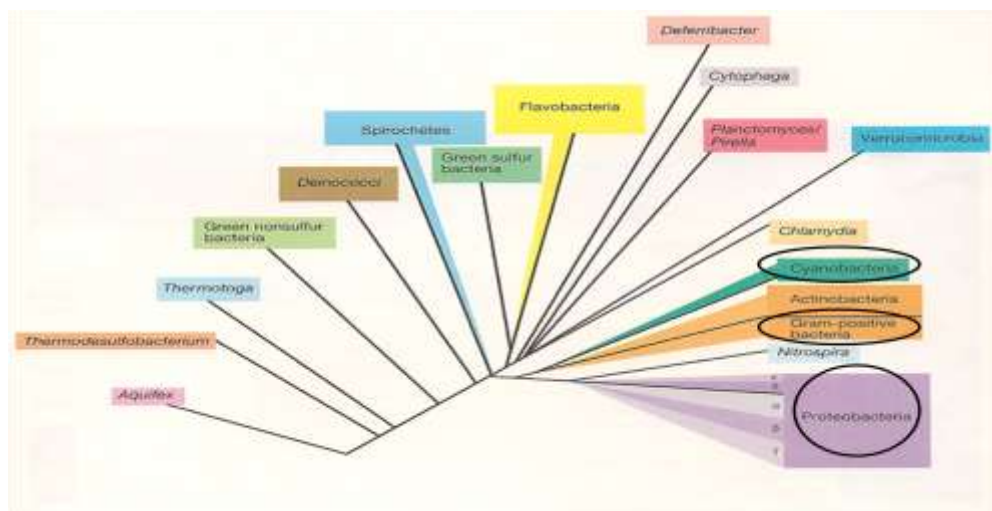


Figure 2.10 The phylogenetic tree of *Bacteria*

General Properties of Bacteria

Cell Size:

In general, microbial cells are very small, particularly prokaryotes. For example, a typical rod-shaped prokaryote is 1-5 μm long and about 1 μm wide and thus is invisible to the naked eye. Eukaryotic cells are typically much larger than prokaryotic cells, but the range of sizes in eukaryotic cells is quite large. On the other hand, viruses, which do not have metabolism so are not considered as living organisms, are also much smaller than prokaryotic cells as seen in Figure 2.11 (Madigan, 2009). Viruses vary in size, with the smallest known viruses being only about 10 nm in diameter.

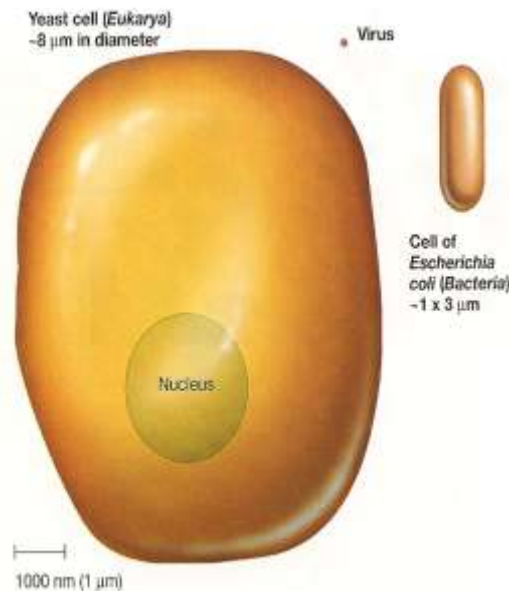


Figure 2.11 Size comparison of viruses, prokaryotic and eukaryotic cells

Cell Morphologies:

Bacteria have different shapes which make them distinguish from each other as seen in Figure 2.12 (Madigan, 2009).

Cell Wall Structure:

Species of bacteria can be divided into two major groups, called gram-positive and gram-negative. The distinction between them is based on the difference in their cell wall structure giving different reaction to **Gram stain** procedure (Section 3.2.3.1). The appearance of cell walls of gram-positive and gram-negative cells in electron microscope differs markedly, as is shown in Figure 2.13. The gram-negative cell wall is a multilayered structure and quite complex, whereas in the gram-positive cell wall is typically much thicker and consists almost entirely of a single type of molecule which is called “peptidoglycan” (Madigan, 2009).

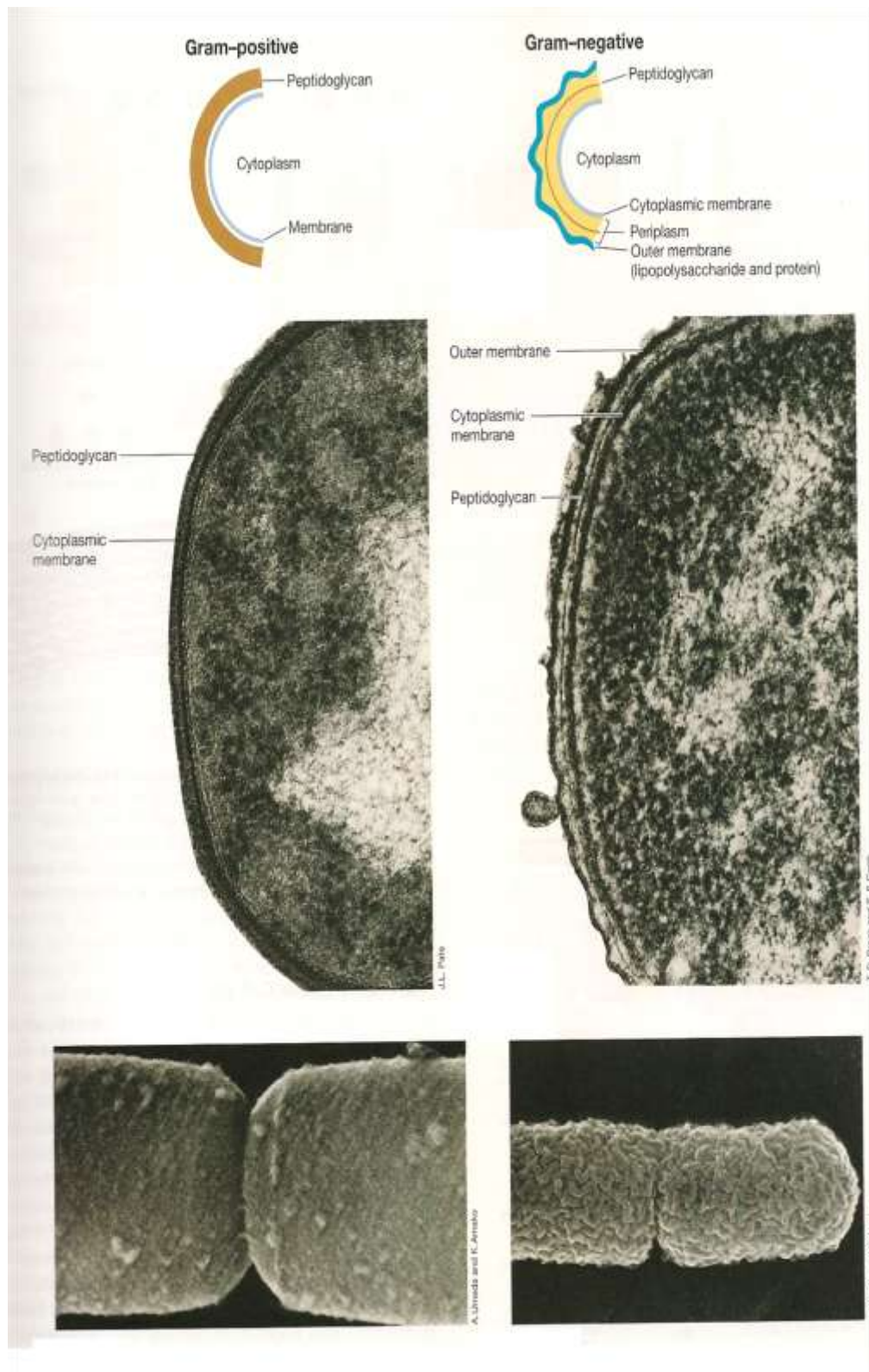


Figure 2.13 Cell walls of *Bacteria*

Life Styles:

Microbial growth can be measured, including microscopic methods, viable counts, and measurements of light scattering (turbidity) by cells suspended in a liquid culture (Madigan, 2009). The turbidimetric measures of bacterial growth assume that cells remain evenly distributed in their liquid growth medium. Under these conditions the optical density of a culture is proportional to the log of the number of cells in suspension (Figure 2.14). The increasing (left to right) optical density (OD_{540}) of each culture is shown below the tube.

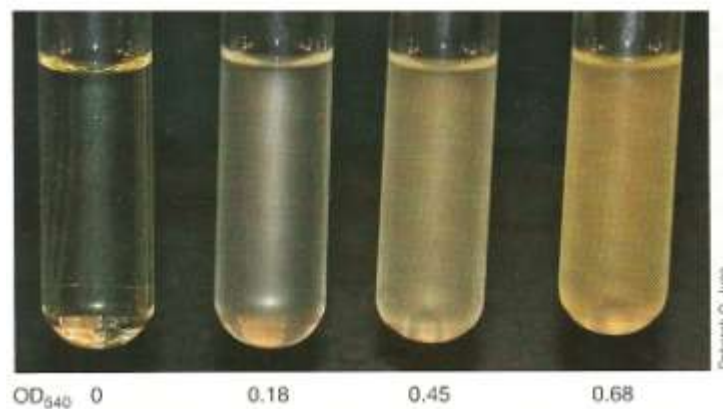


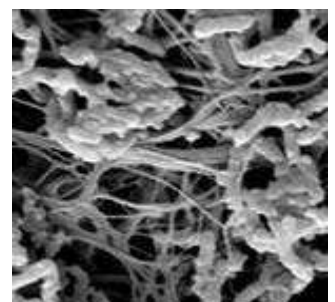
Figure 2.14 Liquid cultures of *Escherichia coli*

This floating lifestyle, called planktonic, is the way some bacteria actually live in nature, for example cells that inhabit the water column of a lake. However, many other microorganisms are sessile, meaning that they grow attached to a surface. These attached cells can then develop into **biofilms** (Madigan, 2009).

Humans encounter bacterial biofilms on a daily basis, for example, when cleaning out a pet's water bowl that has been sitting unattended for a few days or when a person senses with his/her tongue the "film" that develops on his/her unbrushed teeth.

Biofilms: Microbial Growth on Surfaces

Surfaces are important microbial habitats for at least two reasons. For one, nutrients adsorb to surfaces and thus often contain more resources than are available to



planktonic cells. And second, surfaces are areas to which microbial cells can attach. Attachment is a means of cells to remain in a favorable habitat and not be washed away. As a consequence, microbial numbers and activities are typically quite high on surfaces (Madigan, 2009).

Microscope slides can be used as experimental surfaces to which organisms can attach and grow. A slide can be immersed in a microbial habitat, left for a period of time, and then retrieved and examined microscopically (Figure 2.15a). Microcolonies readily develop on such surfaces much as they do on natural surfaces in nature (Madigan, 2009).

A surface may also be a nutrient, such as a particle of organic matter. On such surfaces, the attached microorganisms catabolize nutrients directly from the surface of the particle. For example, plant roots become heavily colonized by soil bacteria living on organic exudates from the plant and fluorescent stains can be used to detect and enumerate them (Figure 2.15b).

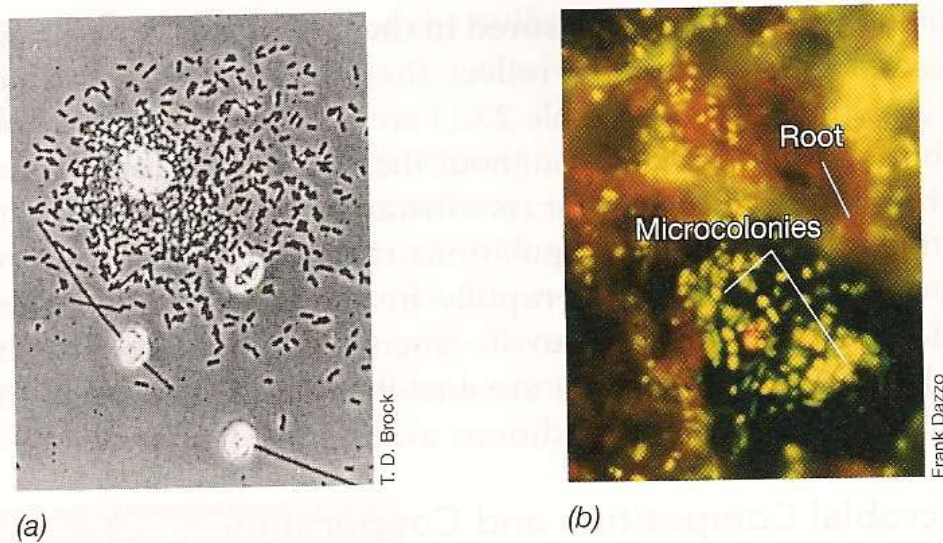


Figure 2.15 Microorganisms on surface

As bacterial cells grow on surface, they tend to form biofilms. A biofilm is an attached polysaccharide matrix containing bacterial cells (Figure 2.16). The matrix is typically a mixture of polysaccharides, but can contain proteins and even nucleic acids.

Figure 2.16a is a cross-sectional view of an experimental biofilm made up of cells of *Pseudomonas aeruginosa*. The yellow layer (about 15 μm in depth) contains cells and is stained by a reaction showing activity of the enzyme alkaline phosphatase.

Biofilms typically contain several porous layers and the cells in each layer can be examined by scanning laser confocal microscopy (Figure 2.16b). It is the top view of a natural biofilm on a leaf surface. The color of the cells indicates their depth in the biofilm: red, cells on the surface; green, 9 μm depth; blue, 18 μm depth.

Biofilms trap nutrients for microbial growth and help prevent the detachment of cells on surfaces present in flowing system (Figure 2.16c). It is the view of a biofilm of iron-oxidizing prokaryotes attached to rocks in iron-rich Rio Tinto in Spain. As Fe^{+2} -rich water passes over and through the biofilm, iron-oxidizing microorganisms oxidize Fe^{+2} to Fe^{+3} to obtain energy.

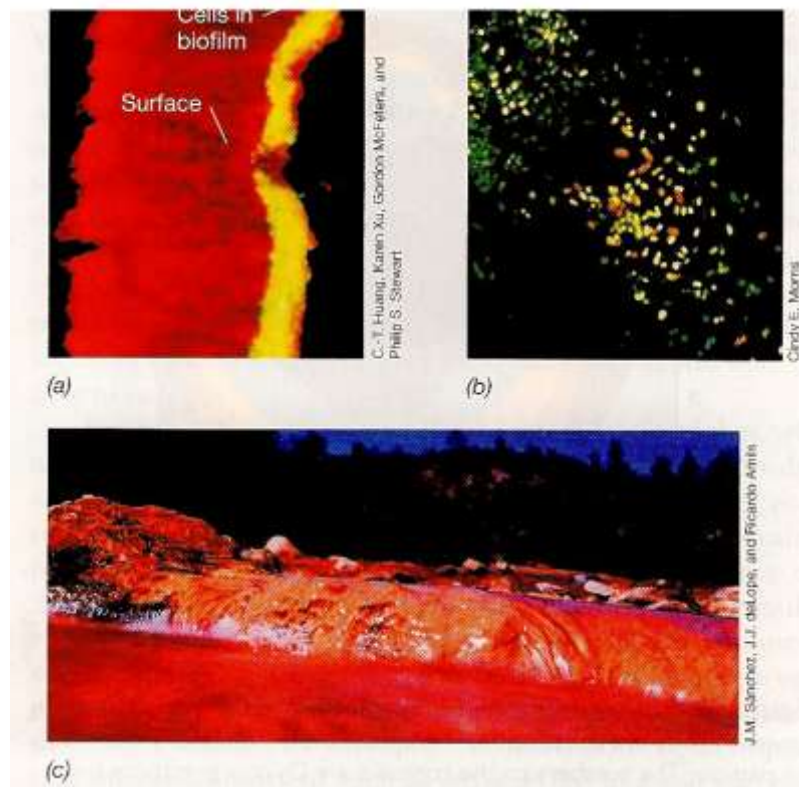


Figure 2.16 Examples of microbial biofilms

Biofilms may contain one or more species as seen in Figure 2.17. It is the SEM image taken of a biofilm consisting of bacteria and yeast strains colonizing on silicone rubber voice prosthesis after being placed for four months in a patient.

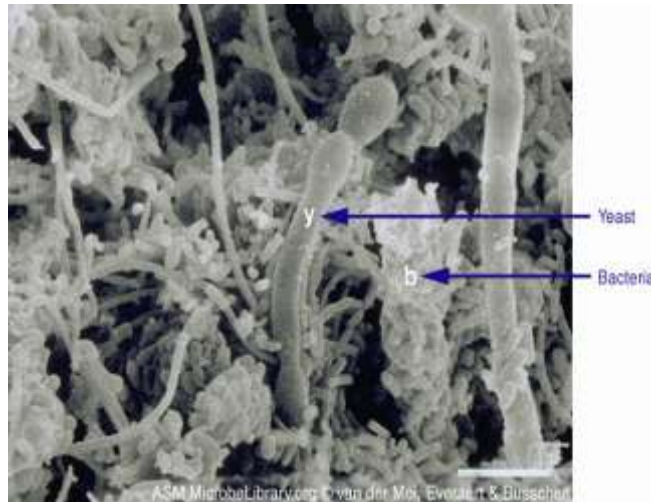


Figure 2.17 The SEM image of a biofilm formed on a voice prosthesis

Biofilm Formation:

Attachment of a cell to a surface is a signal for the expression of biofilm-specific genes. These genes encode proteins that synthesize intercellular signaling molecules and initiate matrix formation. Biofilms begin with the attachment of a few cells that then grow and communicate with other cells. The matrix is formed and becomes more extensive as the biofilm grows as in Figure 2.18a.

Figure 2.18b illustrates the photomicrograph of a DAPI-stained biofilm that developed on a stainless steel pipe. The water channels formed are clearly observed.

Intercellular communication is critical in the development and maintenance of a biofilm. In the early stages of biofilm formation, small buttons of *Pseudomonas aeruginosa* cells are attached to a surface (Figure 2.19a). Over time, large mushrooms of *Pseudomonas aeruginosa* form that are over 100 μm high and contain billions of cells enmeshed in a sticky polysaccharide matrix (Figure 2.19b).

Pseudomonas aeruginosa biofilms can occur in the disease cystic fibrosis. This genetic disorder is often associated with a tenacious biofilm that forms in the lungs, leading to symptoms of pneumonia. However, like most biofilms, cystic fibrosis biofilm contains more than one bacterial species (Madigan, 2009).

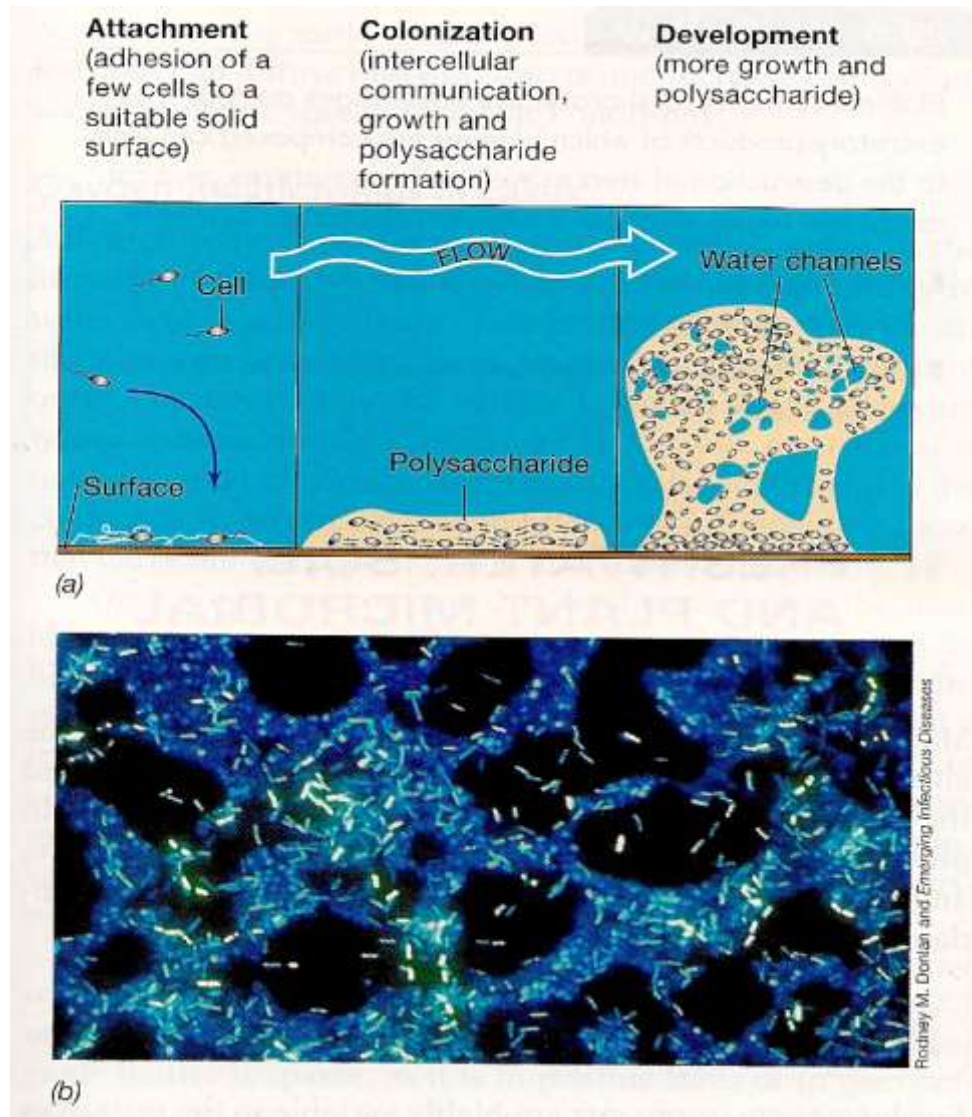


Figure 2.18 Biofilm formation

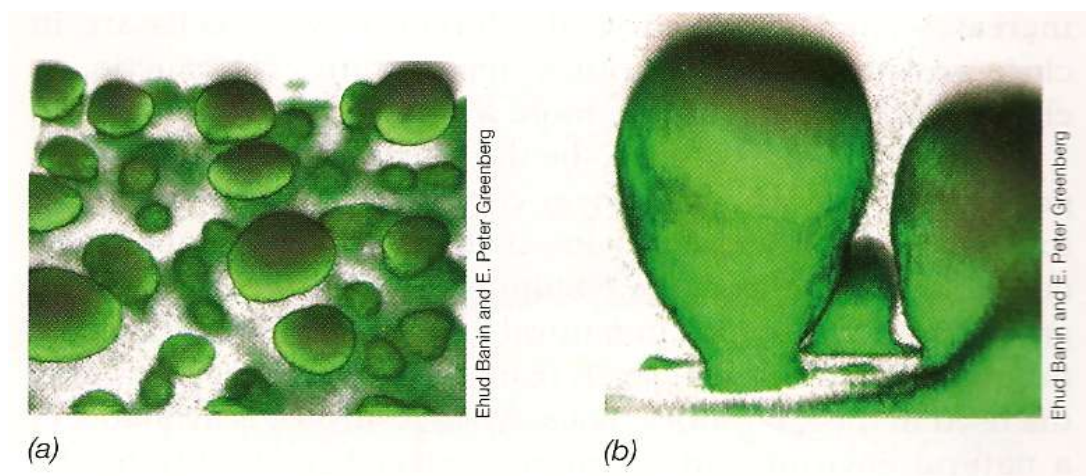


Figure 2.19 Biofilms of *Pseudomonas aeruginosa*

The Advantages of Forming a Biofilm:

There are at least four reasons why bacteria form biofilms. All of these advantages improve the chances for survival of cells in the biofilms (Madigan, 2009).

First, biofilms are means of microbial self-defense. Biofilms resist physical forces that could sweep away unattached cells. Moreover, biofilms resist phagocytosis by cells of the immune system and the penetration of toxic molecules such as antibiotics.

Second, biofilms formation allows cells to remain in a favorable niche. Biofilms attached to nutrient-rich surfaces, such as animal tissues, or to surfaces in flowing systems, such as a rock in a stream as in Figure 2.35c, fix bacterial cells in locations where nutrients are more abundant or are constantly replenished.

Third, biofilms form because they allow bacterial cells to live in close association with each other as in the case of *Pseudomonas aeruginosa* (Figure 2.38). This increases the chances for survival; moreover, when cells are in close proximity to one another, opportunities for genetic exchange are more available.

Finally, biofilms seem to be the typical way bacterial cells grow in nature, where nutrient concentrations are often one to two orders of magnitude lower than they are in laboratory culture media. Indeed, the biofilms may be the default mode of growth for prokaryotes in natural environment, which differ dramatically in nutrient levels from the rich liquid culture media used in the laboratory. Thus, when a surface is available in a natural environment, planktonic growth may only be the norm for these bacteria adapted to life extremely low nutrient concentrations (Madigan, 2009).

Biofilm Control:

Biofilms have significant implications in human medicine and commerce. In the body, bacteria cells within a biofilms are protected from an attack by the immune system, antibiotics and other antimicrobial agents often fail to pierce the

biofilm. Biofilms have been implicated in medical and dental conditions, including periodontal disease, kidney stones, tuberculosis, Legionnaire's disease and *Staphylococcus* infections. Medical implants are excellent surfaces for biofilm development. These include both short-term devices such as a urinary catheter, as well as long-term implants, such as artificial joints. It is estimated that 10 million a year in the United States experience biofilm infections from implants or intrusive medical procedures. Biofilms explain why routine oral hygiene is so important for maintaining dental health. Dental plaque is a typical biofilm and contains acid-producing bacteria responsible for dental carries (Madigan, 2009).

In industrial situations, biofilms can slow the flow of water; oil, or other liquids through pipelines and can accelerate the corrosion of the pipes themselves. Biofilms also initiate the degradation of submerged objects, such as structural components of offshore oil platforms, boats and shoreline installations.

The safety of drinking water may be compromised by biofilms that develop in water distribution pipes, many of which in the United States are nearly one hundred years old. Although water pipe biofilms mostly contain harmless microorganisms, if pathogens successfully colonize the biofilm, standard chlorination practices may be insufficient to kill them. Periodic releases of cells can then lead to outbreaks of disease. There is some concern that *Vibrio cholera*, the causative agent of cholera may be propagated in this manner (Madigan, 2009).

Biofilm control is big business, as thus far, only a limited number of tools exist to fight biofilms. Collectively, industries commit huge financial resources to treating pipes and other surfaces to keep them free of biofilms. New antimicrobial agents that can penetrate biofilms, as well as drugs that prevent biofilm formation by interfering with intercellular communication, are being developed. A class of chemicals called furanones, for example has shown promise as biofilm preventatives in tests on abiotic surfaces. Furanones are also stable some are relatively nontoxic, so they may have applications as antibiofilm agents in human medicine as well.

2.8.3 Studies in Literature Performed with the Microorganisms Used

The microorganisms used in this study are aquarium water, *Gracilaria verrucosa*, *Chlorella sp.*, *Spirulina platensis* and LMG 17238. Similar studies are tabulated in the following tables and they predominantly consist of biosorption studies.

2.8.3.1 Aquarium Water

A well-balanced tank contains organisms that are able to metabolize the waste products of other aquarium residents. Bacteria known as nitrifiers (genus *Nitrosomonas*) metabolize nitrogen waste. Nitrifying bacteria capture ammonia from the water and metabolize it to produce nitrite. Nitrite is toxic to fish in high concentrations. Another type of bacteria, genus *Nitrospira*, converts nitrite into nitrate, a less toxic substance. *Nitrobacter* bacteria were previously believed to fill this role. While biologically they could theoretically fill the same niche as *Nitrospira*, it has recently been found that *Nitrobacter* are not present in detectable levels in established aquaria, while *Nitrospira* are plentiful. In addition to bacteria, aquatic plants also eliminate nitrogen waste by metabolizing ammonia and nitrate. When plants metabolize nitrogen compounds, they remove nitrogen from the water by using it to build biomass that decays more slowly than ammonia-driven plankton already dissolved in the water.

Studies in literature done with aquarium water together with their equilibrium models are tabulated in Table 2.18.

Table 2.18 Studies in literature done with aquarium water and the equilibrium models evaluated

Adsorbent	Adsorbate	Aquarium Water							Reference
		pH and T	Langmuir Isoterm Constants			Freundlich Isoterm Constants			
			Q _{max} (mg/g)	b (L/mg)	R ²	n	K _F ((mg/g)*(mg/L) ⁿ)	R ²	
<i>E.Coli</i>	Arsenic(III)	T=293 K	0.626	0.183	0.886	1.207	0.082	0.962	Wu et al., 2009
		T=303 K	0.908	0.164	0.886	1.261	0.113	0.979	
		T=313 K	1.111	0.162	0.768	1.317	0.141	0.969	
Suspended particulate matter in aquatic ecosystem	Microcystin* LW	pH=7	-	-	-	1.290	1.768	0.967	Liu et al., 2008
	Microcystin* LR	pH=7	-	-	-	1.040	26.252	0.998	

*Microcystins, a group of monocyclic heptapeptide hepatotoxins, are produced by various freshwater cyanobacteria species.

2.8.3.2 *Gracilaria verrucosa*

This is one of dozens or more species of *Gracilaria* known to occur in southern waters. Its size, up to 12 inches or more, it's purple-red to wine red color and its cylindrical tapered branches, noticeably narrowed at their base, readily catch one's attention. In shallow and well lit shores the plant will have yellow branches and a red base, intense sun light will not cause the plant to have yellow tops. Certain species of *Gracilaria* are



used as food in Japan, Hawaii, and the Philippines. The macro algae are known to be both a good aquarium decorative and a good food for algae eating fishes.

Without adding any inoculum, *Gracilaria verrucosa* has its mixed culture naturally. Although it's a red micro alga, it is a source of mixed bacteria culture.

Bacteria in *Gracilaria verrucosa* are detected by Microbiology Laboratory of Karlsruhe Technical University (Germany) and the results are given in Section 4.1.6.

Studies in literature done with *Gracilaria* together with their equilibrium models are tabulated in Table 2.19.

Table 2.19 Studies in literature done with *Gracilaria* and the equilibrium models evaluated

Adsorbent	Adsorbate	Gracilaria							Reference
		Amount of Adsorbent (g)	Langmuir Isoterm Constants			Freundlich Isoterm Constants			
			Q _{max} (mg/g)	b (L/mg)	R ²	n (-)	K _F ((mg/g)* (mg/L) ⁿ)	R ²	
Pretreated biomass of marine alga Gracilaria verrucosa	Cadmium (II)	0.2	0.63	3.600	0.999	-	-	-	Chaisuksant, 2003
	Copper (II)	0.2	0.72	7.890	0.999	-	-	-	
Activated carbon prepared from red alga Gracilaria	Cu(II)	2.0	2.527	0.208	0.997	2.444	0.640	0.992	Esmaeili et al., 2008
		3.0	1.817	0.493	0.646	2.704	0.671	0.995	
		4.0	1.330	0.616	0.999	2.633	0.475	0.974	
Activated carbon prepared from red alga Gracilaria	Ni(II)	4.0	2.686	0.673	0.924	0.144	1.595	0.979	Esmaeili et al., 2009
		6.0	2.305	1.100	0.993	0.175	1.322	0.982	

2.8.3.3 Algae

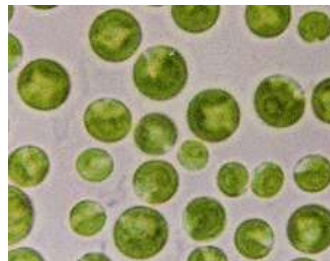
Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. The largest and most complex marine forms are called seaweeds. They are photosynthetic, like plants, and "simple" because they lack the many distinct organs found in land plants.

Though the prokaryotic Cyanobacteria (commonly referred to as blue-green algae) were traditionally included as "algae" in older textbooks, many modern sources regard this as outdated (Nabors, 2004) as they are now considered to be closely related to bacteria. The term *algae* are now restricted to eukaryotic organisms (Allaby, 1992). All true algae therefore have a nucleus enclosed within a membrane and plastids bound in one or more membranes (Nabors, 2004). Diatoms are also examples of algae.

Nearly all algae have photosynthetic machinery ultimately derived from the Cyanobacteria, and so produce oxygen as a by-product of photosynthesis, unlike other photosynthetic bacteria such as purple and green sulfur bacteria.

2.8.3.3.1 *Chlorella* sp.

Chlorella sp. is a natural green micro-alga where sp. stands for the undefined species of *Chlorella*. There are dozens of sub-species. This green colored bacterium has the distinction of having the highest concentration of chlorophyll among any natural substances.



Since it is a natural alga that can easily reproduce with the help of water, sunlight, carbon dioxide and a few naturally obtained minerals, chlorella is very easy to produce.

Studies in literature done with *Chlorella* together with their equilibrium models are tabulated in Table 2.20.

2.8.3.3.2 *Spirulina platensis*

Spirulina is a microscopic blue-green alga in the shape of a perfect spiral coil, living both in sea and fresh water. *Spirulina* is the common name for human and animal food supplements produced primarily from two species of cyanobacteria: *Spirulina platensis*, and *Spirulina maxima* (Figure 2.20). *Spirulina* is cultivated around the world, and is used as a human dietary supplement, as well as a whole food, and is available in tablet, flake, and powder form. It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries.

Studies in literature done with *Spirulina platensis* together with their equilibrium models are tabulated in Table 2.21.

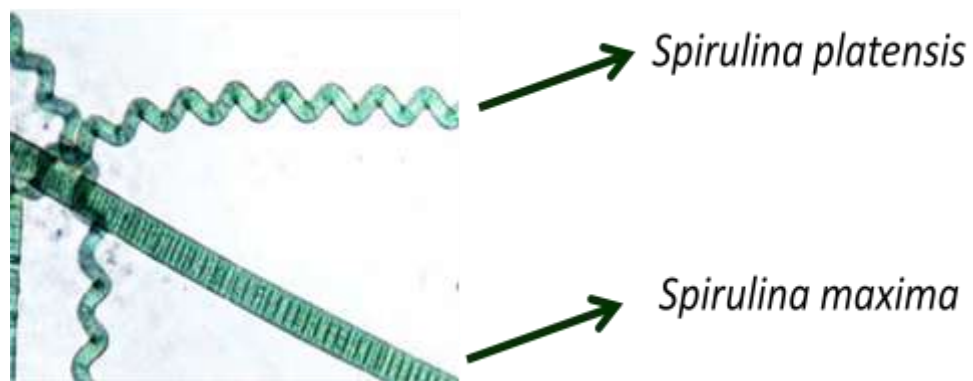


Figure 2.20 Two types of *Spirulina*: *Spirulina platensis* and *Spirulina maxima*

Table 2.20 Studies in literature done with *Chlorella*. and the equilibrium models evaluated

Adsorbent	Adsorbate	Chlorella.							Reference
		Langmuir Isotherm Constants				Freundlich Isotherm Constants			
		pH and T	Q _{max} (mg/g)	b (L/mg)	R ²	n	K _F ((mg/g)*(mg/L) ⁿ)	R ²	
Chlorella sp.	Cr(IV) synthetic wastewater	pH=1	10000.000	0.002	0.992	27.430	1.092	0.995	Nakiboglu et al., 2005
		pH=2	1111.110	0.007	0.969	27.220	1.706	0.986	
Chlorella vulgaris	Au	T=30°C	28.170	0.033	0.970	-	-	-	Nakbanpote et al., 2002
Activated Carbon		T=30°C	73.530	0.240	0.990	-	-	-	

Table 2.21 Studies in literature done with *Spirulina platensis* and the equilibrium models evaluated

Adsorbent	Adsorbate	Spilurina platensis						Reference
		Langmuir Isotherm Constants			Freundlich Isotherm Constants			
		Q _{max} (mg/g)	b (L/mg)	R ²	n	K _F ((mg/g)*(mg/L) ⁿ)	R ²	
Re-hydrated biomass of Spilurina platensis	Cr(III)	36.200	1.430	1.000	6.570	3.910	0.820	Lodi et al., 2007
Spilurina platensis		D–R Isotherm Constants						Seker et al., 2007
	Pb(II)	k	q _m	R ²				
		0.002	0.002	0.887	0.217	0.004	0.855	
	Cd(II)	0.002	0.001	0.946	0.217	0.003	0.931	
	Ni(II)	0.006	0.003	0.902	0.479	0.016	0.920	

2.9 Measurement Techniques

If a beam of white light passes through a glass container (cuvet) filled with liquid, the emergent radiation is always less powerful than that entering. The diminution of power is generally of different extent for different wavelengths. The loss is due in part to reflections at surfaces and to scattering by any suspended particles present, but in the absence of such particles, it is primarily accounted for by the absorption of radiant energy by the liquid (Ewing, 1985).

If the energy absorbed is greater for some visible wavelengths than for others, the emergent beam will appear colored. The apparent color of the solution is always the complement of the color absorbed. Thus a solution absorbing in the blue region will appear yellow; one that absorbs green will appear purple, etc. (Ewing, 1985).

Many organic substances found in raw water sources exhibit characteristics property of absorbing light in the ultraviolet (UV) and visible regions, wavelengths 10 to 400 nm and 400 to 800 nm, respectively. Most of the absorbance occurs in the near UV range of 200 to 400 nm. This UV-absorbance, due mainly to the stimulations of π - electrons, is characteristics for substances with carbon-carbon and carbon-oxygen double bonds. The UV absorbance spectra of naturally occurring substances are featureless due to random interactions and the spectra monotonically decrease with increasing wavelength (Sontheimer et al., 1988).

Ultraviolet-visible Spectroscopy (UV/VIS)

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometer (UV/ VIS) involves the spectroscopy of photons and spectrophotometer. It uses light in the visible and adjacent near ultraviolet (UV) and near infrared (NIR) ranges. In this region of energy space molecules undergo electronic transitions.

Applications of UV

UV/V is spectroscopy is routinely used in quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Solutions of transition metals ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The color of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the color of a dilute solution of copper sulphate is a very light blue; adding ammonia intensifies the color and changes the wavelength of maximum absorption (λ_{max}).

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water soluble compounds, or ethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths.)

While charge transfer complexes also give rise to colors, the colors are often too intense to be used for quantitative measurements.

Mathematical Theory

The absorption of radiant energy by matter can be described quantitatively through the general principle known as **Beer's Law**. Consider a glass cuvet with a plane parallel faces traversed by monochromatic radiation. Losses by reflection at the surfaces and absorption by the glass will be neglected for the moment. Suppose that the cuvet is filled with an absorbing species dissolved in a non-absorbing solvent. The radiation diminishes in power the further it penetrates into the solution and the greater the concentration of solute. More generally stated, the diminution in power is proportional to the number of absorbing molecules in the path of the beam. The quantitative statement of this relation is **Beer's Law (or Beer-Lambert Law)** (Ewing, 1985).

Beer-Lambert Law

The method is most often used in a quantitative way to determine concentrations of an absorbing species in solutions, using the Beer-Lambert Law:

$$A = \log_{10}(I/I_o) = \varepsilon \cdot c \cdot L$$

where A is the measured absorbance, I_o is the intensity of the incident light at a given wavelength, I is the transmitted intensity, L the path length through the sample, and c the concentration of the absorbing species (Figure 2.21). For each species and wavelength, ε is a constant known as the molar absorptivity or extinction coefficient. This constant is a fundamental molecular property in a given solvent, at a particular temperature and pressure, and has units of $1 / M^*cm$ or often AU / M^*cm .

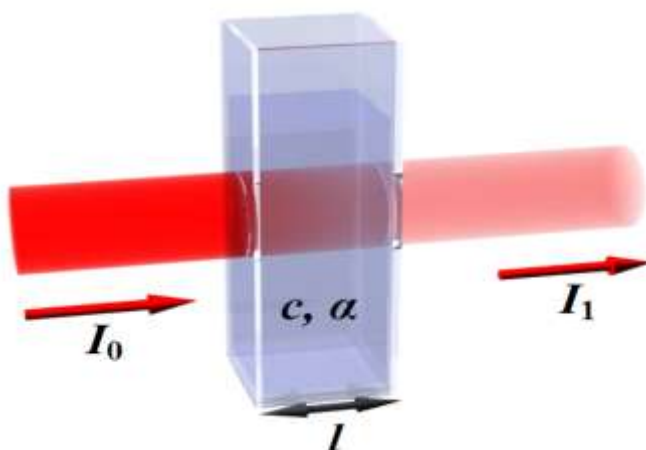


Figure 2.21 Figure of Beer-Lambert Law

In spectroscopy, the absorbance A is defined as;

$$A_\lambda = \log_{10}(I_o/I)$$

where I is the intensity of light at a specified wavelength λ that has passed through a sample (transmitted light intensity) and I_o is the intensity of the light before it enters the sample or incident light intensity. Absorbance measurements are often carried out in analytical chemistry, since the absorbance of a sample is

proportional to the thickness of the sample and the concentration of the absorbing species in the sample, in contrast to the transmittance I / I_o of a sample, which varies exponentially with thickness and concentration.

The absorbance and extinction ϵ are sometimes defined in terms of the natural logarithm instead of the base 10 logarithm.

The Beer-Lambert Law is useful for characterizing many compounds but does not hold as a universal relationship for the concentration and absorption of all substances.

Comparison of UV/V, HPLC, Gas Chromatography

UV/V is spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions can be colored, organic compounds, especially those with a high degree of conjugation . Less volatile and larger samples can be used with HPLC. It was discovered that better separation of the components of the mixture occurs if the particles in the stationary phase are very small. However, it was also found that if very small particles were used in the column, then the liquid passed very slowly through the column. Therefore, a pump is used to force the liquid through the column. This is not necessary in GC but a shorter column can be used in HPLC because the separation is more efficient.

The used measurement techniques of different adsorbates (especially pesticides) on some adsorbents are tabulated in Table 2.22.

Table 2.22 The used measurement techniques of some adsorbate on adsorbents

Adsorbate	Adsorbent	Measurement Techniques	References
Acid dyes	GAC	UV	Walker and Weatherley, 1997
2,4D Carbofuran	Fertilizer industry waste & Steel industry waste	UV	Gupta et al., 2006
2,4D	PAC	UV	Aksu and Kabasakal, 2005
2,4D	PAC	UV	Belmouden et al., 2000
2,4D 2,4DB 2,4DP 2,4,5 T MCPA	Organo-clays	UV	Akçay and Yurdakoc, 2000
Cr(VI) (Chromium)	Pomace (Olive oil industry waste)	UV	Malkoç et al., 2006
2,4D	GAC	UV	Kim et al., 2003
Isoproturon	Alumina(with and without sodium dodecyl sulfate)	UV	Gawade et al., 2004
Thiazafluron Metamitron	Soil –Clay suspensions	UV & HPLC	Cox et al., 1997
2,4D	Dodecylammonium sepiolite (DAS)	UV	Akçay et al., 2004
TNT (trinitrotoluene)	GAC	HPLC	Marinovic et al., 2005
Atrazine	Activated carbon fibres(ACF)	HPLC	Gullon and Font, 2001
2,4D Atrazine	Waste Tyre Rubber Granules	GC	Alam et al., 2004
Atrazine Simazine Terbutryn Diuron	Different soil samples	GC	Csutoras and Kiss, 2007

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

The used chemicals in the experiments were; 2,4-Dichlorophenoxyacetic acid (Sigma Aldrich), GAC (1-2 mm, Aqualine), Ethanol (Kimetsan), Methanol (99.9%, Kimetsan), HCl (Emir Kimya), NaOH (pure pellets, Merck), CaCl_2 (10^{-2} M, pure, Merck), pH=4 and pH=7 buffer (WTW), NaCl (Merck), KCl (3M, Merck), Distilled water, Chromic acid, TSA-Tryptic Soy Agar (Merck), TSB-Tryptic Soy Broth (Criterion), Zarrouk's Medium, Bold's Basal Medium.

Granular Activated Carbon:

Aqualine labeled activated carbon was purchased from Esli and PWG Co., Turkey. It was ground and sieved into desired particle size (1-2mm) in Dokuz Eylul University Mining Engineering Department. Before using, the carbon washed with distilled water a few times to remove carbon powder and impurities, oven dried at 110°C unless obtain stable weight. The GAC was stored in a sealed bottle along a silica gel to prevent the readsorption of moisture before use. GAC's characteristics are summarized in Table 3.1 and its SEM photo is seen in Figure 3.1.

Table 3.1 The properties of aqualine granular activated carbon

BET (m^2/g)*	671
Total Pore volume (cm^3/g)	0.320
Micropore Volume (<2nm) volume, cm^3/g	0.125
Percent (%v)	39.1
Micropore area (m^2/g)	303
Percent (%s.a)	45.2
Mesopore Volume (Percent)	60.9
Particle density (g/cm^3)**	2.35

*The BET areas of activated carbon were estimated by the standard adsorption of N_2 at 77K, using a surface analyzer (QS-7 Quantasorb Surface Analyzer).

**The density was determined using an autopycnometer (AccuPyc He-pycnometer).

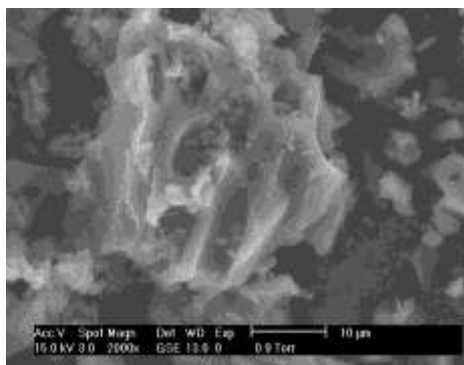


Figure 3.1 The SEM photo of GAC

3.1.2 Equipment

The used equipment in the experiments were; glass columns (ID=1.1cm), shakers (Memmert WNB 7-45, P Selecta Unitronic OR), peristaltic pump (Watson- Marlow 430-VM4 (10rpm) with 4 exits), power supply (Makelsan 2006-1000VA), tubing (Watson–Marlow marprene grey/ grey 1.29 mm and green/green 1.85 mm), petri dishes (Isolab disposal), Schleicher & Schuell Blue Band Filter Papers (pore size=1 µm), vacuum (Sartorius Glass Vacuum Filter Holder), vacuum filter (cellulose filter pore size=0.45 µm), Sartorius GF/C Filter Papers (pore size=0.45 µm), magnetic stirrer (IKAMAG RO5 power, Selectra multimatic-5N), vortex (Bio Vortex V-1 plus), scale (AND HM-200, sensitive to 0.0001 g), pH meter (WTW pH 330/set), spectrophotometer (Jasco Model 7800 UV/VIS Spectrophotometer), microwave (Bosh 812-c), water bath, autoclave (HMC HIRAYAMA Hiclave HV-50L), incubator (Nuve), desicator, electron microscope (Olympus CX31), and centrifuge (TD3).

3.1.3 Microorganisms

Table 3.2 shows the types of microorganisms used and where they are obtained from.

Table 3.2 Microorganisms used and their sources

Type of Microorganisms	Sources
Aquarium Water	Aquarium
<i>Gracilaria verrucosa</i>	İzmir Bay
<i>Acidovorax Avenae Subsp. Avenae</i> LMG 17238	BCCM TM /LMG bacteria collection of the Gent University, Belgium
<i>Spirulina platensis</i>	CCAP-Culture Collection of Algae and Protozoa, UK
<i>Chlorella sp.</i>	CCAP-Culture Collection of Algae and Protozoa, UK

3.1.4 Culture Media

3.1.4.1 Culture Media for *Acidovorax Avenae Subsp. Avenae* LMG 17238

There are two types of culture media used for the inoculation of pure bacteria LMG 17238 which are TSB (Tryptic Soy Broth) and TSA (Tryptic Soy Agar). The contents of these media are tabulated in Table 3.3.

TSB (Tryptic Soy Broth) medium was prepared as 30 g (broth) / 1 liter distilled water where TSA (Tryptic Soy Agar) medium was prepared as 40 g (agar) / 1 liter distilled water. Then either two media were autoclaved for 15 min at 121°C. The media's pH should be 7.3 ± 0.2 at 25°C. After preparation both media were clear and yellowish-brown. 0.86% NaCl solution was prepared by dissolving 0.86 g NaCl in 100 mL distilled water.

Table 3.3 Contents of culture media for *Acidovorax Avenae Subsp. Avenae* LMG 17238

TSB (Tryptic Soy Broth)		TSA (Tryptic Soy Agar)	
Constituents	Composition (g/L)	Constituents	Composition (g/L)
Casein peptone	17	Casein peptone	17
Soy peptone	3	Soy peptone	3
Sodium chloride	5	Sodium chloride	5
Hydrogen phosphate	2.5	Agar agar	15
Glucose	2.5		

3.1.4.2 Culture Medium for *Spirulina platensis*

Spirulina platensis grows best in Zarrouk's medium and its content is tabulated in Table 3.4.

Table 3.4 Content of Zarrouk's medium

Constituents	Composition (g/L)
NaHCO ₃	16.80
K ₂ HPO ₄	0.50
NaNO ₃	2.50
K ₂ SO ₄	1.00
NaCl	1.00
MgSO ₄ .7H ₂ O	0.20
CaCl ₂ .2H ₂ O	0.04
FeSO ₄ .7H ₂ O	0.01
EDTA	0.08
A ₅ micronutrient sol. ^a	1 ml

^aA₅ micronutrient solution consists of H₃BO₃, 2.86 g/L; MnCl₂.4H₂O, 1.81 g/L; ZnSO₄.4H₂O, 0.222 g/L; Na₂MoO₄, 0.0177 g/L; CuSO₄.5H₂O, 0.079 g/L.

3.1.4.3 Culture Medium for *Chlorella* sp.

Chlorella sp. grows best in Bold's Basal medium and its content is tabulated in Table 3.5.

Table 3.5 Content of Bold's Basal medium

Stocks	g/ per 400 ml
(1) NaNO ₃	10.00
(2) MgSO ₄ .7H ₂ O	3.00
(3) NaCl	1.00
(4) K ₂ HPO ₄	3.00
(5) KH ₂ PO ₄	7.00
(6) CaCl ₂ .2H ₂ O	1.00
(7) Trace elements solution (autoclave to dissolve):	g/per 1 L
ZnSO ₄ .7H ₂ O	8.82
MnCl ₂ .4H ₂ O	1.44
MoO ₃	0.71
CuSO ₄ .5H ₂ O	1.57
Co(NO ₃) ₂ .6H ₂ O	0.49
(8) H ₃ BO ₃	11.42
(9) EDTA	50.00
KOH	31.00
(10) FeSO ₄ .7H ₂ O	4.98
H ₂ SO ₄ (conc)	1 ml
Medium	per 1 L
Stock solutions 1-6	10 ml each
Stock solutions 7-10	1 ml each

Make up to 1 liter with glass distilled or deionised water.

3.2 Methods

3.2.1 Microorganism Preparation and Inoculation Methods

Aquarium Water:

1 liter of aquarium water is taken into a sterilized bottle and is waited in the refrigerator for a night to obtain a much more dense mixture in bacterial point of view. It is then ready to be used for both batch and continuous experiments.

Gracilaria verrucosa:

The material is collected from the sea, washed with water, dried under laboratory conditions preserved at room temperature in a container free from moisture. Before it is used, it is immersed in a beaker full of distilled water and waited until it gives its reddish color to the water. It is then ready to be used for both batch and continuous experiments.

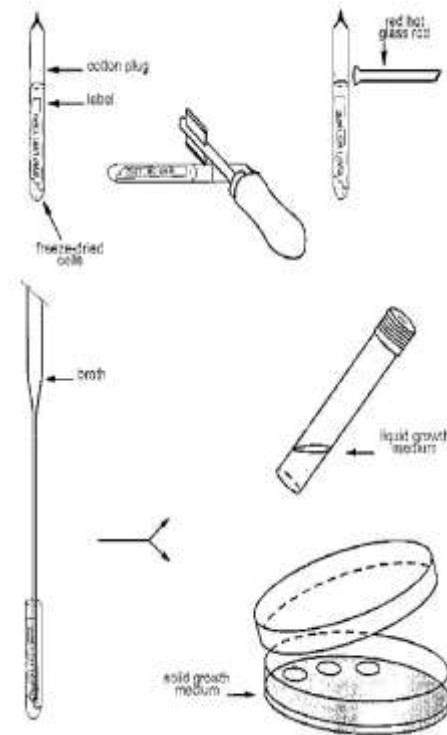


Acidovorax Avenae Subsp. Avenae LMG 17238:

The pure bacteria studied in this project *Acidovorax avenae* subsp. (*Avenae* LMG 17238) is obtained from the BCCMTM/LMG bacteria collection of the Gent University (Gent/Belgium) which is classified as *Pseudomonas* gram negative. The recipe of the bacteria has been sent with the recommended media written on it (Figure A.1). This bacterium is produced for the biodegradation of the synthetic-based polyester based plastics, poly ϵ -caprolactone (PCL), poly (butylene succinate-cobutylene adipate) and a starch –PCL blend.

Inoculation of *LMG* bacteria requires several steps. The procedure that needs to be followed for inoculating the bacteria is:

1. The strain is received in the form of freeze-dried culture in a tube (cells are in suspension). Schematic view of the reviving of *LMG 17238* can be seen in Figure 3.2.



2. Prepare TSB (30 g/L) in an erlenmayer flask, close its top with cotton, sterilize it in an autoclave at 121°C for 20 minutes.
3. Break the tube and put 500 μ L

from this sterilized TSB by the help of a micropipette into the tube.

Figure 3.2 Reviving the pure bacteria culture *LMG 17238*

4. Vortex and keep it at 28°C overnight in the incubator.
5. By the way, in an autoclavable bottle, prepare TSA (40 g/L).
6. Try to dissolve it by heating in a microwave.
7. Pour 5 mL of this hot TSA to 10 mL 10 tubes.
8. Close the tubes and sterilize it in an autoclave. By this way, TSA is also sterilized.
9. Decline the tubes 45 degree and wait for it to be cooled and hardened. (Therefore, it is called “sloped agar”) By this way, the surface area for bacterial growth is increased.

10. Take the tube containing the culture and 500 μL TSB and put 50 μL to each of the 10 tubes. While transferring the bacteria, it is important to work beside flame because of aseptic considerations as seen in Figure 3.3.
11. Vortex and keep it at 28°C for 48 hours in the incubator. Meanwhile, the bacteria grow.
12. Keep the sloped-agar tubes in the refrigerator.
13. If the bacteria will be used in an experiment in liquid form, then prepare TSB (30 g/L), sterilize it in an autoclave and wait until it cools down to 50°C .
14. Applying the aseptic technique, take 1 loop of bacteria from sloped agar and mix it into TSB.



Figure 3.3 Transferring the bacteria in TSB from one tube to another

15. Take bacteria in liquid medium as much as needed in the experiment.

Spirulina platensis and Chlorella sp.:

The steps followed for the growth of the algae:

1. The algae are received in a 50 ml tube within the necessary media, Bold's Basal Medium for *Chlorella sp.* and Zarrouk's Medium for *Spirulina platensis*.



Figure 3.4 *Chlorella sp.* in 150 ml beaker

2. 10 ml of these liquid cultures and 40 ml of prepared media are added into 150 ml beaker as seen in Figure 3.4, so by this way, %20 inoculation is done.
3. One week later, 200 ml of each media and 50 ml solution are added into a 500 ml beaker. (%20 inoculation)
4. Two weeks later, they are inoculated to 1 L and at last to 2 L.
5. Both are kept under light in the incubator at 25°C and shake frequently in order to prevent sedimentation.

3.2.2 Experimental Programme and Methods

3.2.2.1 Batch Mode Adsorption Studies

Blank (without microorganism) sorption studies were conducted in a routine manner by the batch technique. A number of erlenmeyer flasks containing a definite volume (50 mL in each case) of solutions of 2,4-D of desired concentration (100 ppm) and pH (6.5-7.0) were placed in a thermostatic shaking assembly. For the studies of activated carbon, 0.01 g of activated carbon was treated with 50 mL of 2,4-D solution at a defined pH and temperature.

The sample bottles were agitated on a mechanical shaker at a 90 rpm constant shaking rate for 5 days to ensure equilibrium was reached (Figure 3.5) and afterwards, filtration process was applied (Figure 3.6).



Figure 3.5 Internal view of the shaker



Figure 3.6 Filtration

For the biological contamination studies, five different microorganisms- Aquarium water, *Gracilaria verrucosa*, *Chlorella sp.*, *Spirulina platensis* and *Acidovorax Avenae Subsp. Avenae* LMG 17238- were used for different concentrations of 2,4-D. The volume of microorganisms per 50 ml 2,4-D solution were changed. The sample solutions that contained bacteria solution was filtered by vacuum pump using cellulose filter with pore size ($0.45\ \mu\text{m}$) (Figure 3.7).



Figure 3.7 Vacuum filtration

For thermodynamic studies, the sample bottles were agitated on a mechanical shaker at three different temperatures such as 25°C , 35°C and 45°C . In order to compare the results with the blank condition, 2 ml of *Chlorella sp.* is added to the 50 mL 2,4-D solution.

The studies were conducted in duplicate for all samples to ensure the reproducibility of results and the average value is reported in all the cases. The

amount of 2,4-D adsorbed by GAC was calculated from the difference between the initial and equilibrium 2,4-D concentration in solution.

3.2.2.2 Continuous Mode Adsorption Studies

In order to get information about column performance of GAC on removal of 2,4-D, continuous mode adsorption studies were performed. Figure 3.8 shows the photograph of continuous system set-up that is used in the laboratory.

In these studies, a glass column with an internal diameter of 1.1 cm is used. The column was operated in upflow mode at room temperature using a peristaltic pump with no effluent recycle. The column was cleaned with dilute chromic acid and rinsed with distilled water before the experiments. Distilled water was pumped in an upflow mode to remove any trapped air from the bed.

The effect of important parameters such as influent concentration, flow rate, mass of the adsorbent and different biological contaminants were investigated. For the case of biological contamination load, microorganisms were loaded for a certain loading time using a peristaltic pump with effluent microorganism recycle (Figure 3.9). After loading the column with microorganisms, a stock solution of 2,4-D was pumped with a flow rate 0.4 mL/min.



Figure 3.8 Photograph of continuous mode set-up that is used in the laboratory



Figure 3.9 Biological contamination load to the continuous system

3.2.3 Microorganism Identification and Counting Methods

There are several methods for identifying and counting microorganisms and they are shown in Figure 3.10.

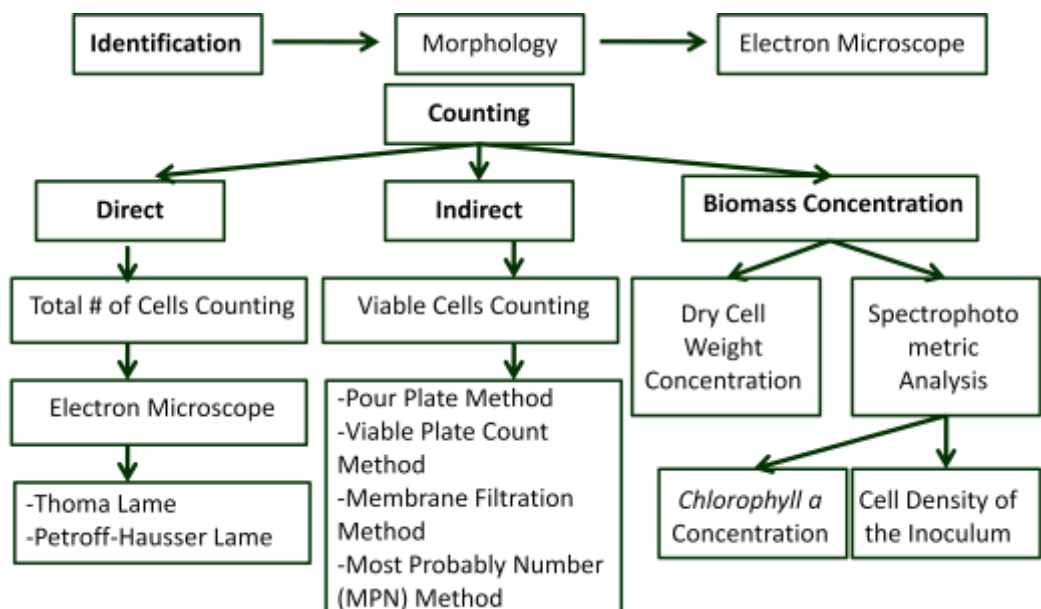


Figure 3.10 Methods for identifying and counting microorganisms

3.2.3.1 Gram-Stain Procedure

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (1853-1938), who developed the technique in 1884 (Gram 1884).

The steps of the procedure are as follows and summarized in Figure 3.11.

1. First, a loopful of a pure culture is smeared on a slide and allowed to air dry. The culture can come from a thick suspension of a liquid culture or a pure colony from a plate suspended in water on the microscope slide.

Important considerations:

- Take a small inoculum – don't make a thick smear that cannot be completely decolorized. This could make gram-negative organisms appear to be gram-positive or gram-variable.

- Take a fresh culture – old cultures stain erratically.

2. Fix the cells to the slide by heat or by exposure to methanol. Heat fix the slide by passing it (cell side up) through a flame to warm the glass. Do not let the glass become hot to the touch.

3. Crystal violet (a basic dye) is then added by covering the heat-fixed cells with a prepared solution. Allow to stain for approximately 1 minute.

4. Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.

5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye, making it more difficult to decolorize and reducing some of the variability of the test.

6. Briefly rinse with water.

7. Decolorize the sample by applying 95% ethanol or a mixture of acetone and alcohol. This can be done in a steady stream, or a series of washes. The important aspect is to ensure that all the color has come out that will do so easily. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple with Gram-negative organisms colorless. The decolorization of the cells is the most “operator-dependent” step of the process and the one that is most likely to be performed incorrectly.

8. Rinse with water to stop decolorization.

9. Rinse the slide with a counterstain (safranin or carbol fuchsin) which stains all cells red. The counterstain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain, its effect is only seen in the previously colorless gram-negative cells which now appear pink/red.

10. Blot gently and allow the slide to dry. Do not smear.

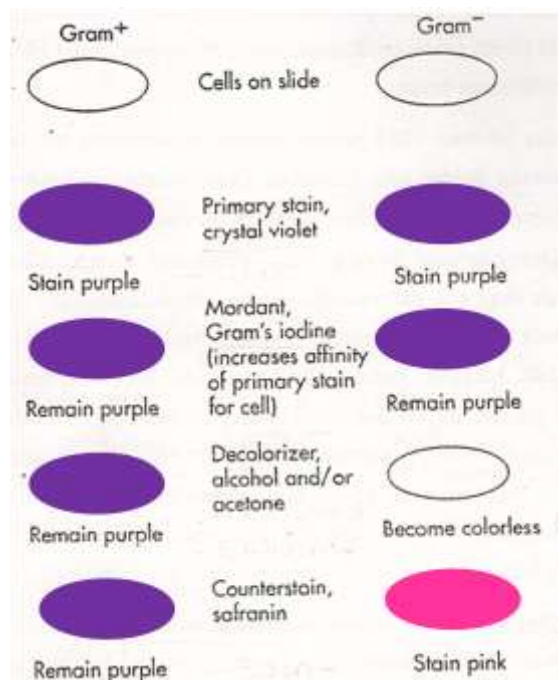


Figure 3.11 The Gram-stain procedure

3.2.3.2 Optical Microscopic Views

The optical microscobic views of *Spirulina platensis*, *Chlorella sp.*, aquarium water, *Gracilaria verrucosa* and *Acidovorax Avenae Subsp. Avenae LMG 17238* are obtained by Olympus CX31.

3.2.3.3 Determination of Inoculums' Dry Cell Weight Concentrations

The procedure of this analysis is as follows:

1. Samples of microorganisms in liquid media are taken to the 15 ml Pyrex tubes.
2. Sartorius GF/C filter papers, which has 0.45 μm pore size, are dried at 105°C in the oven for 2 hours and kept in the desicator for 20 minutes.
3. Taking out the desicator, their tares are recorded.
4. This study is conducted in duplicate for all samples to ensure the reproducibility of results and the average value is reported for each sample.
5. 5 ml of each sample solutions that contain the microorganisms are filtered by vacuum pump on top of the dried Sartorius GF/C filter papers.
6. Filter papers are dried at 105°C in the oven for 2 hours and kept in the desicator for 20 minutes. The samples are taken out of the desicator, ready to be weighed as seen in Figure 3.12.
7. The samples are weighed by the scale sensitive to 0.0001 g and the results are recorded.



Figure 3.12 The filter papers with the dry weight of microorganisms on them

3.2.3.4 Spectrophotometric Analyses

3.2.3.4.1 Determination of *Chlorophyll a* Concentration

The procedure of this analysis is as follows:

1. Samples of microorganisms in liquid media are taken to the 15 ml Pyrex tubes.
2. 5 mL of each sample is transferred to the capped centrifugal tubes.
3. The samples are centrifuged at 4000 rpm for 5 minutes.
4. Without touching the pellet settled at the bottom, supernatant is taken by the help of Pasteur pipette.
5. 5 ml of 99.9% methanol is added to the supernatant.
6. Water bath is heated to 60-70°C and the tubes are immersed in it.
7. After 5 minutes, they are again centrifuged at 4000 rpm.
8. Without touching the pellet at the bottom, supernatant, which includes the extracted *chlorophyll a* with methanol, is taken by the help of Pasteur pipette and put into 15 ml Pyrex tubes as in Figure 3.13.
9. Spectrophotometric reading is done at 750 nm and 665 nm and methanol is used as the blank.
10. Calculation is done according to the formula below:

$$\text{Chlorophyll-a concentration (mg/L)} = (A_{750} - A_{665}) * 13.9$$



Figure 3.13 The supernatants including *chlorophyll a* in the Pyrex tubes

3.2.3.4.2 Determination of the Cell Density of the Inoculums

The procedure of this analysis is as follows: The growths of the algae are followed during the experiment by reading their absorbances at 560 nm. Culture media are used as the blanks.

3.2.3.5 Viable Plate Count Method

A viable cell is one that is able to divide and form offspring, and in most cell-counting situations, these are the cells looked for. For these purposes, a viable counting method can be used. Usually, the number of cells in the sample capable of forming colonies on a suitable agar medium is determined. For this reason, the viable count is also called a "plate count". The assumption made in the viable counting procedure is that each viable cell can grow and divide to yield one colony. Thus, colony numbers and cell numbers are proportional (Madigan, 2009). Many of the bacteria present in a sample may grow on the medium used at the pH and the temperature employed or in the time inoculated. It is often necessary to report on the size of bacterial population in sample, in every inoculation. Accuracy is often demanded where it is not needed. And it should be said that, in viable count methods, it is recognized that large errors are inevitable, even if numbers of replicate plates are used (Collins and Lyne, 1976).

There are at least two ways of performing a plate count: the spread-plate method and the pour-plate method (Figure 3.14). In the spread-plate method, a volume (usually 0.1 ml or less) of an appropriately diluted culture is spread over the surface of an agar plate using a sterile glass spreader. The plate is then incubated until colonies appear, and the number of colonies is counted. The surface of the plate must not be too moist so that the liquid soaks in and the cells remain stationary. Volumes greater than about 0.1 ml are avoided in this method because the excess liquid does not soak in and may cause the colonies to coalesce as they form, making them difficult to count.

In the pour-plate method (Figure 3.14), a known volume (usually 0.1-1.0 ml) of culture is pipette into a sterile Petri plate. Melted agar medium is then

added and mixed well by gently swirling the plate on the benchtop. Because the sample is mixed with the molten agar medium, a larger volume can be used than with spread-plate. However, with this method the organism to be counted must be able to withstand brief exposure to the temperature of the molten agar (nearly 45-50°C). Here colonies form throughout the plate and not just on the agar surface as in the spread-plate method. The plate must therefore be examined closely to make sure all colonies are counted. If the pour-plate method is used to enumerate cells from a natural sample, another problem may arise; any debris in the sample must be distinguishable from actual bacterial colonies or the count will be erroneous (Madigar, 2009).

In Figure 3.14, the fourth column represents colonies of *Escherichia coli* formed from the cells plated by the spread-plate method (top) or the pour-plate method (bottom).

With both the spread-plate and pour-plate methods, it is important that the number of colonies developing on the plate not be too many or too few. On crowded plates some cells may not form colonies and some colonies may fuse, leading to erroneous measurements. If the number of colonies is too small, the statistical significance of the calculated count will be low. The usual practice, which is statistically most valid, is to count colonies only on plates that have between 30 and 300 colonies.

To obtain appropriate colony number, the sample to be counted must almost always be diluted. Because one may not know the approximate viable count ahead of time, it is usually necessary to make more than one dilution. Several 10-fold dilutions of the sample are commonly used (Figure 3.15). To make a 10-fold (10^{-1}) dilution, one can mix 0.5 ml of the sample with 4.5 ml of the diluents, or 1.0 ml of the sample with 9 ml of the diluents. If a 100-fold (10^{-2}) dilution is needed, 0.05 ml can be mixed with 4.95 ml diluents, or 0.1 ml with 9.9 ml diluents. Alternatively, a 10^{-2} dilution can be made by making two successive 10-fold dilutions. With dense cultures, such serial dilutions are needed to reach a suitable dilution. Thus, if a 10^{-6} ($1/10^6$) dilution is needed, it can be achieved by making three successive 10^{-2} ($1/10^2$) dilutions or six successive 10^{-1} dilutions (Figure 3.15)

The sterile liquid used for making dilutions can simply be water, but a balanced salt solution or growth medium may yield a higher recovery. The dilution factor is the reciprocal of the dilution (Madigar, 2009).

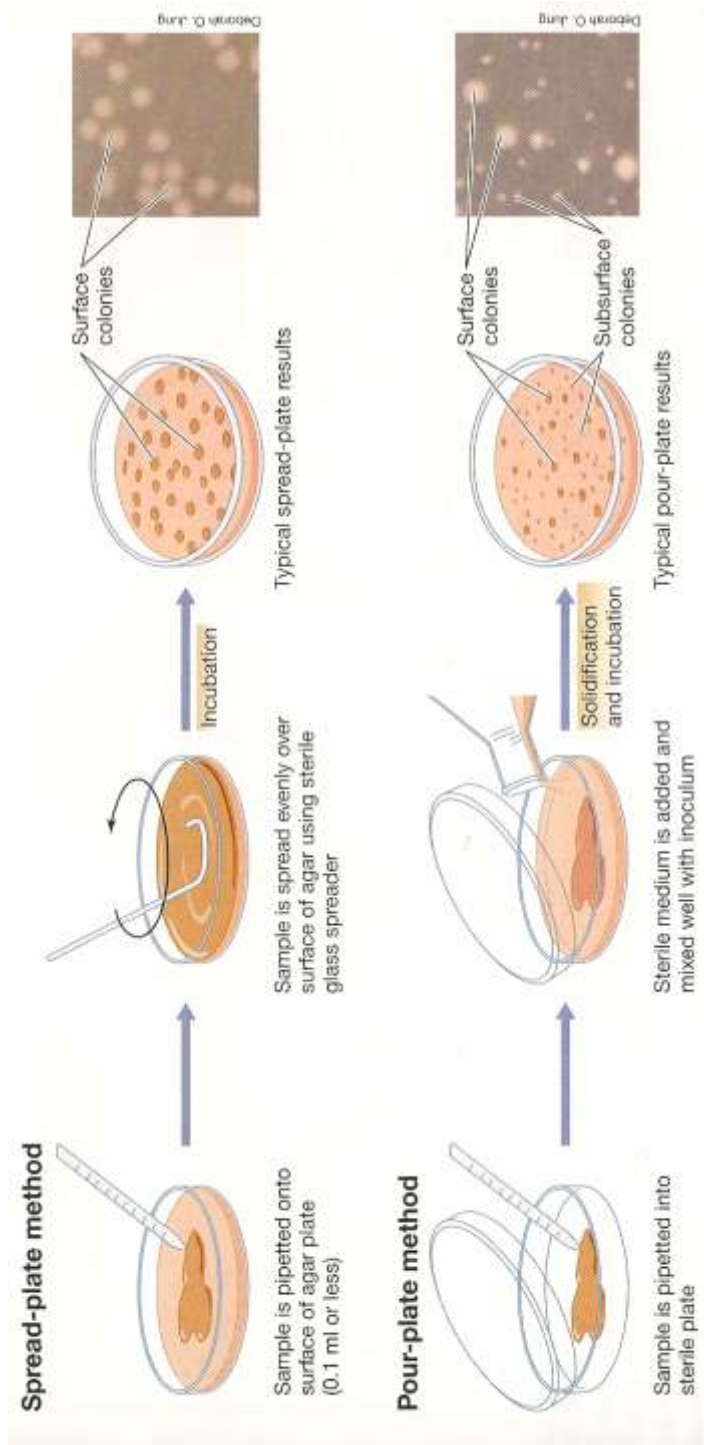


Figure 3.14 Two methods for the viable plate count

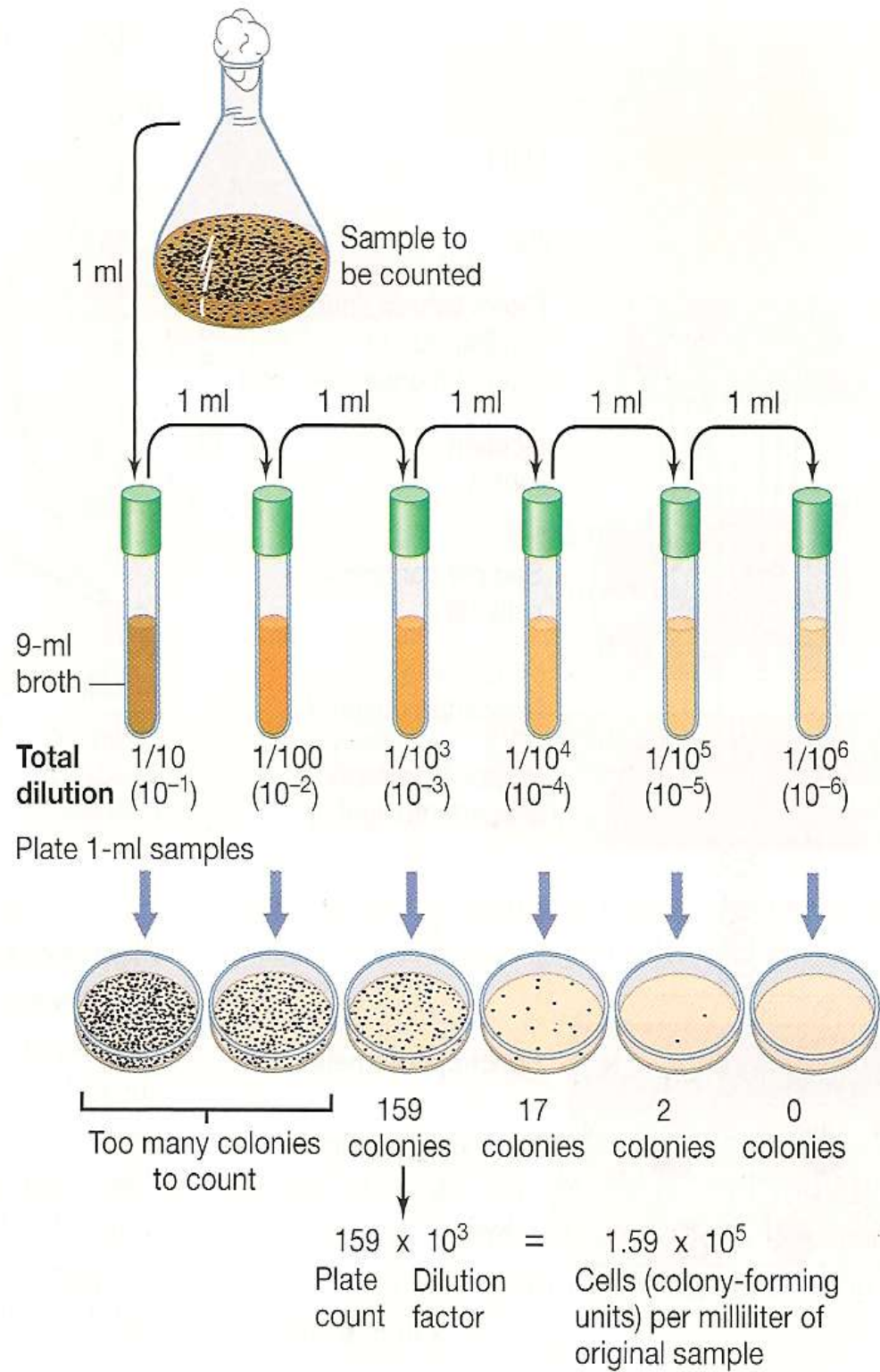


Figure 3.15 Procedure for viable plate counting using several solutions of the sample

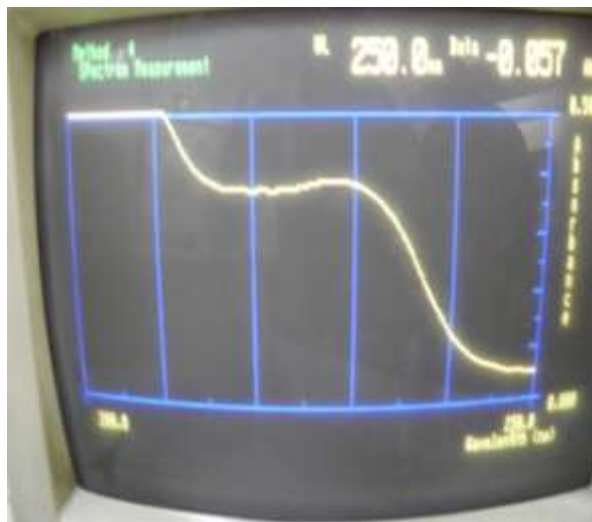
3.2.3.6 Denaturing Gradient Gel Electrophoresis (DGGE) and Polymerase Chain Reaction (PCR) Method

Microbiology Laboratory of Karlsruhe Technical University (Germany) studied a *Gracilaria verrucosa* sample and here is the methodological approach they followed:

1. Total DNA (prokaryotic and eukaryotic) is extracted from the sample.
2. Prokaryotic ribosomal DNA (important for taxonomical identification of bacteria) is amplified from the total DNA via PCR working with universal primers for Eubacteria.
3. So called DGGE (Denaturing gradient gel electrophoresis) experiments are run with the PCR products for population analysis (DNA finger print technique).
4. DGGE results in a very clear DNA band pattern with some dominant DNA bands in a bacterial community.
5. These dominant DNA bands is excised from the gel and this DNA is cloned in an appropriate vector for sequencing and subsequent taxonomical identification.
6. About 30 clones are analysed and identified via ribosomal genebank alignment.
7. The bacteria are identified which are associated with *Gracilaria verrucosa*.

3.2.4 Analysis of 2,4-Dichlorophenoxyacetic Acid

Absorbance of samples were measured at $\lambda_{\max} = 228 \text{ nm}$ by means of Jasco Model 7800 UV/VIS Spectrophotometer where 2,4-D gives a peak (Figure 3.16).



Before beginning measurement of absorbances of the samples, the absorbances of 2.0, 4.0, 6.0, 8.0, 10.0 and 20.0 ppm 2,4-D of standard solutions were measured to obtain the calibration curve (Figure 3.17). The calibration curve was obtained before every measurement. After the calibration curve was determined, absorbances of samples were measured.

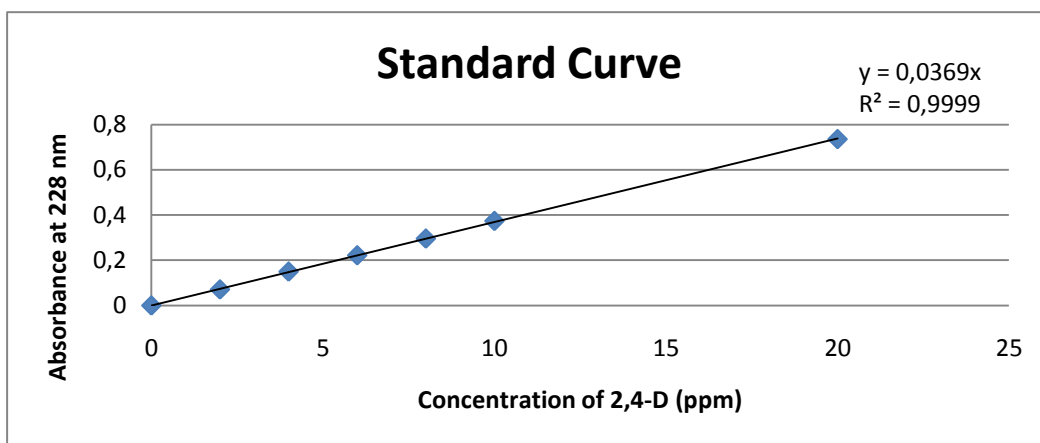


Figure 3.17 The calibration curve for 2,4-Dichlorophenoxyacetic Acid

3.2.5 Computer Modeling Methods-MATLAB

All modeling by non-linear regression is done and the model parameters are evaluated by the help of MATLAB 7.7.0 Curve Fitting Tool Box. Upper and lower limits of the model constants are changing according to each model, but

since they are all physical quantities, it is certain that they all should be positive. The user interface looks like as in Figure 3.18.

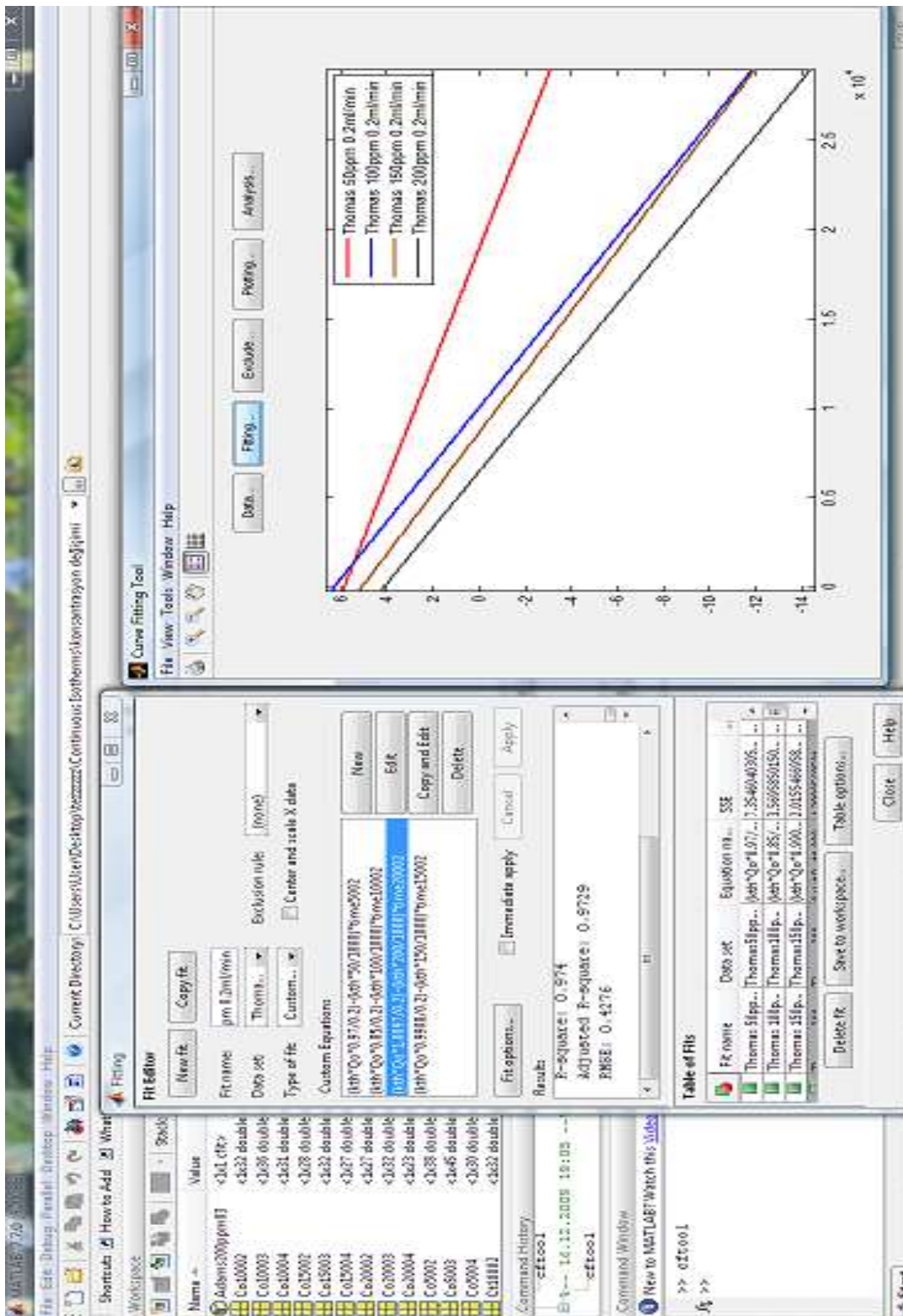


Figure 3.18 The user interface of MATLAB 7.7.0 Curve Fitting Tool Box

4. RESULTS AND DISCUSSION

4.1 Microorganism Identification and Counting Results

4.1.1 Optical Microscopic Views

The optical microscobic views of *Spirulina platensis*, *Chlorella sp.*, aquarium water, *Gracilaria verrucosa* and *Acidovorax Avenae Subsp. Avenae LMG 17238* are obtained by Olympus CX31 with 10x, 40x and 100x magnification.

Microscopic views of *Spirulina platensis* before starting the experiment is seen in Figure 4.1. It is a dense culture with long chains interfering with each other. The size of *Spirulina platensis* is determined to be 40 μm .



Figure 4.1 Microscopic views of *Spirulina platensis* before the experiment (10x)

The following views also belong to *Spirulina platensis* after the time dependent experiment has started. The three-dimensional structure of *Spirulina platensis* after 8 hours of shaking can be seen in Figure 4.2. Algae are together with pesticide molecules in a 100 ppm of 2,4-D solution as it can be observed in Figure 4.3 after 40 hours of shaking. It is important to notice that spiral chains are shorter than before starting the experiment.



Figure 4.2 Three dimensional structure of *Spirulina platensis*, 8 h., 40x



Figure 4.3 *Spirulina platensis* in 100 ppm 2,4-D, 40 h., 10x

After 72 hours of shaking, deformation is observed. The spirals start to open and split up from the long chain is observed as seen in Figure 4.4.



Figure 4.4 Deformation of *Spirulina platensis*, 72h., 10x

Microscopic views of *Chlorella sp.* before starting the experiment is seen in Figure 4.5. It is a dense culture with no contamination. The size of *Chlorella sp.* is determined to be 40 μm .



Figure 4.5 Microscopic views of *Chlorella sp.* before the experiment (40x, 100x, 100x respectively)

The following views also belong to *Chlorella sp.*, but after the time dependent experiment has started. *Chlorella sp.* is a type that can easily reproduce no matter what the conditions are, so no decrease in the culture density is observed. However, there is some contamination observed. In the views, green cells are *Chlorella sp.*, grey are pesticide molecules, white cells are contaminants which belong to some other type of microorganism, other white cells moving with a flagella and *Chlorella sp.* trapped inside are paramecium. This is a very typical view, in every culture of *Chlorella*, paramecium occurs because it feeds on *Chlorella* (Figure 4.6).

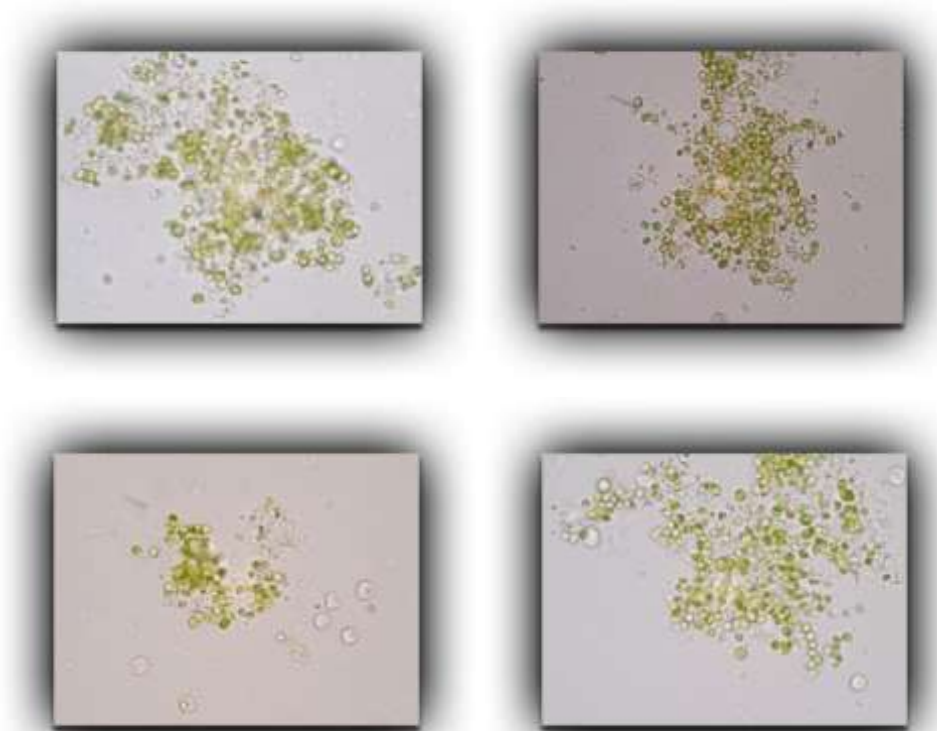


Figure 4.6 *Chlorella* sp. in 100 ppm 2,4-D with contamination; 2 h., 8h., 40 h., 72 h. respectively, 100x

Bacteria in aquarium water after 20 hours of shaking are observed in Figure 4.7 and bacteria in *Gracilaria verrucosa* 72 hours of shaking are seen in Figure 3.18. The sizes of bacteria are determined to be about 1 μ m.



Figure 4.7 Bacteria in aquarium water, 20h., 40x



Figure 4.8 Bacteria in *Gracilaria verrucosa*, 72 h., 100x

Mixed cultures of bacteria are observed in both samples, but it is impossible to identify the types of bacteria only with these optical microscopic views. Further investigation should be done to understand the types of bacteria present.

Microscopic view of *Acidovorax Avenae Subsp. Avenae* LMG 17238 after the Gram-stain procedure is seen in Figure 4.9. The size of LMG bacteria is determined as 2 μm . LMG 17238 is classified as a *Pseudomonas* gram-negative group of bacteria, so after the Gram-Stain Procedure, the color is pink as expected. There is no any contamination observed and it is proved that it is a pure culture.



Figure 4.9 Microscopic view of *Acidovorax Avenae Subsp. Avenae* LMG 17238, 100x

4.1.2 Determination of Inoculums's Dry Cell Weight Concentration Analysis Results

The results of this analysis are as shown in Table 4.1:

Table 4.1 The results of inoculums'dry cell weight concentration analysis

Sample	g Tare	g After Filtration	Dry Cell Weight (g/L)	Dry Cell Weight (mg/L)
Aquarium water	0.0442	0.0442	-	-
<i>Gracilaria verrucosa</i>	0.0443	0.0462	0.380	380
<i>Chlorella sp.</i>	0.0476	0.0480	0.080	80
<i>Spirulina platensis</i>	0.0474	0.0501	0.540	540

Spirulina platensis has the highest dry cell weight; *Gracilaria verrucosa* follows it, and then comes *Chlorella sp.* where there is no dry cell weight detected in aquarium water. This means that the size of the bacteria present in the aquarium water is not big enough for filter papers having 0.45 μm pore size to filter.

4.1.3 Determination of *Chlorophyll a* Concentration Analysis Results

The results of this analysis are as shown in Table 4.2:

Table 4.2 The results of *chlorophyll a* concentration analysis

Sample	A665	A750*	<i>Chlorophyll a</i> (mg/L)
Aquarium water	-	-	-
<i>Gracilaria verrucosa</i>	0.0190	0.005	0.195
<i>Chlorella sp.</i>	0.0177	0.005	2.391
<i>Spirulina platensis</i>	0.0225	0.005	3.058

*A₇₅₀ is a turbidity correction factor. It should be smaller or equal to 0.005. Otherwise, it means that there is mistake and it should not be included in the formula.

Spirulina platensis has the highest *chlorophyll a* concentration, and then comes *Chlorella sp.* where *Gracilaria verrucosa* has the least. This result is obvious from the colors of their supernatants.

4.1.4 Determination of the Cell Density of the Inoculums Analysis

Results

The results of this analysis are tabulated in Table 4.3:

Table 4.3 The results of cell density of the inoculums

Sample	1. day	2. day	3. day	4. day	5. day
<i>Chlorella sp.</i>	0.278	0.413	0.421	0.576	0.739
<i>Spirulina platensis</i>	0.391	0.398	0.433	0.465	0.418

Chlorella sp. can reproduce easily and doubling has occurred in between 1. day and 2. day. *Spirulina platensis* has an adaptation time of 24 hours; after that, it grows faster. However, a decrease in growth is observed from 4.day to 5.day. This may be because *Spirulina platensis* grows best in Zarrouk's medium which is an alkali medium. So if the amount of salt decreases in the medium, the growth of *Spirulina platensis* is affected negatively.

4.1.5 Viable Plate Count Results

Viable plate count method is applied for aquarium water, *Gracilaria verrucosa* and *Acidovorax Avenae Subsp. Avenae LMG 17238* for this study. According to the counting procedure followed by the TARIŞ Research and Development Microbiology Laboratory; *Enterobacter*, *Staphylococcus aureus*, *Total Coliform (E.coli)*, *Bacillus cereus*, *Salmonella* are detected in aquarium water as seen in Figure 4.10. *Salmonella* is detected via test kit. It is a positive or negative test; it does not show the number. If the color in the right bottom room is black, then the test is positive.



Enterobacter



Staphylococcus aureus



Total Coliform, E.coli



Bacillus cereus

*Salmonella***Figure 4.10** Bacteria detected in aquarium water

Staphylococcus aureus, *Enterobacter*, *Total Coliform (E.coli)* and *Salmonella* are detected in *Gracilaria verrucosa* sample as seen in Figure 4.11.

*Enterobacter**Staphylococcus aureus**Total Coliform, E.coli**Salmonella***Figure 4.11** Bacteria detected in *Gracilaria verrucosa*

The results are tabulated in Table 4.4:

Table 4.4 The results of viable plate count method for aquarium water and *Gracilaria verrucosa*

Sample	Total CFU*/Plate $\times 10^3$	<i>Staphylococcus aureus</i> (CFU/Plate) $\times 10^3$	<i>Bacillus cereus</i> (CFU/Plate) $\times 10^3$	Total Coliform, <i>E.coli</i> (CFU/Plate) $\times 10^3$	<i>Enterobacter</i> (CFU/Plate) $\times 10^3$
Aquarium Water**	193	3.2	1.2	81.0	36.9
<i>Gracilaria verrucosa</i> **	135	4.4	0	250	360

*CFU: colony forming unit

**Salmonella is positive for both samples

This study is conducted in duplicate for all samples to ensure the reproducibility of results and the average value is reported for each sample. For optimum accuracy of a count, the preferred range for total CFU/plate is between 30-300 colonies/plate. The results show that the density of bacteria in both samples is very high.

TSB was chosen as inoculation medium for *Acidovorax Avenae Subsp. Avenae LMG 17238* because the higher results were obtained in the counts. The viable plate count results of inoculated bacteria with TSB are tabulated in Table 4.5 and shown in Figure 4.12.

Table 4.5 The results of viable plate count method for LMG 17238

# of inoculation	Inoculated bacteria with TSB $\times 10^8$ (cfu / mL)
1.	8.25
2.	3.30
3.	11.2
4.	11.8
5.(1 st day)	13.3
5.(2 nd day)	16.0
5.(3 rd day)	13.5
5.(4 th day)	14.0
5.(5 th day)	16.2
6.	6.80
7.	0.77

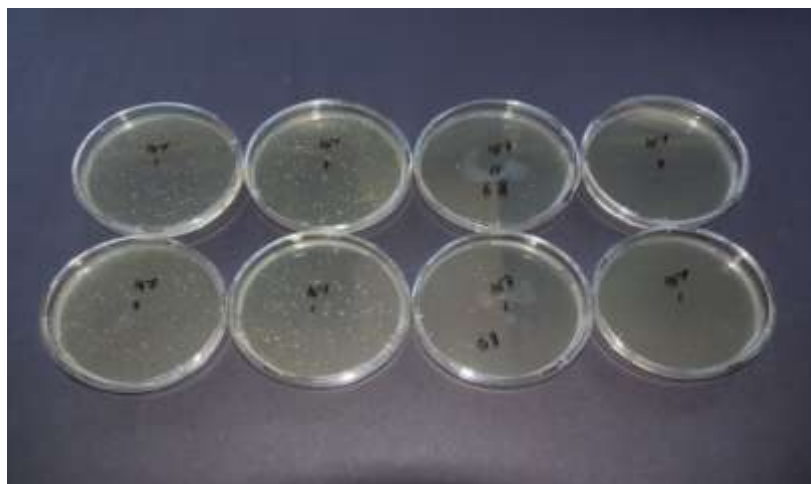


Figure 4.12 Photograph of one of viable plate count techniques for *LMG 17238*

4.1.6 Denaturing Gradient Gel Electrophoresis (DGGE) and Polymerase Chain Reaction (PCR) Method Results

According to the procedure followed by the Microbiology Laboratory of Karlsruhe Technical University (Germany) , the bacteria detected in *Gracilaria verrucosa* sample are:

Bacteria that perform nitrate reduction

Paracoccus spp.(most frequently identified)

Pseudomonas sp.

Pseudomonas putida

Arthrobacter sp.

Others

Planococcus spp.

Palnococcus citreus

Aeromonas sp.

Aeromonas punctata

Stenotrophomonas maltophilia

Vibrio harveyi

Exiguobacterium acetylicum
(may be involved in carbon source production)

Exiguobacterium sp.

Most of the identified bacteria are able to perform nitrate reduction. It is certain that this alga is associated with nitrate reducing and can be used in biological denitrification studies as a carbon source.

4.2 Batch Mode Adsorption Studies

Analysis of adsorption data is important for developing kinetic, equilibrium, and thermodynamic equations that can be used for design purposes. The adsorption of 2,4-D onto GAC was investigated as a function of with and without microorganisms in comparison.

First of all, the time-dependent experiments were done in order to determine the equilibrium time for GAC (1-2mm) at 25°C. Secondly, concentration-dependent experiments were carried out in the concentration range of 10-100 ppm of 2,4-D. In the case of blank experiments, 50 ml of 2,4-D was used for each concentration, but for the case of biological contamination, volume of 2,4-D/volume of microorganism ratio was changed. Lastly, for thermodynamic studies, 2 ml *Chlorella sp.*/50ml 2,4-D ratio was valid for 25°C, 35°C and 45°C.

The result of the time-dependent experiment done with 2 ml microorganism and without microorganism for the adsorption of 2,4-D at 25°C is shown in Figure 4.13.

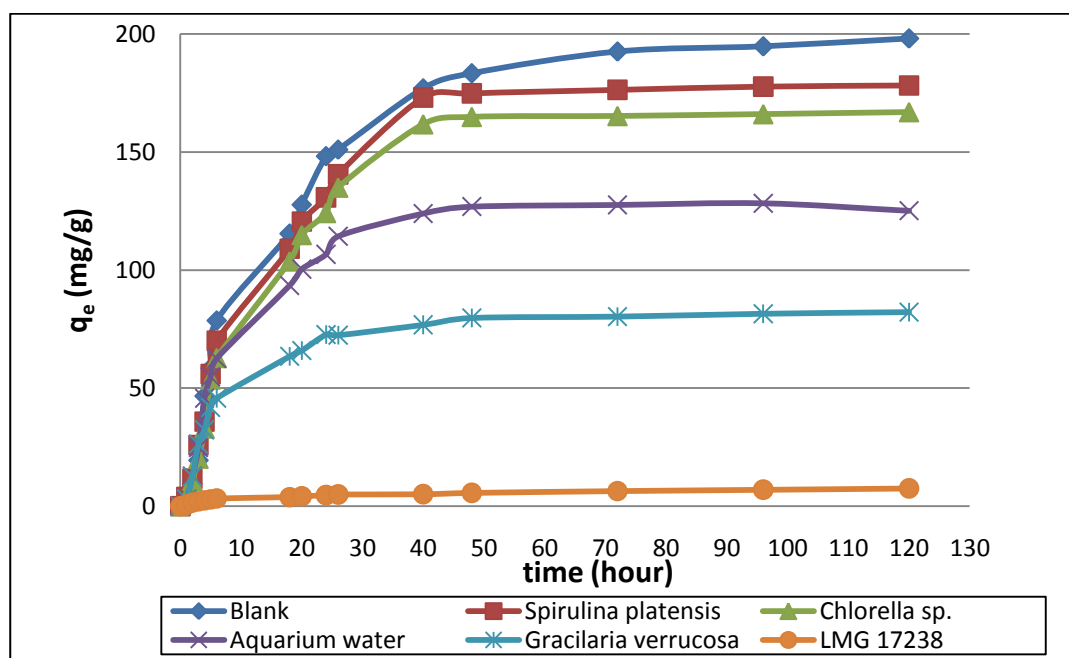


Figure 4.13 The time-dependent experiment at 25°C with and without microorganism

In the similar manner, the equilibrium isotherms are drawn for blank and all microorganisms at 25°C as seen in Figure 4.14.

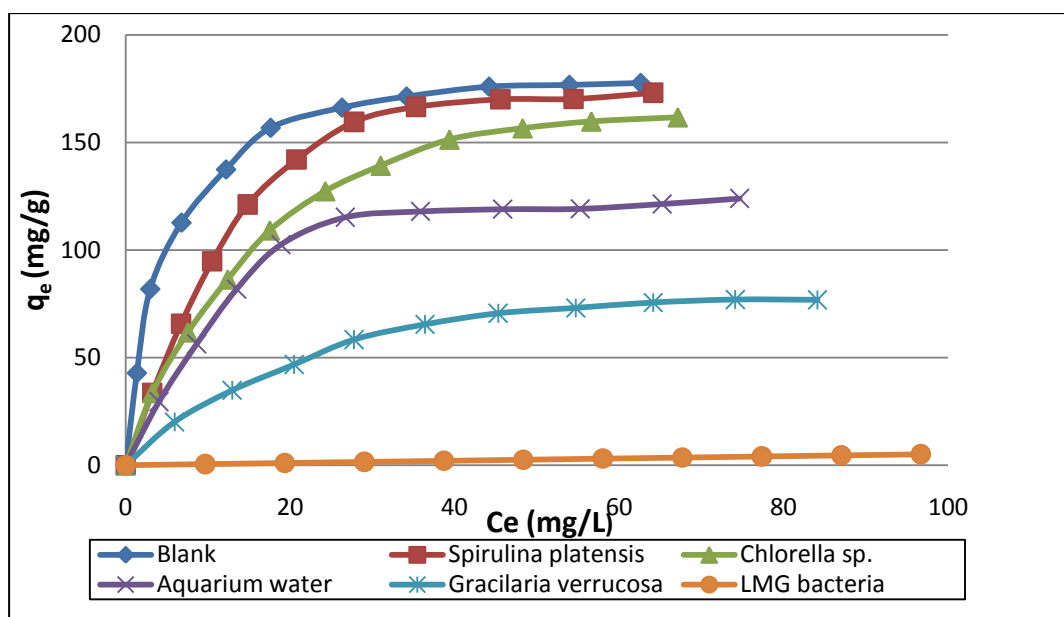


Figure 4.14 The equilibrium isotherms for blank and all microorganisms at 25°C

According to the results of the experiments done with microorganisms, the order of the adsorption 2,4-D in terms of maximum adsorption capacity (mg/g) from the least to the most is:

Acidovorax Avenae Subsp. Avenae LMG 17238 < *Gracilaria verrucosa* < Aquarium water < *Chlorella sp.* < *Spirulina platensis* < Blank

There can be three interpretations of this order:

1. First of all, it is certain that in none of the conditions with microorganism, the adsorption capacity cannot reach to the one in the no-microorganism situation.
2. Bacteria are small compared to the algae and they can fill the mesapores and micropores of GAC better. Since LMG is a dense culture full of small bacteria, this is an expected result. It was the first time, LMG bacteria, which were produced for PCL, had been used with the activated carbon.
3. *Chlorella sp.* is a smaller alga compared to *Spirulina platensis* and its shape is rounded; better fill the pores of GAC. *Spirulina platensis* is a spiraled alga, so it cannot exactly fill the pores and the result is closest to the one without microorganism.

Also, the adsorption concentration study was carried out with 0.25, 0.50, 0.75, 1, and 2 mL of LMG bacteria/50 mL 2,4-D solution at 25°C and the equilibrium relationship is shown in Figure 4.15.

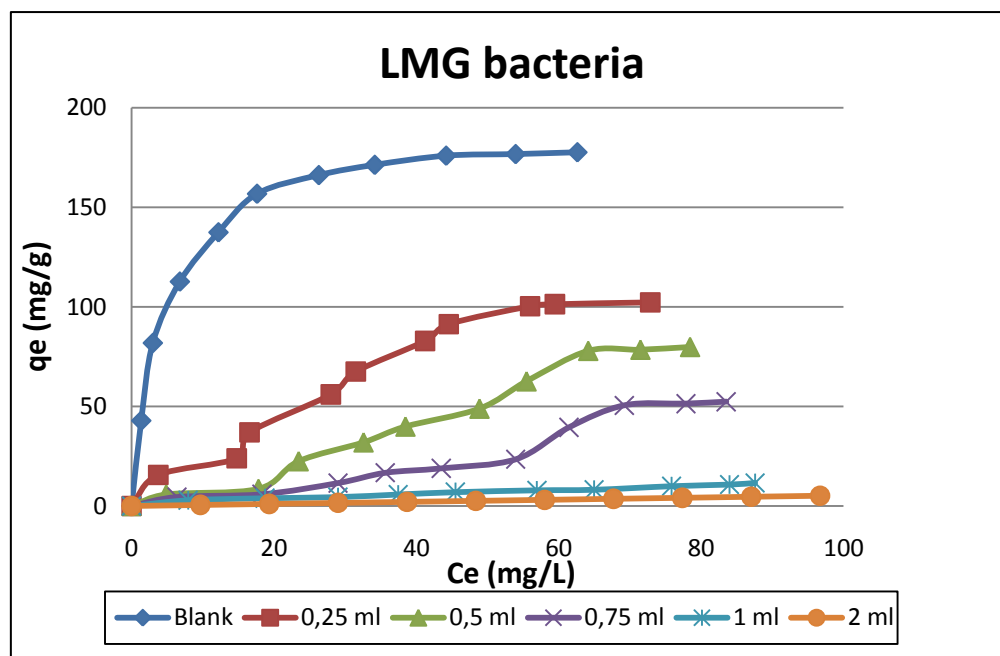


Figure 4.15 The comparison of equilibrium isotherm of GAC with different volumes of *LMG Bacteria* and without any biological contamination for adsorption of 2,4-D

As seen from Figure 4.15, the removal percentage of 2,4-D decreased with increasing bacteria concentration and lastly in 2 mL bacteria/ 50 mL 2,4-D solution equilibrium study, no 2,4-D removal was observed. The decrease in the removal of 2,4-D occurred because the surface of the carbon particle was covered with biofilm. In batch mode adsorption studies, the same solution remains in contact with a given quantity of adsorbent, so the bacteria solution contacted the carbon particle during the shaking time. When the particle surface is covered with the solution, the mesopores and micropores of carbon were filled with the bacteria solution by the time the biofilm occurred. So the number of adsorption sites decreased. As a result, removal of 2,4-D decreased. When the bacteria-2,4-D solution ratio is increased, the startup time of occurring biofilm decreases. This is the reason, why no removal in the 2 mL bacteria/ 50 mL 2,4-D solution ratio was observed.

The equilibrium isotherms are drawn for 1, 2, and 3 ml of *Chlorella sp.* at 25°C to investigate the effect of the algae volume in 50 ml 2,4-D solution on the adsorption is observed in Figure 4.16. The same result with LMG bacteria can be driven for an alga, *Chlorella sp.*

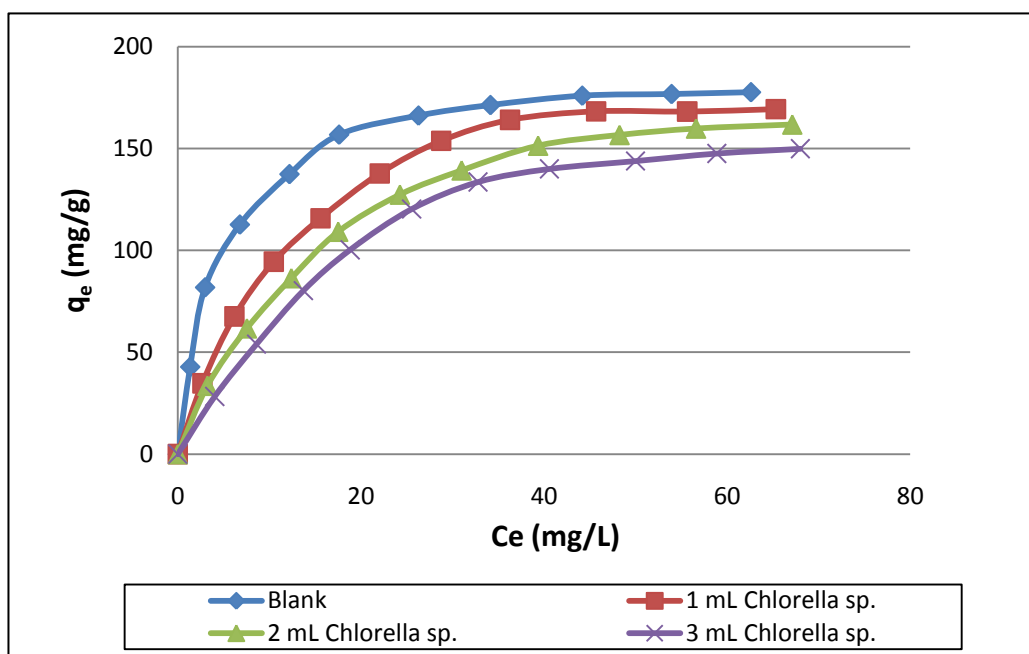


Figure 4.16 The comparison of equilibrium isotherm of GAC with different volumes of *Chlorella sp.* and without any biological contamination for adsorption of 2,4-D

4.2.1 Kinetic Modeling of Batch Adsorption

Kinetic models are used to determine the rate of the adsorption process. Four kinetic models -film diffusion, intraparticle diffusion, pseudo-first order, pseudo-second order models- were used to investigate the adsorption process of 2,4-D on GAC without microorganism and in the presence of microorganisms which are *Spirulina platensis*, *Chlorella sp.*, aquarium water, *Gracilaria verrucosa* and LMG 17238.

If the movement of organic pollutant molecule from the bulk liquid to the liquid film or boundary layer surrounding the adsorbent is ignored, the following sequence of steps can take place in the adsorption process of a porous adsorbent: transport of solute molecules from the boundary film to the external surface of the adsorbent (film diffusion), transfer of molecules from the surface to the intraparticle active sites and uptake of molecules by the active sites of sorbent.

In the removal of organics from wastewater, it is important for design purposes to investigate the mechanisms of adsorption and potential rate controlling steps which control the adsorption rate. In order to find the contribution of rate controlling steps such as external mass transfer, intraparticle diffusion, and adsorption process and also, mass transfer and kinetic models have been used to test the experimental data.

Application of the Film Diffusion Model

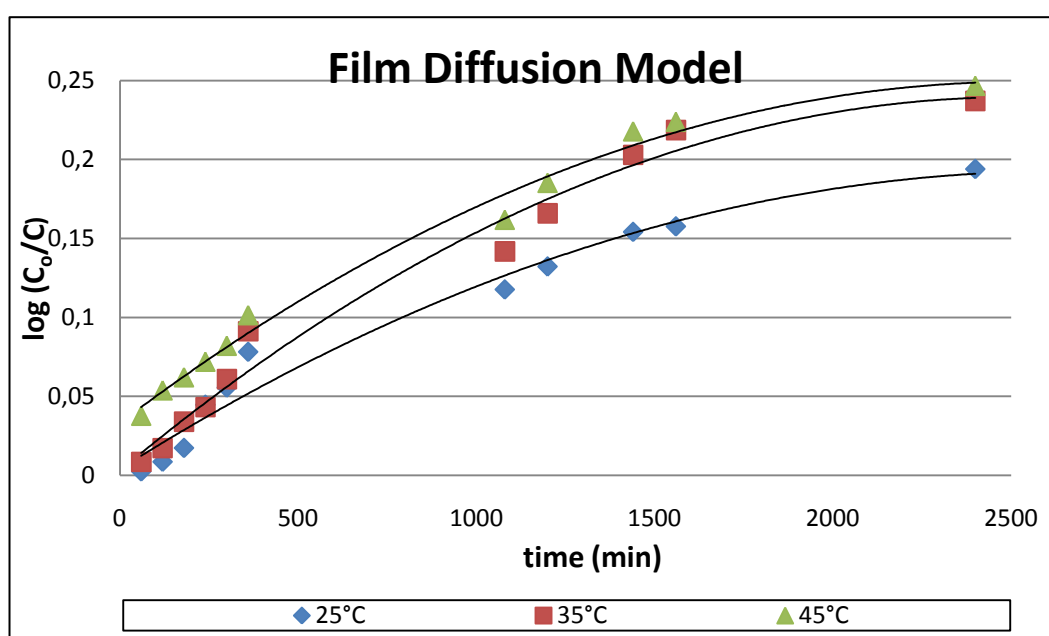


Figure 4.17 The film diffusion graph for GAC in blank experiment

In Figure 4.17, the film diffusion graph of GAC for adsorption of 2,4-D is shown. From the slope of the $\log (C_0/C)$ versus time graph, the film diffusion adsorption rate can be evaluated. If the film diffusion would be the rate controlling step, the line must be passed through the origin and a linear trend line should be obtained from the experimental data. But in Figure 4.17, it is seen that the obtained trend line was nonlinear and did not pass through the origin. As a result, it can be said that the film diffusion was not the controlling step and the adsorption rate was not proportional to the first order power of the concentration.

Application of the Intraparticle Diffusion Model

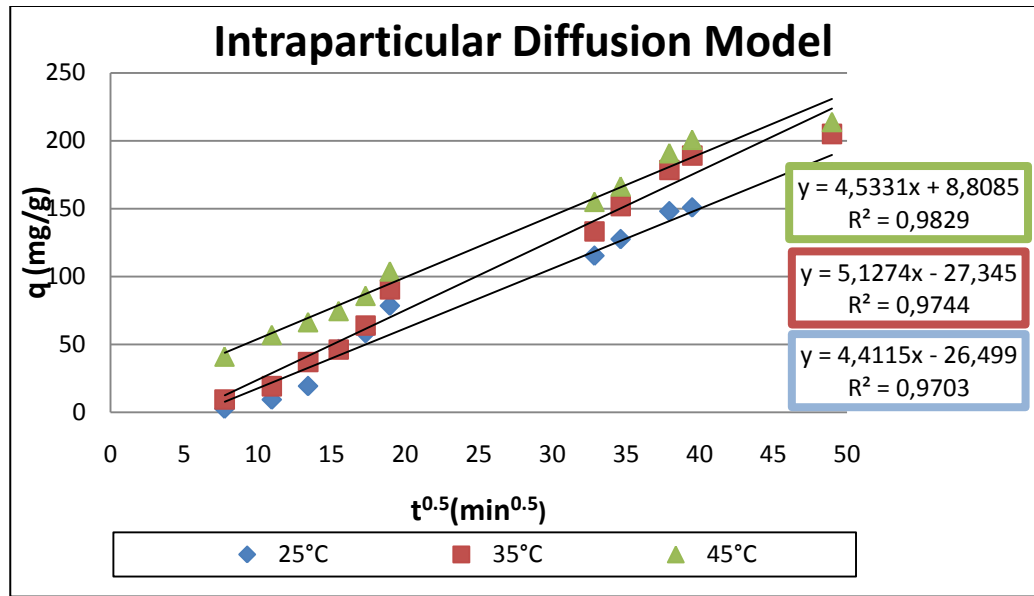


Figure 4.18 The intraparticle diffusion graph for GAC in blank experiment

The intraparticle diffusion graph of GAC for adsorption of 2,4-D is shown in Figure 4.18. The linear plot of the intraparticle diffusion equation [$q_t = k_{id}t^{1/2} + C$] was attributed to the pore diffusion (probably macrospores) that is the accessible sites of adsorption. This is attributed to the instantaneous utilization of the most readily available adsorbing sites on the adsorbent surface. From the slope of the q versus $t^{0.5}$ graph, the intraparticle diffusion rate constant (k_{id} (mg/g $\times$ min $^{0.5}$)) can be evaluated. According to that, the k_{id} value was found as 4.412 (mg/g $\times$ min $^{0.5}$), and the value of intercept “C” was found to be 26.499 at 25°C. It can be said that the boundary layer effect was great or in other words the thickness of the boundary layer was high.

Also by assuming the spherical geometry for the active carbon particle and the diameter taken as 0.15cm, from the pore diffusion equation [$t_{1/2} = 0.03r_0^2 / D_p$], where $t_{1/2}$ is the time for half adsorption (s), r_0 is the radius of the adsorbent particle (cm) and D_p is the diffusion coefficient (cm ^{2} /s)], the pore diffusion coefficient was calculated as 2.34×10^{-9} cm ^{2} /s.

Constants found at three different temperatures are compared with the studies in literature in Table 4.6.

Table 4.6 Intraparticle diffusion constants at different temperatures in comparison with literature

Adsorbent	Adsorbate	Temperature (°C)	k_{id} (mg/g x min ^{0.5})	D_p (cm ² /s)	References
Samla coal	Phenol	25	0.0353	1.36×10^{-10}	Ahmaruzzaman and Sharma (2005)
Activate carbon	Phenol	25	0.0268	3.72×10^{-11}	Ahmaruzzaman and Sharma (2005)
GAC (1-2 mm)	2,4-D	25	4.412	2.34×10^{-9}	Thesis
		35	5.127		
		45	4.533		

When the k_{id} values of 2,4-D adsorption onto GAC were compared with respect to the phenol adsorption onto samla coal and activated carbon, it can be said that in the 2,4-D adsorption, intraparticle diffusion occurred faster.

The value of the pore diffusion rate constant was found to be on the order of 10^{-9} cm²/s indicating that the pore diffusion was significant in 2,4-D adsorption on GAC whereas, in phenol adsorption it was not because of 10^{-10} - 10^{-11} cm²/s orders as proved by Faust and Alay (1986) for phenol adsorption onto active carbon.

Application of the Pseudo-First Order and the Pseudo-Second Order Kinetic Models

The obtained kinetic data was fitted to the sorption kinetics using the pseudo first order and pseudo second order kinetic model equations for GAC for blank and *Chlorella sp.* at 25°C. Figure A.2 (in Appendix) was plotted in order to observe first order reaction orders by plotting the log ($q_e - q$) versus time for blank and *Chlorella sp.* conditions where Figure A.3 (in Appendix) was plotted in order to observe second order reaction orders by plotting t/q versus time for blank and *Chlorella sp.* conditions. Predicted q_e values were also calculated from the graphs. Theoretical and experimental comparisons are given in Figure A.4 (in Appendix) for the first order and second order kinetics for both conditions at 25°C and the results are tabulated in Table 4.7.

Table 4.7 The pseudo-first order, pseudo-second order constants and calculated q_e values for blank and *Chlorella sp.* conditions

Condition	R^2		Calculated Constants		$q_{e, \text{calculated}}$ (mg/g)	
	Pseudo-first order	Pseudo-second order	$k_{1, \text{ads}}$ (1/min)	$k_{2, \text{ads}}$ (g/mg x min)	Pseudo-first order	Pseudo-second order
Blank	0.9716	0.9808	11.52×10^{-4}	0.35×10^{-5}	181.68	256.41
<i>Chlorella sp.</i>	0.9816	0.9693	11.52×10^{-4}	0.44×10^{-5}	164.17	222.22

Experimental data fit to the Ho pseudo-second-order mechanism in the case of blank since the correlation coefficient is higher ($R^2 = 0.9808$). In the case of *Chlorella sp.*, the process turns out to obey the pseudo-first order model ($R^2 = 0.9816$ for first order kinetics and 0.9693 for second order kinetics). The first order kinetic constant has not changed, but the second order kinetic constant has increased slightly. Also $q_{e, \text{calculated}}$ (mg/g) has decreased in both cases.

The comparison of the experimental and theoretical curves for the second order kinetics showed similarity as a proof of the fitting to the Ho pseudo-second-order mechanism for blank condition where it fitted the first-order kinetic model for *Chlorella sp.*

With the comparison of obtained results from different adsorption processes of 2,4-D, the first and second order kinetic constants are tabulated in Table 4.8.

Table 4.8 The comparison of the pseudo-first order, pseudo-second order kinetic constants and calculated q_e values at 25°C with the values in the literature

Adsorbent	pH	$k_{1, \text{ads}}$ (1/min)	$q_{e, \text{calc}}$ (mg/g)	$k_{2, \text{ads}}$ [g/(mg x min)]	$q_{e, \text{calc}}$ (mg/g)	Reference
GAC (1.19-3.36mm)	2.0	39.12×10^{-4}	96.60	18.29×10^{-5}	102.00	Aksu and Kabasakal, 2004
PAC (<0.15mm)	2.0	15.34×10^{-2}	50.80	0.86×10^{-5}	102.00	Aksu and Kabasakal, 2005
Carbonaceous adsorbent	6.5-7.5	31.90×10^{-3}	48.67	2.18×10^{-3}	81.96	Gupta et al., 2006
GAC-blank (1-2mm)	6.5-7.0	11.52×10^{-4}	181.68	0.35×10^{-5}	256.41	Thesis
GAC-with microorganisms (1-2mm)	6.5-7.0	11.52×10^{-4}	164.17	0.44×10^{-5}	222.22	Thesis

As seen in Table 4.8, although the rate constant of GAC (1.19-3.36mm) was nearly half of the GAC (1-2mm), the amount of solute adsorbed at equilibrium (q_e) was nearly 100 mg/g because of the studied pH. For the second order model, $k_{2,ads}$ (rate constant) and the amount of solute adsorbed at equilibrium (q_e) of PAC were nearly half of the GAC (1-2mm) even if PAC had more surface area than GAC. The rate constant of GAC (1.19-3.36mm) was nearly ten times that of the GAC (1-2mm), but the q_e value was not too small when compared with the rate constants ratio. The reason for this result might be the studied pH.

Some other kinetic studies in literature are tabulated in Table 4.9:

Table 4.9 The comparison of the kinetic values calculated with the studies in literature

Adsorbent	BET Surface Area of Adsorbent (m ² /g)	Adsorbed Materials	T & pH	Kinetic Model	Kinetic Constant	Control Mechansim	Reference
F400-AC F400AN-Mod. AC	790 960	2,4-D Benazolin	25°C pH=5	Pseudo-second order k ₂ (g/mg x min)	3.29 x 10 ⁻⁴ 5.44 x 10 ⁻⁴	Film Diffusion	Chingombe et. al., 2006
		2,4-D Benazolin			5.35 x 10 ⁻⁴ 3.01 x 10 ⁻⁴		
Activated carbon derived from date stones	763.40	2,4-D	30°C pH=3.6	Pseudo-first-order k ₁ (1/h)	33.16 x 10 ⁻²	Intraparticle diffusion	Hameed et. al., 2009
Hazelnut shell activated carbon	441	Copper	25°C pH=6	Pseudo-second order k ₂ (g/ g x min)	0.18 x 10 ⁻²	Intraparticle diffusion	Demirbas et. al., 2009
XAD-4 resin	-	4-chlorophenol	30°C	Pseudo-second order k ₂ (g/ g x min)	2.14 x 10 ⁻²	Intraparticle diffusion	Bilgili, 2006
Cyclodextrin-based	3.1	C.I. Basic Green 4	25°C pH=8	Pseudo-second order k ₂ (g/ g x min)	0.25×10 ⁻²	Intraparticle diffusion	Crini et. al., 2007
GAC	671	2,4-D	25°C pH=6.5-7	Pseudo-second order k ₂ (g/ mg x min)	0.35 x 10 ⁻⁵	Intraparticle diffusion	Thesis

4.2.2 Equilibrium Modeling of Batch Adsorption

Equilibrium data were obtained for 2,4-D adsorption onto GAC without microorganisms and in the presence of *Chlorella sp.* at 25°C. The results are tabulated in Table 4.10. The equilibrium relationship was shown in Figure 4.14. The linearized fitting curves for blank and *Chlorella sp.* are given for Langmuir and Freundlich models in Figure 4.19 and Figure 4.20 respectively.

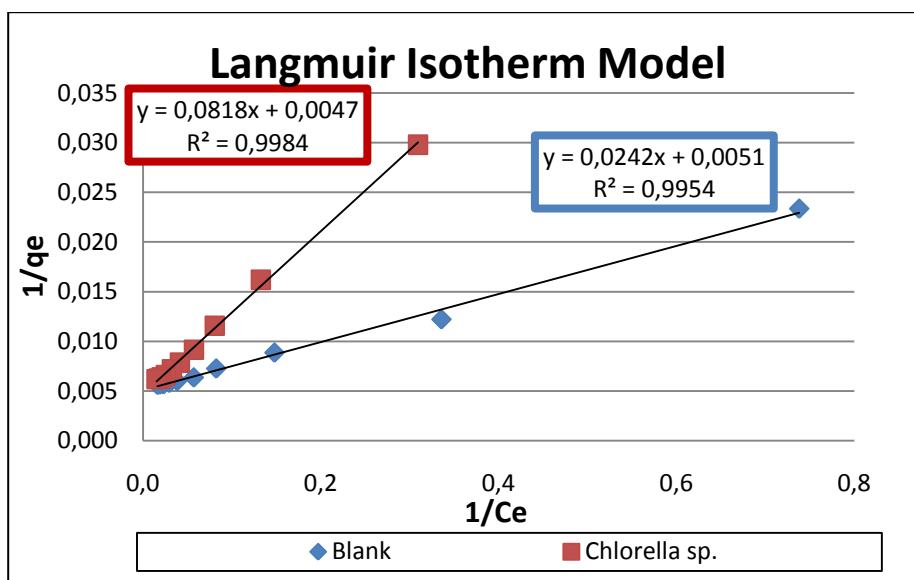


Figure 4.19 Linearized form of Langmuir isotherm model for blank and *Chlorella sp.*

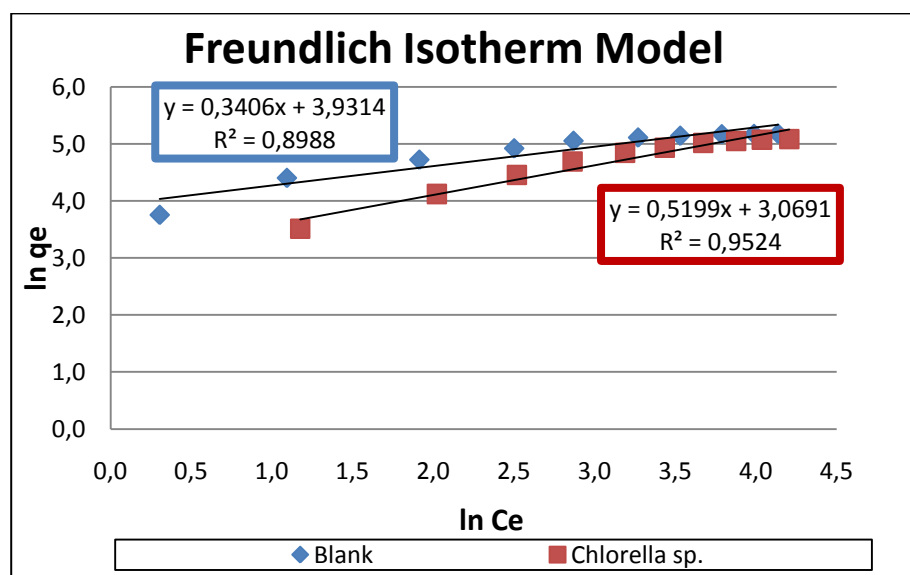


Figure 4.20 Linearized form of Freundlich isotherm model for blank and *Chlorella sp.*

Table 4.10 Langmuir and Freundlich constant values for blank and *Chlorella sp.* conditions

Condition	R^2		Langmuir Constants		Freundlich Constants	
	Langmuir	Freundlich	Q_0 (mg/g)	b (L/mg)	K_F [(mg/g) x (mg/L) ⁿ]	n (-)
Blank	0.9954	0.8988	196.08	21.07×10^{-3}	50.98	2.94
<i>Chlorella sp.</i>	0.9984	0.9524	212.77	57.46×10^{-3}	21.52	1.92

Experimental data fit the Langmuir model in the case of blank since the correlation coefficient is higher ($R^2 = 0.9954$). In the case of *Chlorella sp.*, the correlation coefficients for the linear regression fit the Langmuir and Freundlich model were found to be 0.9984 and 0.9524 respectively. Looking at the correlation coefficients, the adsorbent showed a better fit for the Langmuir model than the Freundlich model both for blank and with *Chlorella sp.* studies. All the results show that the Langmuir model fit the results quite well suggesting that the surface of the sorbent is homogeneous.

In the presence of *Chlorella sp.*, the Langmuir constants have increased and the Freundlich constants have decreased compared to the blank condition.

The Langmuir parameter can be used to predict the affinity between the sorbent and sorbate using the dimensionless separation factor (R_L). Figure 4.21 shows that the values of R_L ($R_L = 1/(1+bC_0)$) where C_0 is the initial concentration of adsorbate (mg/L), and b is the Langmuir constant for the adsorption of 2,4-D without microorganism. R_L values were between 0 and 1, which indicates the favorable adsorption of 2,4-D on GAC as Ahmaruzzaman and Sharma (2005) found for the adsorption of phenols from wastewater and Vadivelan and Kumar (2005) found for sorption of methylene blue onto rice husk. And also it can be said that the higher R_L values at lower concentrations showed that the adsorption was more favourable at lower 2,4-D concentrations.

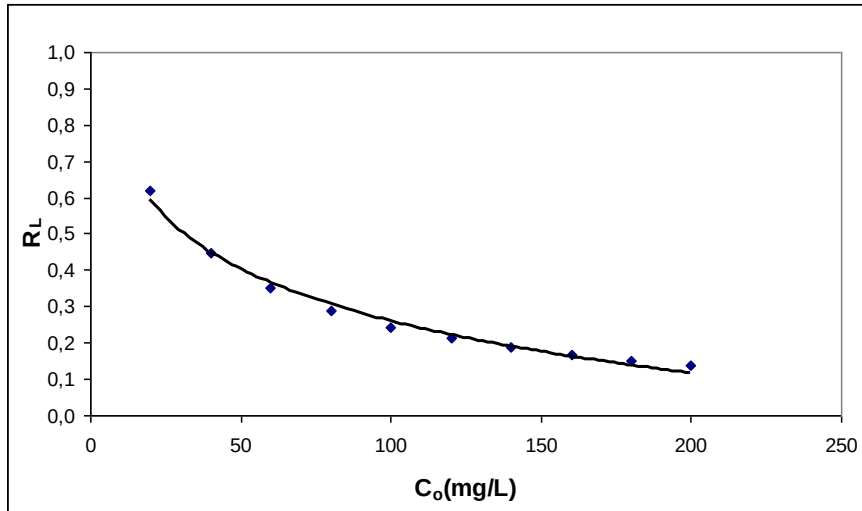


Figure 4.21 Separation factor of 2,4-D sorbed on GAC for blank condition

The comparison of the Langmuir parameters (Q_0 and b) of different adsorbents that are used in the removal of 2,4-D is tabulated in Table 4.11.

Table 4.11 Langmuir parameters of different adsorbents that are used in removal of 2,4-D

Adsorbent	pH	Q_0 (mg/g)	b (L/mg)	Reference
GAC (1.19-3.36mm)	2.0	555.00	28.00×10^{-3}	Aksu and Kabasakal, 2004
PAC (<0.15mm)	2.0	333.30	29.00×10^{-3}	Aksu and Kabasakal, 2005
GAC F400 (55-65mm)	7.0	135.92	40.69×10^{-3}	Kim et al., 2005; Kim et al., 2002; Cho et al., 2003
GAC SLS103 (55-65mm)	7.0	142.77	38.30×10^{-3}	Kim et al., 2005; Kim et al., 2002; Cho et al., 2003
GAC WWL (55-65mm)	7.0	126.19	6.50×10^{-3}	Kim et al., 2005; Kim et al., 2002; Cho et al., 2003
Carbonaceous adsorbent	6.5-7.5	322.50	22.50×10^{-3}	Gupta et al., 2006
Organo-clays	2.8	4.60	0.14×10^{-3}	Akçay and Yurdakoç, 2000
Calcinated Hydrotalcite	6.5-7.0	179.67	12.67×10^{-3}	Pavlovic et al., 2005
Zinc-aluminium-chloride layered double hydroxides	7.0	(115.8±11.05) (735.93±6.63) (587.86±6.63)	(57.1±0.361) $\times 10^{-3}$ (56.6±0.361) $\times 10^{-3}$ (55.8±0.361) $\times 10^{-3}$	Legrouiri et al., 2005
GAC-blank (1-2mm)	6.5-7.0	196.08	21.07×10^{-3}	Thesis
GAC- with microorganism (1-2mm)	6.5-7.0	212.77	57.46×10^{-3}	Thesis

As seen from Table 4.11, the Q_0 (maximum adsorption capacity (mg/g)) of PAC was higher than GAC (1-2mm) as a result of the high studied pH even if b (bonding energy of adsorption (L/mg)) was nearly the same. Although the bonding energy of adsorption values (b) of GAC F400 (55-65mm) and GAC SLS103 (55-65mm) were higher than GAC (1-2mm), maximum adsorption capacities (Q_0) were lower because of the particle size, in spite of the same pH that was studied. It was not a surprise that the maximum adsorption capacity of organo-clay was very low in respect to activated carbon because of the surface area, even if the optimum pH level was studied for 2,4-D adsorption onto organo-clays.

The comparison of the Langmuir parameters (Q_0 and b) of different adsorbents that are used in the removal of 2,4-D at 25°C is tabulated in Table 4.12.

Table 4.12 Freundlich parameters of different adsorbents that are used in removal of 2,4-D

Adsorbent	pH	K_F [(mg/g)x(mg/L) ⁿ]	n (-)	Reference
GAC (1.19-3.36mm)	2.0	42.48	2.06	Aksu and Kabasakal, 2004
PAC (<0.15mm)	2.0	43.93	2.91	Aksu and Kabasakal, 2005
GAC SLS103 (55-65mm)	7.0	0.89	3.56	Kim et al., 2005; Kim et al., 2002; Cho et al., 2003
GAC WWL (55-65mm)	7.0	0.69	1.99	Kim et al., 2005; Kim et al., 2002; Cho et al., 2003
GAC-blank (1-2mm)	6.5-7.0	50.98	2.94	Thesis
GAC- with microorganism (1-2mm)	6.5-7.0	21.52	1.92	Thesis

As seen in Table 4.12, the adsorption intensity, n and adsorption capacity, K_F values of GAC WWL (55-65 mm) is least compared to the other adsorbents. At the studied pH and with GAC (1-2 mm), the values of the constants are close to each other.

Besides linear regression, the non-linear regression is done with the MATLAB 7.7.0 Curve Fitting Tool Box and the q_e versus C_e graphs for different models for blank and *Chlorella sp.* at 25°C are as seen in Figure 4.22 and 4.23 respectively. Also the model constants are evaluated and tabulated in Table 4.13.

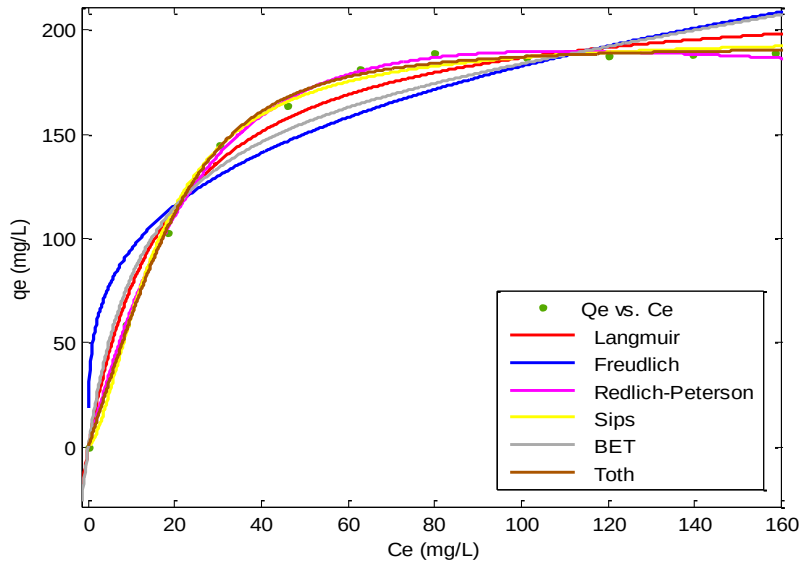


Figure 4.22 The equilibrium curves for different models for blank condition

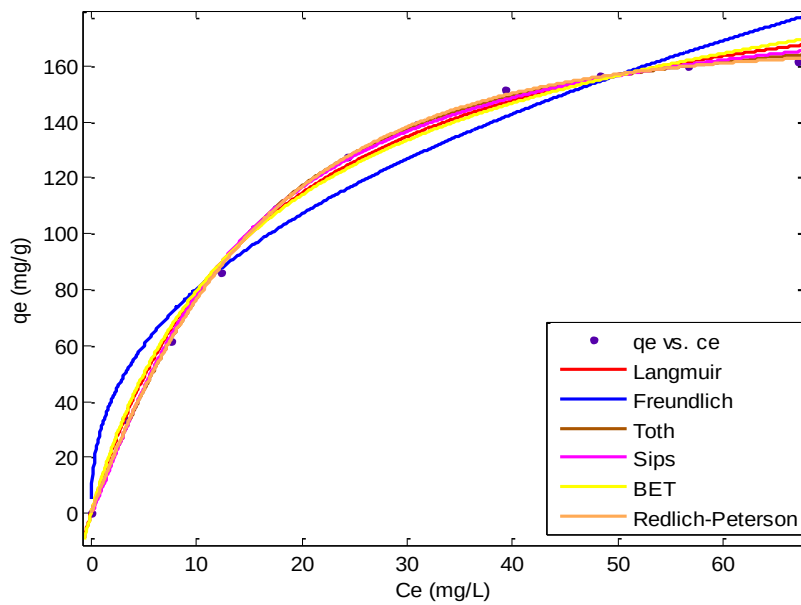


Figure 4.23 The equilibrium curves for different models for *Chlorella sp.* condition

Table 4.13 Linear and non-linear model constants calculated by MATLAB for blank and *Chlorella sp.* in comparison

Batch Isotherm Models	Model Equations	Blank			With <i>Chlorella sp.</i>		
		Constants		R ²	Constants		R ²
Langmuir	$q_e = \frac{b * Q_0 * C_e}{1 + b * C_e}$	b (L/mg)	Q₀ (mg/g)	0.9845	b (L/mg)	Q₀ (mg/g)	0.9984
		21.07 x 10 ⁻³	196.08		57.46 x 10 ⁻³	212.77	
Freundlich	$q_e = K_F * C_e^{1/n}$	K_F ((mg/g)x(mg/L) ⁿ)	n (-)	0.9271	K_F ((mg/g)x(mg/L) ⁿ)	n (-)	0.9524
		50.98	2.94		21.52	1.92	
Toth	$q_e = \frac{A * C_e}{(B + C_e^D)^{1/D}}$	A	B	0.9975	A	B	0.9988
		192.30	2155		179.60	82.99	
Redlich-Peterson	$q_e = \frac{k_{rp} * C_e}{1 + a_{rx} * C_e^\beta}$	k_{rp}	a_{rx}	0.9959	k_{rp}	a_{rx}	0.9993
		7.72	8.20 x 10 ⁻³		10.01	1.86 x 10 ⁻²	
Sips	$q_e = \frac{K_s * C_e^{1/bs}}{1 + a_s * C_e^{1/bs}}$	K_s	a_s	0.9968	K_s	a_s	0.9981
		2.50	1.27 x 10 ⁻²		8.84	4.64 x 10 ⁻²	
BET	$q_e = \frac{B * Q_0 * C_s * C_e}{(C_s - C_e) * [C_s + (B - 1) * C_e]}$	B	Q₀	0.9617	B	Q₀	0.9944
		71.81	181.70		64.77	186.70	

In equilibrium modeling done by the linear and nonlinear regression in MATLAB, among all the models, the experimental data best fit in the order of Toth, Sips, and then Redlich-Peterson for the blank case and in the order of Redlich-Peterson, Toth and then Langmuir for *Chlorella sp.* case regarding the correlation coefficients. This result is not surprising since these four models are derived from the same origin as it can be seen from the model equations.

The studies in literature done by different adsorbent and adsorbate materials and the equilibrium models used to model the experimental data are tabulated in between Tables 4.14 and 4.19 for Langmuir, Freundlich, Toth, Redlich-Peterson, Sips, and BET models respectively.

Table 4.14 Langmuir model constants evaluated in literature studies done with different adsorbents and adsorbates without microorganism

Adsorbent	Adsorbate	Constants			Reference
		b (L/mg)	Q ₀ (mg/g)	R ²	
GAC-blank	2,4-D	0.002	196.08	0.984	Thesis
GAC-with microorganism	2,4-D	0.006	212.77	0.998	Thesis
Activated Carbon derived from date stones	2,4-D	0.052	238.10	0.994	Hameed et al., 2009
GAC	2,4-D	0.029	625.00	0.900	Aksu et al., 2004
Activated Carbon derived from olive bagasse	Cr(VI)	0.539	104.10	0.992	Demiral et al., 2008
Hazelnut shell Activated Carbon	Cu (II)	0.005	51.52	0.999	Demirbas et al., 2009
GAC	Phenol	0.109	49.72	0.994	Ozkaya, 2006
Hectorite clay	Ni(II)	0.608	22.64	0.978	Ramamurthi et al., 2009

Table 4.15 Freundlich model constants evaluated in literature studies done with different adsorbents and adsorbates without microorganism

Adsorbent	Adsorbate	Constants			Reference
		$K_F ((\text{mg/g}) \times (\text{mg/L})^n)$	$n(-)$	R^2	
GAC-blank	2,4-D	50.98	2.94	0.927	Thesis
GAC-with microorganism	2,4-D	21.52	1.92	0.952	Thesis
Activated Carbon derived from date stones	2,4-D	2.53	32.06	0.922	Hameed et al., 2009
GAC	2,4-D	43.57	1.94	0.968	Aksu et al., 2004
Activated Carbon derived from olive bagasse	Cr (VI)	65.02	6.36	0.983	Demiral et al., 2008
Hazelnut shell Activated Carbon	Cu (II)	11.45	4.08	0.943	Demirbas et al., 2009
GAC	Phenol	6.19	0.61	0.983	Ozkaya, 2006
Hectorite clay	Ni(II)	8.82	0.32	0.976	Ramamurthi et al., 2009

Table 4.16 Toth model constants evaluated in literature studies done with different adsorbents and adsorbates without microorganism

Adsorbent	Adsorbate	Constants				Reference
		A	B	D	R ²	
GAC-blank	2,4-D	192.30	2155	2.27	0.997	Thesis
GAC-with microorganism	2,4-D	179.60	82.99	1.51	0.999	Thesis
GAC	Phenol	60.05	6.81	0.83	0.994	Ozkaya, 2006
Peat	Basic dye	896.99	0.68	0.27	0.989	Allen et al., 2004
GAC	Phenol	1139.86	56.17	0.54	0.997	Dabhade et al., 2009

Table 4.17 Redlich-Peterson model constants evaluated in literature studies done with different adsorbents and adsorbates without microorganism

Adsorbent	Adsorbate	Constants				Reference
		k _{rp}	a _{rp}	β	R ²	
GAC-blank	2,4-D	7.72	0.009	1.290	0.995	Thesis
GAC-with microorganism	2,4-D	10.01	0.019	1.220	0.999	Thesis
GAC	Phenol	6.70	0.244	0.818	0.993	Ozkaya, 2006
Hectorite clay	Ni(II)	12.43	0.442	0.688	0.985	Ramamurthi et al., 2009
GAC	2,4-D	20.00	0.070	0.839	0.930	Aksu et al., 2004

Table 4.18 Sips model constants evaluated in literature studies done with different adsorbents and adsorbates without microorganism

Adsorbent	Adsorbate	Sips				Reference
		K_s	a_s	β_s	R^2	
GAC-blank	2,4-D	2.50	0.013	0.636	0.996	Thesis
GAC-with microorganism	2,4-D	8.84	0.046	0.850	0.998	Thesis
Hectorite Clay	Ni	5.87	0.137	0.500	0.985	Ramamurthi et al., 2009
Green alga <i>Pithophora oedogonia</i>	Cu(II)	0.43	2.963	0.306	0.999	Kumar et al., 2008
Composite Alginate Bead	Chromium	0.93	0.130	0.970	0.951	Kim et al., 2008

Table 4.19 BET model constants evaluated in literature studies done with different adsorbents and adsorbates without microorganism

Adsorbent	Adsorbate	BET			Reference
		B (mg/g)	Qo	R^2	
GAC-blank	2,4-D	71.81	181.70	0.961	Thesis
GAC-with microorganism	2,4-D	64.77	186.70	0.994	Thesis
<i>Rhizopus arrhizus</i>	Cu(II)	94.44	19.99	0.926	İleri, 2006
Activated H ₃ PO ₄	Atrazine	-1.89	0.38×10^{-3}	0.894	Udoji et al., 2010

4.2.3 Thermodynamic Studies in Batch Adsorption

Under the title of thermodynamic studies, the adsorption characteristics of 2,4-D on GAC were investigated at three different temperatures (25°C, 35°C, and 45°C) for blank experiments and with-microorganism experiments.

The results of the time-dependent blank experiment for different temperatures such as 25°C, 35°C, and 45°C are seen in Figure 4.24.

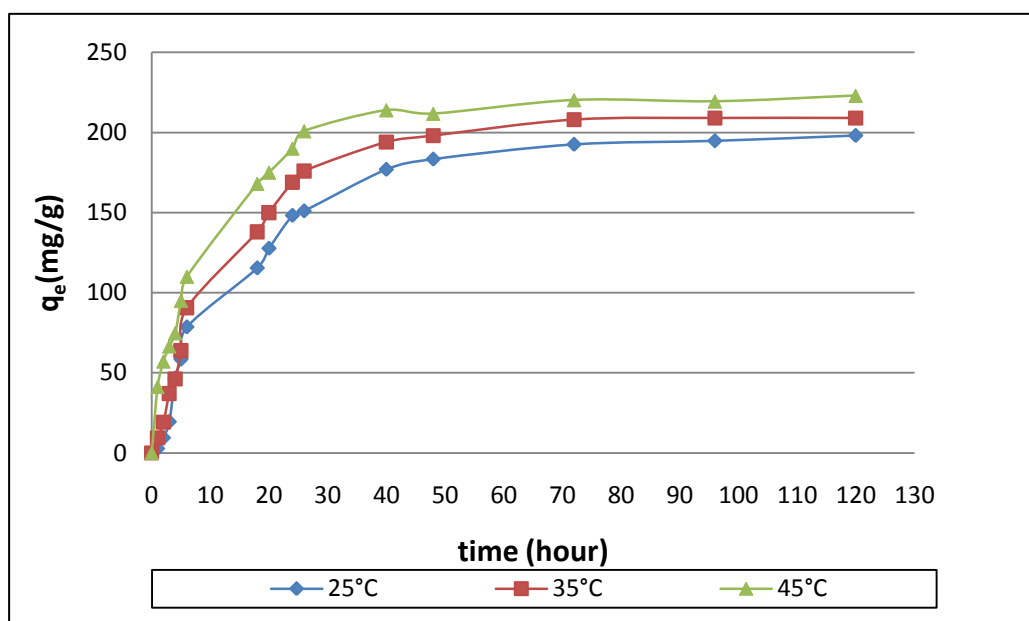


Figure 4.24 Time dependent blank experiment for different temperatures, 25°C, 35°C and 45°C

In endothermic processes, as the temperature increases, the adsorption capacity increases. This is directly related to the nature of the adsorption process.

In the same manner, the equilibrium curve is drawn for blank and *Chlorella* sp. conditions at 25°C, 35°C and 45°C and shown in Figure 4.25.

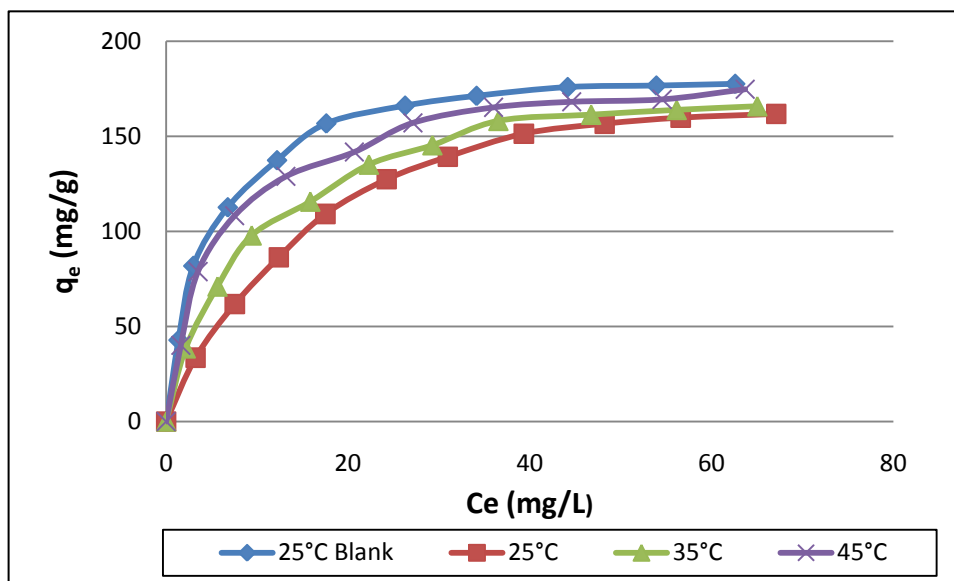


Figure 4.25 The equilibrium curves for blank and *Chlorella sp.* at three different temperatures

As seen in Figure 4.25, the adsorption capacity increases as temperature increases, but its value for *Chlorella sp.* condition cannot reach to the adsorption capacity in the without microorganism condition at 25°C. However, it is dangerous to increase the temperature further because it causes deformation in the protein structure of the microorganisms, so it may not be easy to observe pesticide adsorption since some other effects related to microorganism will arise.

A plot of ΔG° vs. temperature T is drawn for blank and *Chlorella sp.* at 25°C and found to be linear as seen in Figure 4.26 and Figure 4.27 respectively.

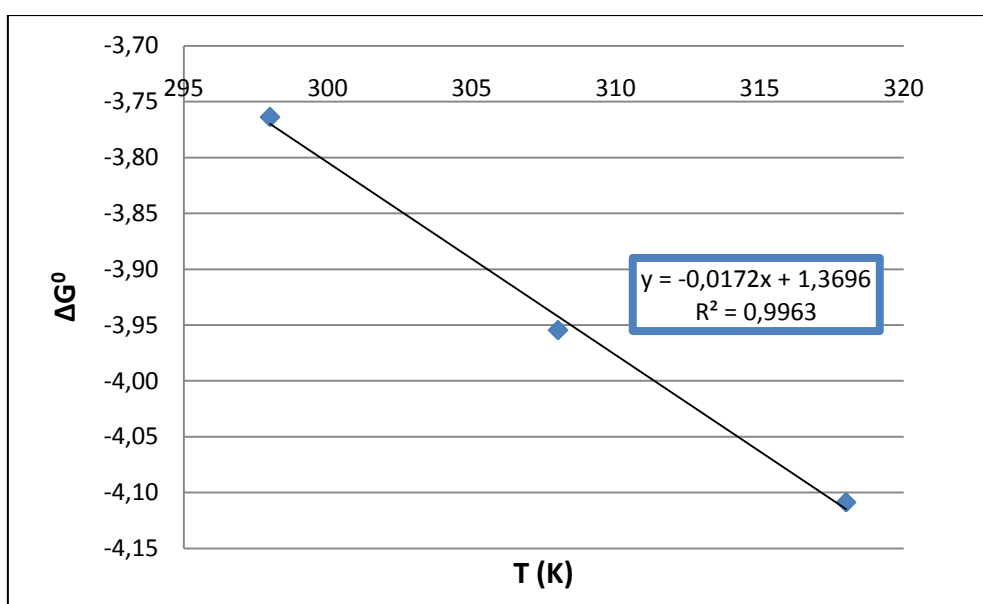


Figure 4.26 ΔG° vs. temperature T graph for blank

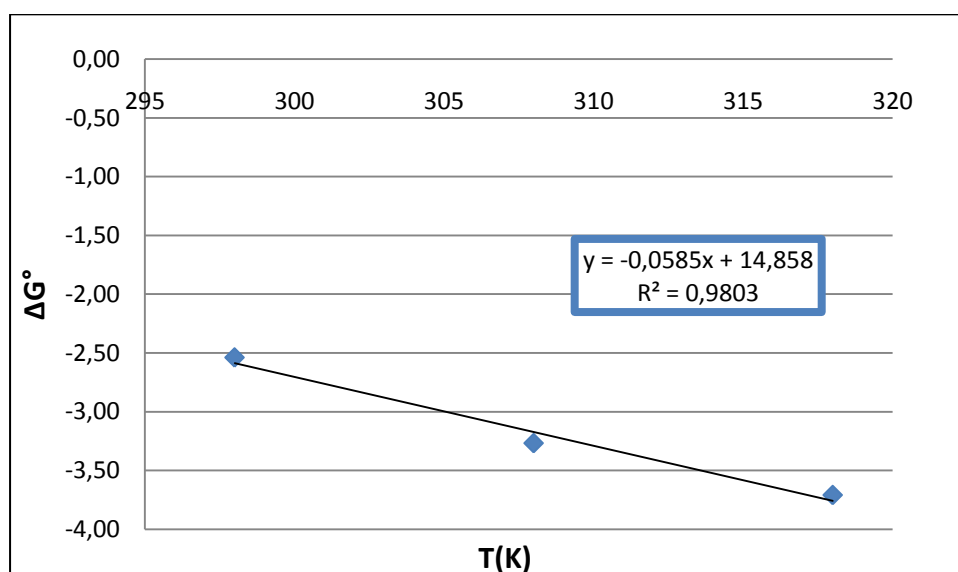


Figure 4.27 ΔG° vs. temperature T graph for *Chlorella sp.*

The results are tabulated for blank and *Chlorella sp.* at three different temperatures in Table 4.20 and Table 4.21 respectively.

Table 4.20 ΔG° , ΔH° and ΔS° values for blank condition

Temperature (°C)	ΔG° (kJ/mol)	R^2	ΔH° (kJ/mol)	ΔS° (kJ/molK)
25	-3.764	0.9963	1.370	0.017
35	-3.955			
45	-4.109			

Table 4.21 ΔG° , ΔH° and ΔS° values for *Chlorella sp.* condition

Temperature (°C)	ΔG° (kJ/mol)	R^2	ΔH° (kJ/mol)	ΔS° (kJ/molK)
25	-2.540	0.9803	14.858	0.059
35	-3.269			
45	-3.711			

The negative value of ΔG° confirms the spontaneous nature of adsorption, while the positive value of ΔH° indicates that it is an endothermic process. In the previous calculations, it has been found that as temperature increases, adsorption

capacity increases and it is known that this happens in endothermic processes. Here with these results, it is proven that the process is endothermic.

Comparing the results of two situations, the quantities of ΔG° values have decreased with the presence of microorganism. The value of ΔH° has increased greatly where the value of ΔS° has stayed nearly close to zero.

In the research article of Ovez et al. (2008), the ΔG° values were found to be -2.103 kJ/ mol for 25°C , -2.059 kJ/ mol for 35°C and -2.041 kJ/ mol for 45°C and hence, the numerical values of ΔS° and ΔH° were obtained as 3.089 J/molK and -3.019 kJ/mol respectively for the adsorption of atrazine on polycaprolactone. For simazine, the ΔG° values were found to be -2.027 kJ/ mol for 25°C , -2.046 kJ/ mol for 35°C and -2.087 kJ/ mol for 45°C and hence, the numerical values of ΔS° and ΔH° were obtained as 3.018 J/molK and -1.124 kJ/mol respectively.

These enthalpy values are of an order of magnitude corresponding to physisorption (-10 kJ/mol) and in the range of hydrogen bonding energy ($10 - 20$ kJ mol $^{-1}$) [20]. Since atrazine and simazine are weak bases with free electron pairs on the N ring and alkyl atoms, it can be assumed that hydrogen bonds are formed between PCL molecules and the nitrogen atoms of the atrazine and simazine molecules (Ovez et al., 2008).

Aksu and Kabasakal (2004) obtained a free energy value of -6.8 kJ/mol for the batch adsorption of 2,4-D onto granular activated carbon. This difference is mainly attributed to the difference in experimental conditions. However, in all cases, it was noted that the adsorption of 2,4-D was a spontaneous process. The positive values of the enthalpy change could suggest the adsorption process is endothermic, a phenomenon that was observed by Akcay et al. (2005) and Aksu and Kabasakal (2004). Bhattacharyya and Sharma (2005) reported that such small values in enthalpy change are not consistent with the formation of strong chemical bonds between the adsorbent and the adsorbate. The positive value of the entropy is an indication of increased randomness at the solid-liquid interface and an affinity of the adsorbents for the pesticides.

4.3 Continuous Mode Adsorption Studies

The column performance of GAC was investigated in a fixed bed column (column diameter ID=1.1cm). The effect of important parameters, such as influent concentration, flow rate, adsorbent mass and biological contamination load were studied.

4.3.1 Effect of Inlet Concentration

In order to examine the change in influent 2,4-D concentration, the flow rate was kept constant at 0.4 mL/min and the mass of adsorbent was 1 g for all conditions. Four different 2,4-D influent concentrations (50 ppm, 100 ppm, 150 ppm, and 200 ppm) were used. Breakthrough curves are shown in Figure 4.28.

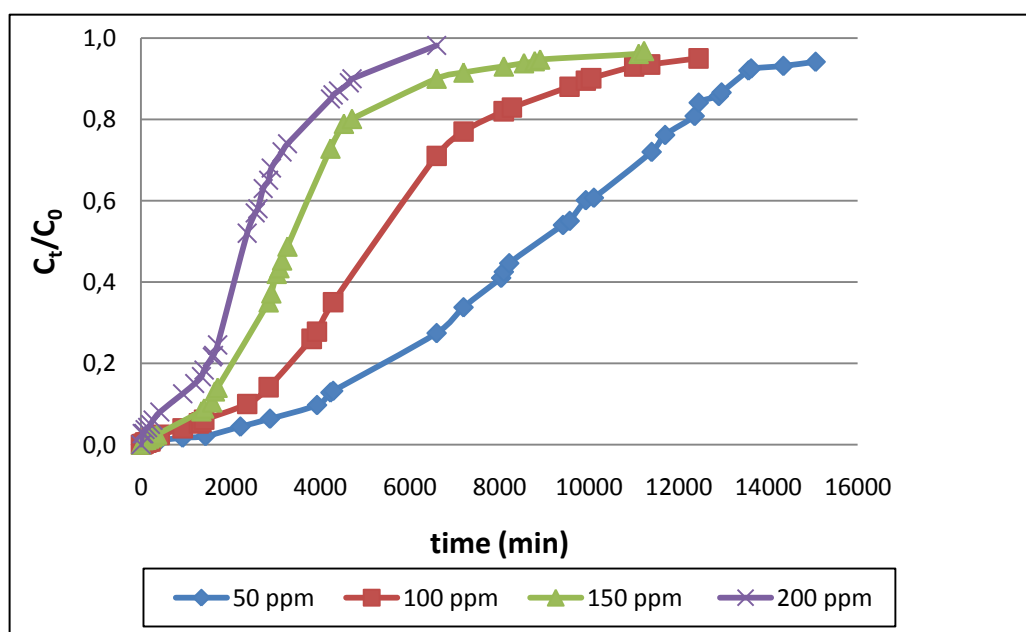


Figure 4.28 Breakthrough curves of GAC obtained at different concentrations for 2,4-D adsorption

As shown in the breakthrough curves in Figure 4.28, the larger the influent concentration, the steeper the slope of the breakthrough curve and the smaller is the breakthrough time. These results demonstrate that the change of concentration gradient affects the saturation rate and breakthrough time or in other words, the diffusion process is concentration dependent. As the influent concentration increases, the 2,4-D loading rate increases, but so does the driving force for mass

transfer, which in a decrease in the adsorption zone length. The net effect is an appreciable increase in adsorption capacity as proved by Goel et al. (2005) for the removal of lead (II) by adsorption using treated granular activated carbon in column studies.

4.3.2 Effect of Flow Rate

In order to examine the effect of the flow rate of the pump, the initial concentration of 2,4-D was kept constant 100 ppm and the mass of adsorbent was 1 g for all conditions. Three different flow rates (0.2 ml/min, 0.3 ml/min, 0.4 ml/min) were used. Breakthrough curves are shown in Figure 4.29.

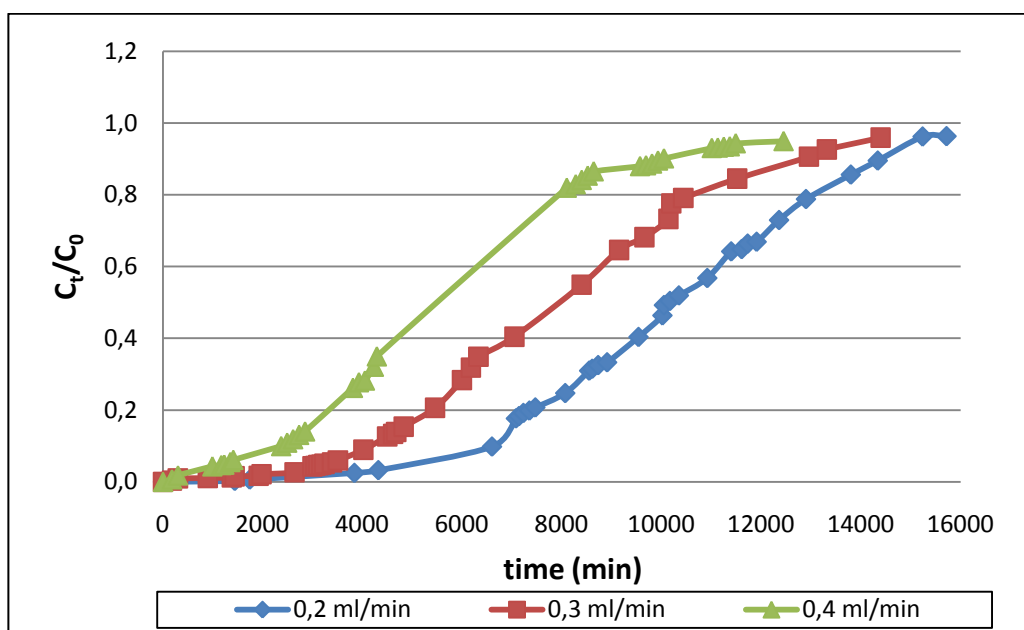


Figure 4.29 Breakthrough curves of GAC obtained at different flow rates for 2,4-D adsorption

It was shown in the breakthrough curves in Figure 4.32 that breakthrough generally occurred faster with faster flow rate. Breakthrough time reaching saturation increased significantly with a decrease in the flow rate. At a low rate of influent, pesticide ions had more time to contact with the carbon that resulted in a higher removal of 2,4-D ions in the column. While increasing the flow rate, the results indicated that the adsorption capacity would reach the equilibrium value faster, which may cause a negative effect on the mass transfer efficiency of 2,4-D ions. In other word, as the flowrate increased, the breakthrough curve becomes

steeper. The break point time and adsorbed ion concentration decreases. The reason for this behavior can be explained in the following way: if the residence time of the solute in the column is not long enough for adsorption equilibrium to be reached at that flowrate, the pesticide solution leaves the column before equilibrium occurs.

To sum up; it can be said that for reaching the maximum adsorption capacity in the column, it should be studied with a lower flowrate and a higher influent concentration and a higher flowrate and a lower influent concentration may have a disadvantage in the adsorption of 2,4-D on GAC in the column.

4.3.3 Effect of Adsorbent Mass

To investigate the effect of bed height on the adsorption of 2,4-D by GAC, the adsorbent mass was changed from 0.75 g to 1.5 g, while the influent 2,4-D concentration and the flow rate was held constant at 100 ppm and 0.4 mL/min, respectively. Breakthrough curves of different bed heights with the same influent concentration of 2,4-D and flow rate are shown in Figure 4.30.

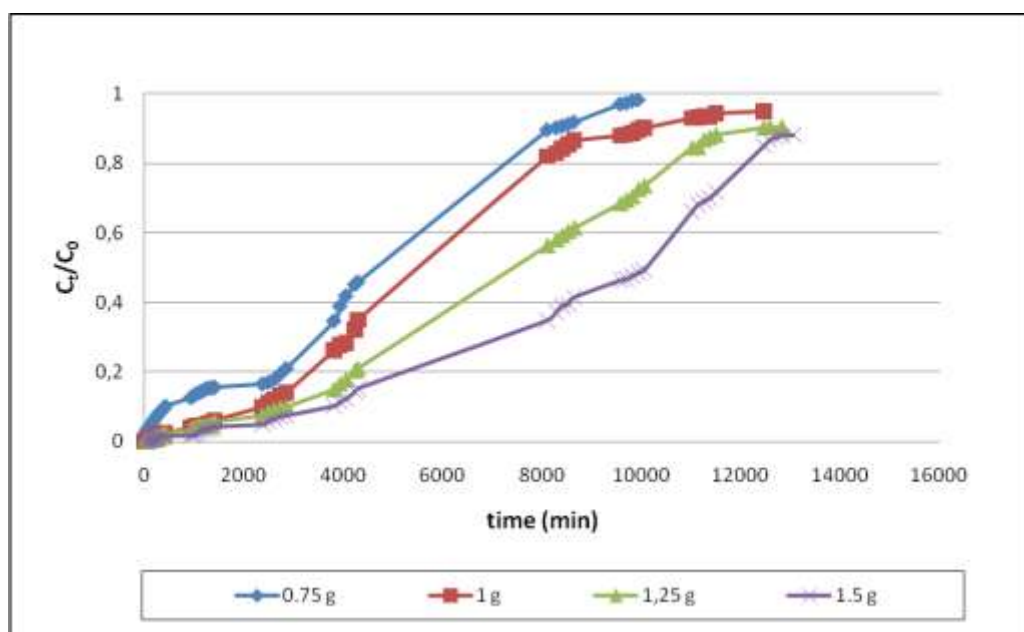


Figure 4.30 Breakthrough curves of GAC obtained at different adsorbent masses for 2,4-D adsorption

It was shown in Figure 4.30 that breakthrough generally occurred steeper with a smaller adsorbent mass. Increasing the mass of adsorbent, in other words

bed height, resulted in the increase in the breakthrough time. Not only the total bed capacity, but also the retention time increases. Because of these reasons, longer breakthrough times and larger bed capacities were obtained with bigger adsorbent masses.

Application of the Thomas, Yoon-Nelson and Adams-Bohart Models

The column data were fitted to the Thomas model to determine the Thomas rate constant, ($k_{Th} = \text{ml/mg} \times \text{min}$), and the maximum adsorption capacity of the bed ($q_0 = \text{mg/g}$); the Yoon and Nelson model to determine the Yoon and Nelson rate constant ($k_{YN} = \text{L/min}$) and the time required for 50% adsorbate breakthrough ($\tau = \text{min}$) and the Adams–Bohart sorption model to estimate the characteristic parameters, such as maximum adsorption capacity ($N_o = \text{mg/L}$) and kinetic constant ($k_{AB} = \text{L/mg} \times \text{min}$).

The constants that are obtained from the applied continuous models at different influent concentrations, different flow rates and different adsorbent masses are tabulated in Table 4.22 for comparison.

Table 4.22 Comparison of the constants predicted from the continuous models of 2,4-D adsorption at different influent concentrations, flow rates and adsorbent masses

C_0 (mg/L)	v (mL/min)	Mass of adsorbent (g)	Thomas Model			Yoon-Nelson Model			Adams-Bohart Model		
			$k_{TH} \times 10^{-6}$ (mL/min x mg)	q_0 (mg/g)	R^2	k_{YN} (L/min)	τ (min)	R^2	$k_{AB} \times 10^{-6}$ (L/min x mg)	N_0 (mg/L)	R^2
50	0.4	1.0	10.00	172.85	0.9916	0.0005	8643	0.9916	6.00	133.19	0.9036
100	0.2	1.0	6.00	198.58	0.9861	0.0006	9929	0.9861	4.00	252.76	0.8522
	0.3		6.00	242.79	0.9826	0.0006	8093	0.9826	4.00	226.48	0.8735
	0.4	0.75	7.00	207.26	0.9559	0.0006	4695	0.9809	3.00	198.77	0.8906
		1.00	7.00	241.03	0.9397	0.0007	6026	0.9397	4.00	209.58	0.8019
		1.25	5.00	274.30	0.9347	0.0005	8572	0.9347	4.00	196.31	0.8305
		1.50	5.00	247.69	0.9353	0.0005	9288	0.9353	4.00	196.69	0.8672
150	0.4	0.75	6.00	229.02	0.9506	0.0009	2863	0.8506	2.70	187.99	0.6157
		1.00	4.67	253.37	0.8526	0.0007	4306	0.8526	2.67	219.37	0.6036
		1.25	4.00	306,32	0.8523	0.0006	6382	0.8523	2.67	256.87	0.6854
		1.50	3.33	355.46	0.9458	0.0005	8886	0.9458	2.67	272.16	0.8381

As seen in Table 4.22, as concentration increased, the values of k_{Th} decreased and the values of q_0 increased and as flowrate increased, the values of k_{Th} increased and the values of q_0 decreased. These results were expected because; increasing flowrate causes decreasing in retention time so pesticide ions do not have more time to contact with carbon as a result bed capacity decreases and increasing concentration causes increasing the opportunity of come across of the pesticide ion and adsorbate so bed capacity increases. The maximum bed adsorption capacity (q_0) was observed at minimum flowrate and maximum influent concentration. The minimum bed adsorption capacity (q_0) was observed at maximum flowrate and minimum influent concentration. Those results were evidences for validity of the mechanism of flowrate and concentration differences. The same conclusions were found by Han et al. (2005) for the sorption of the copper (II) and lead (II) from aqueous solutions by chaff in a fixed bed column and by Malkoc and Nuhoglu (2006) for the removal of Ni (II) ions from aqueous solutions using waste of tea factory. Also as the mass of adsorbent increased, the values of k_{Th} decreased and the values of q_0 increased. Maximum bed adsorption capacity (q_0) was obtained at maximum bed height with the lowest rate constant of Thomas because of the increase in the adsorbate weight and retention time.

Generally the values of k_{YN} increased and the values of τ decreased both with increasing in the influent concentration and flowrate. The time required for 50% adsorbate breakthrough (τ) was minimum at operational parameters of 200 ppm influent concentration and 0.4mL/min flowrate. Because of (1) the high flowrate, the retention time of the pesticide solution in column was decreased; (2) the high concentration, the opportunity of the encounter of the adsorbate and the pesticide solution was increased so bed capacity filled steeper. And τ was maximum at operational parameters of 50 ppm concentration and 0.2mL/min. Because of (1) the low flowrate, the retention time of the pesticide solution in column was increased; (2) the low concentration, the opportunity of the encounter of the adsorbate and the pesticide solution was decreased so bed capacity filled slower. The same conclusions were obtained by Malkoc et al. (2006) for Cr(VI) adsorption onto waste acorn in fixed beds and by Aksu and Gönen (2004) for phenol sorption by immobilized activated sludge. Also as the mass of the adsorbent increased, the values of k_{YN} decreased and the values of τ increased. Maximum

value of time required for 50% adsorbate breakthrough (τ) was obtained in a maximum bed height with the lowest rate constant.

The values of the Adams-Bohart kinetic constant was influenced by the flowrate, increased with increasing flowrate. This showed that the overall system kinetics is dominated by external mass transfer in the initial part of the adsorption in column. As it was expected, maximum adsorption capacity (N_o) increased with increasing influent concentration. This was the reason for obtaining the maximum adsorption capacity at minimum flowrate 0.2mL/min and maximum influent concentration 200 ppm as proved by Aksu and Gonen (2004) for phenol sorption by immobilized activated sludge. Also maximum adsorption capacity (N_o) and the kinetic constant (k_{AB}) decreases with increasing the mass of adsorbent. Maximum adsorption capacity was obtained by the highest mass of adsorbent with the lowest k_{AB} value.

4.3.4 Effect of Biological Contamination Load

Breakthrough curves of 2,4-D, after loading at 20 hour, 44 hour and 68 hour bacteria and the comparison of breakthrough curves of bacteria loading is shown in Figure 4.31.

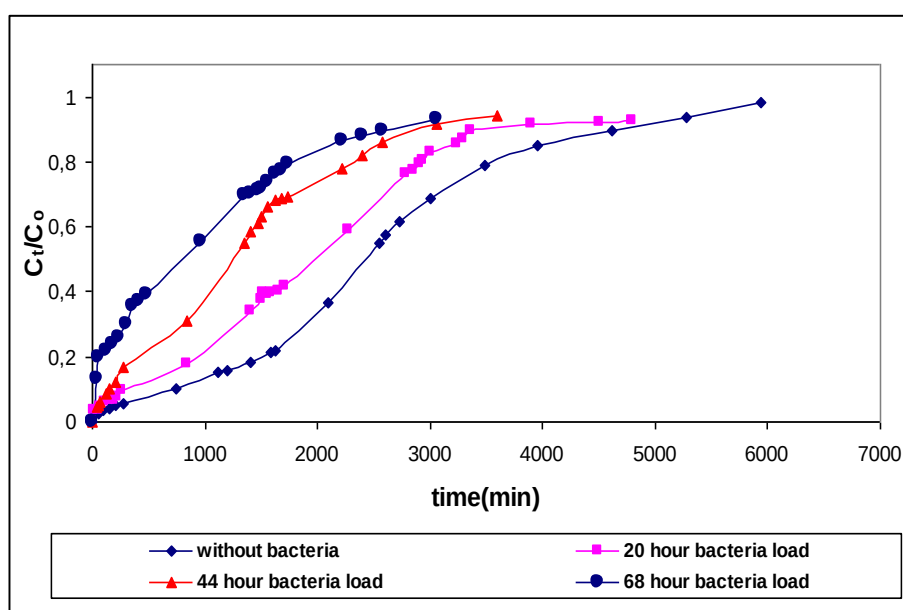


Figure 4.31 Comparison of the breakthrough curves obtained at different bacteria loading times for 2,4-D adsorption

To investigate the effect of the biological contamination load on the adsorption of 2,4-D by GAC, the loading time was changed from 20 to 68 hour. This was tried with the pure bacteria *LMG 17238*. The influent concentration of 2,4-D was 200 ppm with a flow rate of 0,4 mL/min.

In the column studies, solution continuously enters and leaves the column. As a result, as seen from Figure 4.31, after loading, bacteria drifted with the effluent 2,4-D solution within 20 hour. This shows that bacteria could not hang on to the surface because of the stable structure of the carbon particle. But it is clear from the breakthrough curves that although bacteria could not hang on to the particle surface completely, they filled the mesapores and micropores of the carbon particle which caused the breakthrough time to decrease in respect to a without bacteria study. Although in the batch study at 1 mL bacteria/ 50 mL 2,4-D solution) ratio, no 2,4-D removal was observed, in the column study 2,4-D removal was seen because of being dynamic. As the bacteria loading time increased, the bacteria had more opportunity onto hang on the carbon surface, so the probability of occurring biofilm increased. To sum up, increasing the bacteria loading time, causes decrease in breakthrough time.

The breakthrough curves of GAC obtained for adsorption of 2,4-D in the presence of different microorganisms are seen in Figure 4.32.

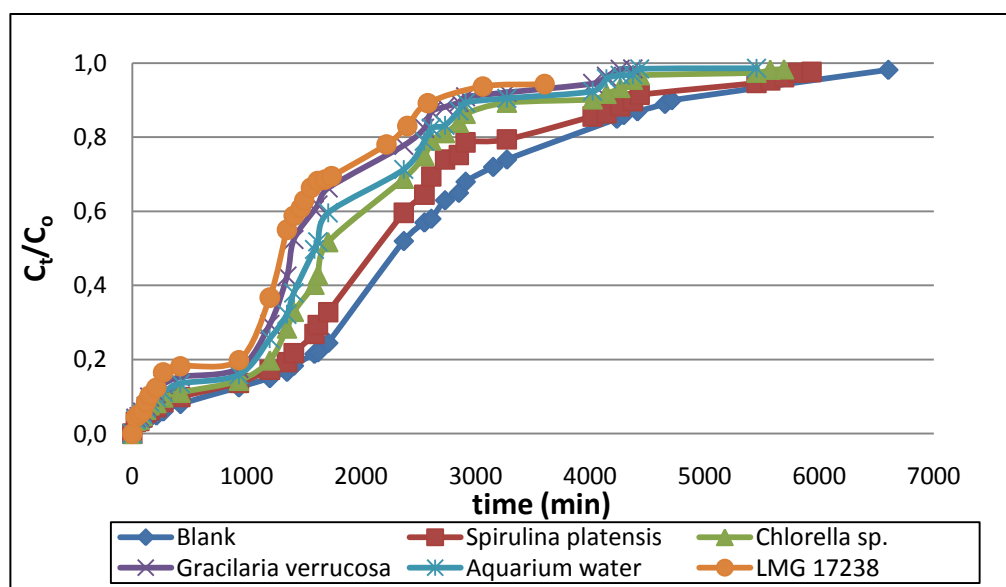


Figure 4.32 The breakthrough curves of GAC obtained for adsorption of 2,4-D in the presence of different microorganisms

According to the results of the experiments done by all the microorganisms loaded for 44 hours, the order of adsorption of 2,4-D in terms of maximum adsorption capacity (mg/g) from the least to the most is:

$$\text{Blank} < \textit{Spirulina platensis} < \textit{Chlorella sp.} < \textit{Aquarium water} < \textit{Gracilaria verrucosa} < \textit{Acidovorax Avenae Subsp. Avenae LMG 17238}$$

The operating principles of batch and continuous systems and the logic behind them are totally different. The result obtained in the continuous system is just the opposite of the batch systems with the same microorganisms.

Application of the Thomas Model

Table 4.23 shows the parameters together with the correlation coefficients for blank and in the presence of microorganisms where $C_0 = 200$ ppm, $v = 0.4$ ml/min, adsorbent mass = 1 g.

Table 4.23 Parameters predicted from Thomas model of 2,4-D adsorption for different microorganisms

Microorganisms	$k_{Th} \times 10^{-6}$ (mL /min x mg)	q_0 (mg/g)	R^2
Blank	6.00	207.55	0.9782
<i>Spirulina platensis</i>	5.50	180.69	0.9618
<i>Chlorella sp.</i>	6.50	143.79	0.9629
Aquarium water	7.50	126.49	0.9818
<i>Gracilaria verrucosa</i>	7.50	117.11	0.9760
<i>LMG 17238</i>	9.50	97.10	0.9417

As in the order of Blank - *Spirulina platensis* - *Chlorella sp.* - Aquarium water - *Gracilaria verrucosa* - *LMG 17238*, the Thomas rate constant, (k_{Th} = ml/mg x min) increases and the maximum adsorption capacity of the bed (q_0 = mg/g) decreases.

Application of the Yoon-Nelson Model

Table 4.24 shows the parameters of Yoon-Nelson together with the correlation coefficients for blank and in the presence of microorganisms where $C_0 = 200$ ppm, $v = 0.4$ ml/min, adsorbent mass = 1 g.

Table 4.24 Parameters predicted from Yoon-Nelson model of 2,4-D adsorption for different microorganisms

Microorganisms	$k_{YN} \times 10^{-4}$ (L/min)	τ (min)	R^2
Blank	12.00	2599	0.9782
<i>Spirulina platensis</i>	11.00	2259	0.9618
<i>Chlorella sp.</i>	13.00	1797	0.9629
Aquarium water	15.00	1571	0.9818
<i>Gracilaria verrucosa</i>	15.00	1464	0.9760
LMG 17238	17.00	1418	0.9545

As in the order of Blank - *Spirulina platensis* - *Chlorella sp.* - Aquarium water - *Gracilaria verrucosa* - LMG 17238, the Yoon-Nelson rate constant, ($k_{YN} = \text{L min}^{-1}$) increases and the time required for 50% adsorbate breakthrough ($\tau = \text{min}$) decreases.

Application of the Adams-Bohart Model

After applying to the experimental data for different microorganisms, the results are tabulated in Table 4.25 together with the correlation coefficients for blank and in the presence of microorganisms where $C_0 = 200$ ppm, $v = 0.4$ ml/min, adsorbent mass = 1 g.

The correlation coefficients are very low as seen in Table 4.25 and the kinetic rate constant of Adams-Bohart (k_{AB}) and maximum adsorption capacity (N_0) vary dramatically.

Table 4.25 Parameters predicted from Adams-Bohart model of 2,4-D adsorption for different microorganisms

Microorganisms	$k_{AB} \times 10^{-6}$ (L/min x mg)	N_0 (g/L)	R^2
Blank	3.00	133.19	0.9036
<i>Spirulina platensis</i>	2.50	186.95	0.7853
<i>Chlorella sp.</i>	2.50	174.85	0.7591
Aquarium water	3.00	144.59	0.7925
<i>Gracilaria verrucosa</i>	3.00	137.92	0.7720
LMG 17238	4.50	107.61	0.7919

According to the correlation coefficients, among the three kinetic models, the Thomas, Yoon-Nelson and Adams-Bohart models, it was observed that the Thomas and Yoon-Nelson kinetic models better fit the experimental data.

4.3.5 Comparison of the Column Results with Pesticide Standards

The WHO guidelines for drinking-water quality on 1993 set the standard pesticide range between 0.03 µg/L and 100 µg/L. These levels are detected according to the consequence of any potential health problems. For example, the level for 2,4-D is set to be 30 µg/L.

In the EU Drinking Water Directive (1998), the values for individual pesticides and for total pesticides were retained 0.1µg/L and 0.5µg/L respectively, and more stringent values were introduced for certain pesticides 0.03µg/L.

When the column performances for the adsorption study are compared with respect to these pesticide standards; with minimum flowrate, minimum influent concentration, and maximum adsorbent mass operational parameters, better results are obtained.

The comparison of columns with minimum effluent concentration and time reaching to those concentrations are tabulated in Table 4.26.

Table 4.26 Comparison of the column data with the pesticide standards

C _o (mg/L)	v (mL/min)	Mass of the Adsorbent (g)	EU pesticide standards (1998)	WHO 2,4-D standard (1993)	Time reaching for min eff.conc. (min)	Min effluent Conc. (mg/L)
50	0.4	1.00	0.1 µg/L for individual pesticides and 0 0.5 µg/L for total of all pesticides present	30 µg/L	240	0.515
100	0.2	1.00			1440	0.260
	0.3				180	3.049
	0.4	0.75			30	0.258
		1.00			180	0.732
		1.25			30	0.122
		1.50			30	0.122
150	0.4	0.75			60	3.620
		1.00			60	1.584
		1.25			60	0.905
		1.50			60	0.792
200	0.4	1.00				

5. CONCLUSIONS

Water sources are going to be distinct and water pollution by organic and inorganic chemical pollutants has become a primary public concern in the last few years.

In this thesis, removal of 2,4-D as a member of the phenoxy herbicide group by adsorption technique was investigated. The adsorbent used was aqualine labeled GAC (1-2mm). Five types of microorganisms were involved in all of the experiments: two of them belong to mixed bacteria sources which are aquarium water and *Gracilaria verrucosa*, one is pure bacteria culture- *Acidovorax Avenae Subsp. Avenae LMG 17238*- and the other two are algae which are *Chlorella sp.* and *Spirulina platensis*.

In the batch mode studies, done with all microorganisms, the order of adsorption of 2,4-D in terms of maximum adsorption capacity (mg/g) from the least to the most is:

Acidovorax Avenae Subsp. Avenae LMG 17238 < *Gracilaria verrucosa* < Aquarium water < *Chlorella sp.* < *Spirulina platensis* < Blank

There can be three interpretations of this order. First of all, it is certain that in none of the conditions with microorganism, the adsorption capacity cannot reach to the one in the blank situation. Secondly, bacteria are small compared to the algae and they can fill the mesapores and micropores of GAC better. Since LMG is a dense culture full of small bacteria, this is an expected result. Thirdly, *Chlorella sp.* is a smaller alga compared to *Spirulina platensis* and its shape is rounded, a proper shape to fill the pores of GAC. *Spirulina platensis* is a spiraled alga, so it cannot exactly fill the pores and the result is closest to the one in without microorganism.

Also it was observed that an increase in the removal of 2,4-D is achieved by decreasing the volume of bacteria in the solution because if more bacteria are

in contact with the carbon, the thickness of the biofilm increased. The same result was obtained with different volumes of *Chlorella sp.*

In batch mode kinetic studies, the intraparticle diffusion is the rate controlling step. Also it can be said that the thickness of the boundary layer is high since the value of intercept “C” is high. Blank experimental data obtained fit to the pseudo-second-order mechanism where in the case of *Chlorella sp.*, the process turns out to obey pseudo-first order model. The first order kinetic constant has not changed, but the second order kinetic constant has increased slightly. Also $q_{e, \text{calculated}}$ has decreased in both cases.

In equilibrium modeling done by the nonlinear regression in MATLAB, among all the nonlinear models, the experimental data best fit in the order of Toth, Sips and then Redlich-Peterson for the blank case and in the order of Redlich-Peterson, Toth and then Langmuir for *Chlorella sp.* case regarding the correlation coefficients. This result is not surprising since these four models are derived from the same origin as it can be realized from the model equations.

In the batch mode thermodynamic studies, as the temperature increases, adsorption capacity, q (mg/g) increases. The value of maximum adsorption capacity in the presence of microorganism cannot reach to the one in the without microorganism condition at 25°C. It is dangerous to increase the temperature further because of the possible deformation in the protein structure of microorganisms. The negative values of ΔG° confirm the spontaneous nature of adsorption where the positive value of ΔH° indicates that it is an endothermic process. In an endothermic adsorption, as temperature increases, adsorption capacity, q (mg/g) increases, so this proves all the results. The quantities of ΔG° values have decreased with the presence of microorganism compared to the ones without microorganism. The value of ΔH° has increased very much where the value of ΔS° has stayed nearly close to zero.

In the continuous mode experiments, the capacity of the bed increases when the initial concentration of 2,4-D solution is increased, the flow rate is decreased and the mass of adsorbent is increased. Also it is obvious that bacteria could not

hang on to surface because of the stable structure of the carbon particle. But it is clear from the breakthrough curves that although bacteria could not hang on the particle surface completely, they filled the mesapores and micropores of the carbon particle. The breakthrough time decreases with the increase in the bacteria loading time. When the bacteria load time was increased, the bacteria had more opportunity to hang onto the carbon surface, so the probability of occurring biofilm increased. The bacteria –LMG 17238- could not hang on the carbon surface because this bacteria is produced for the biodegradation of the synthetic-based polyester based plastics, poly ϵ -caprolactone (PCL), poly (butylene succinate-cobutylene adipate) and a starch –PCL blend. So there should be an external bond effect applied to attach the LMG bacteria on a carbon surface. And in further studies, the column studies of PCL and the effect of biological contamination on the PCL column should be investigated.

The results obtained in the continuous system are just the opposite of the batch systems with the same microorganisms. The order of adsorption of 2,4-D in terms of maximum adsorption capacity (mg/g) from the least to the most is:

Blank < *Spirulina platensis* < *Chlorella sp.* < Aquarium water < *Gracilaria verrucosa* < *Acidovorax Avenae Subsp. Avenae* LMG 17238

The experimental data were analyzed regarding the three continuous mathematical models, the Thomas, Yoon-Nelson, and Adams-Bohart kinetic models. According to the correlation coefficients, among these three kinetic models, the Thomas and Yoon-Nelson kinetic models better fit the experimental data. As in the order of blank - *Spirulina platensis* - *Chlorella sp.* - Aquarium water - *Gracilaria verrucosa* - *Acidovorax Avenae Subsp. Avenae* LMG 17238, the kinetic constants of the Thomas and Yoon-Nelson models have increased and the maximum adsorption capacity together with half saturation time decreased whereas the constants of Adams-Bohart model vary dramatically.

As a conclusion, the microorganisms present in drinking water decrease the efficiency of 2,4-D adsorption, so before treating the water with activated carbon, the microorganisms should be eliminated somehow.

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APPENDIX

BCCM™/LMG**BACTERIA COLLECTION**

Laboratorium voor Microbiologie
Universiteit Gent

K.L. Ledeganckstraat, 35 B-9000 Gent BELGIUM
Phone: +32-9-2645108 / Fax: +32-9-2645346
E-mail: bccm.lmg@UGent.be
Website: <http://www.belspo.be/bccm>

DELIVERY NOTE

GENT, 13 December 2005

YOUR CUSTOMER NUMBER 3859
Must be mentioned on all orders submitted
by e-mail or via the Website

Attn. Prof. Dr. Bikem Ovez
Ege University
Engineering Faculty
Chemical Engineering Department
35100 Bornova/Izmir
Turkey

YOUR REF.: E-mail dd. 24/11/05
OUR REF.:

Please find enclosed following freeze dried BCCM™ bacterial culture:

Acidovorax avenae* subsp. *avenae (Manns 1909) Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992^{VP}
Obj. syn.: *Pseudomonas avenae* Manns 1909^{AL}
Obj. syn.: *Pseudomonas avenae* subsp. *avenae* Manns 1909^{AL}
Subj. syn.: *Pseudomonas rubrilineans* (Lee, Purdy, Barnum and Martin 1925) Stapp 1928^{AL}
Medium 2, 28°C.
LMG 17238 = PHA 1183.
← 1996, J. Mergaert University Ghent Belgium, Ghent Belgium, botanical garden, freshwater.
Biohazard group 1

Recommended media:

1. **Nutrient Agar (Oxoid CM3)**

'Lab-Lemco' beef extract	1 g
Yeast extract	2 g
Peptone	5 g
NaCl	5 g
Agar	15 g
Distilled water	1 L
pH	7,4
2.

KH ₂ PO ₄	0,45 g
Na ₂ HPO ₄ x 12H ₂ O	2,39 g
Medium 1	1 L
pH	6,8

ir. C. Vereecke
Public Collection Curator

The BCCM™/LMG Bacteria Collection will be closed from 23/12/2005 until 2/1/2006.

GRATIS-COOPERATION

FREEZE DRIED CULTURES STORED IN THEIR INTACT GLASS AMPOLLES AT +4°C TO 8°C IN THE DARK, GENERALLY REMAIN VIABLE FOR MANY YEARS AFTER PRODUCTION
BCCM™/LMG is a node of the Belgian Coordinated Collections of Microorganisms (BCCM™), a consortium of research-based culture collections
supported by the Belgian Federal Science Policy.

Figure A.1 The recipe of *Acidovorax Avenae* Subsp. *Avenae* LMG 17238

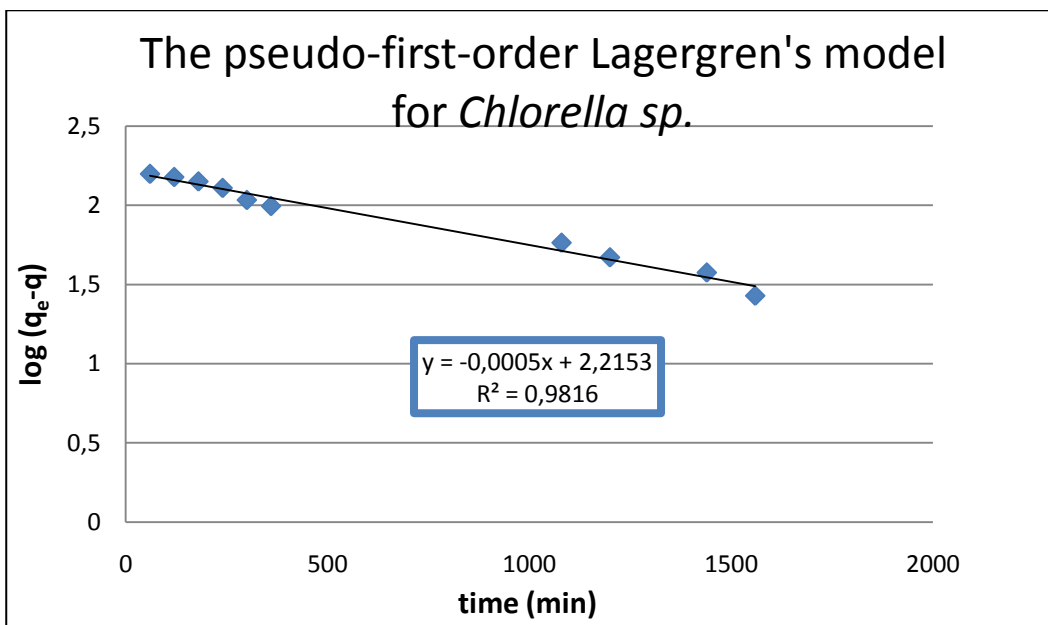
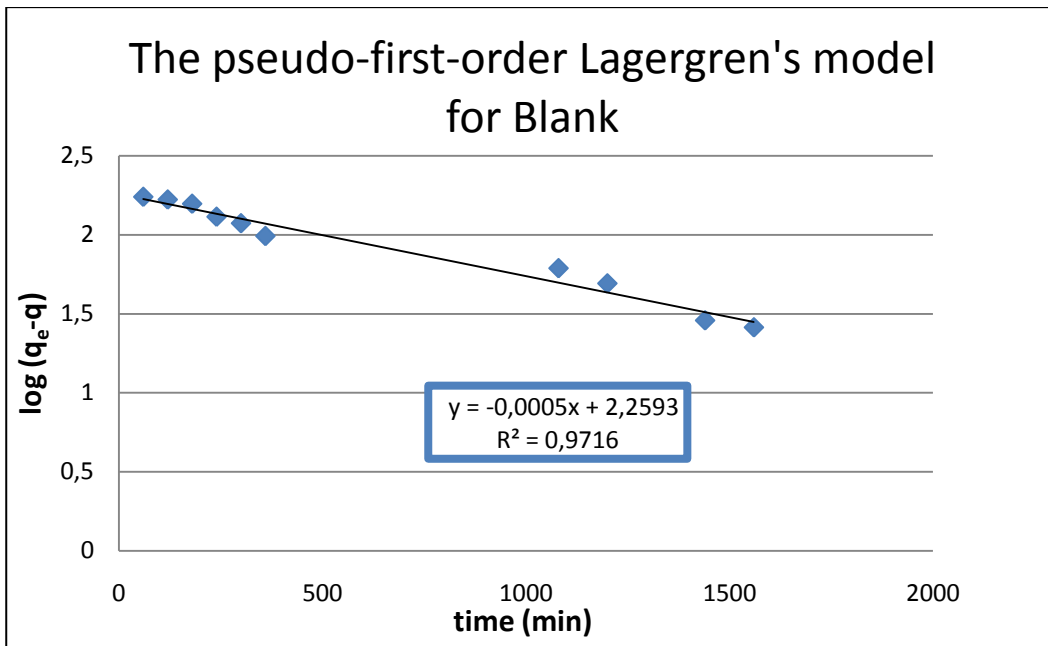


Figure A.2 Linearized form of pseudo-first-order kinetics for 2,4-D adsorption on GAC for blank and *Chlorella sp* at 25°C

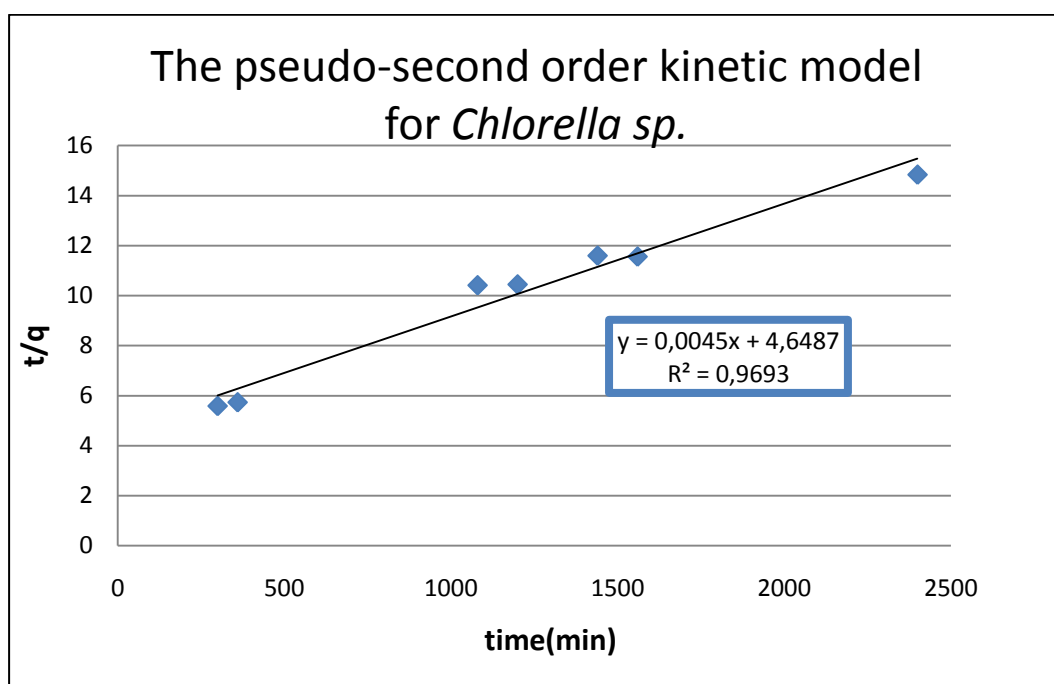
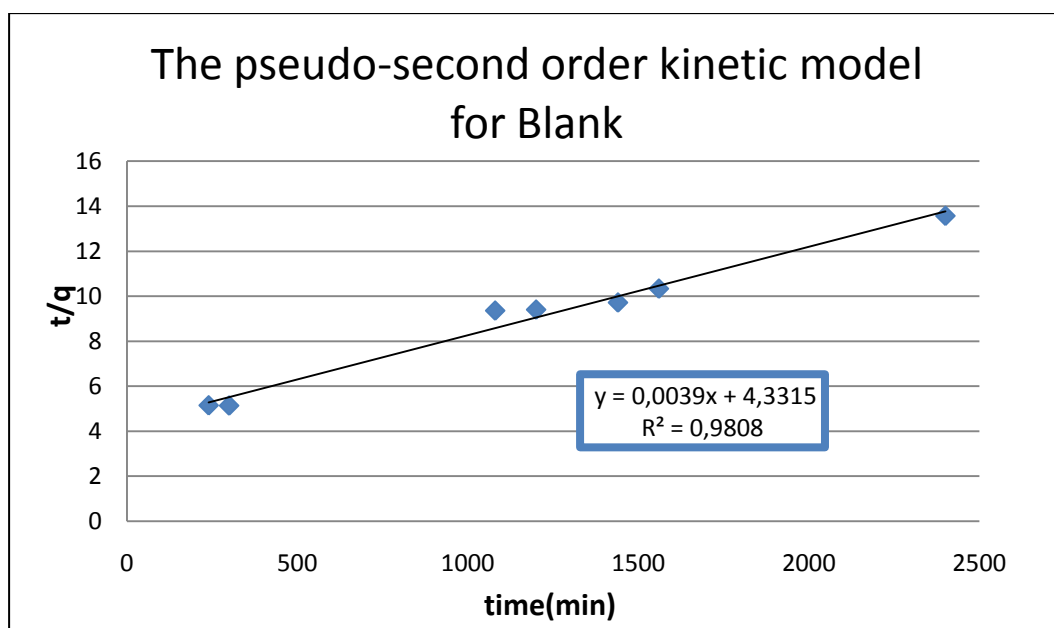


Figure A.3 Linearized form of pseudo-second-order kinetics for 2,4-D adsorption on GAC for blank and *Chlorella sp.* at 25°C

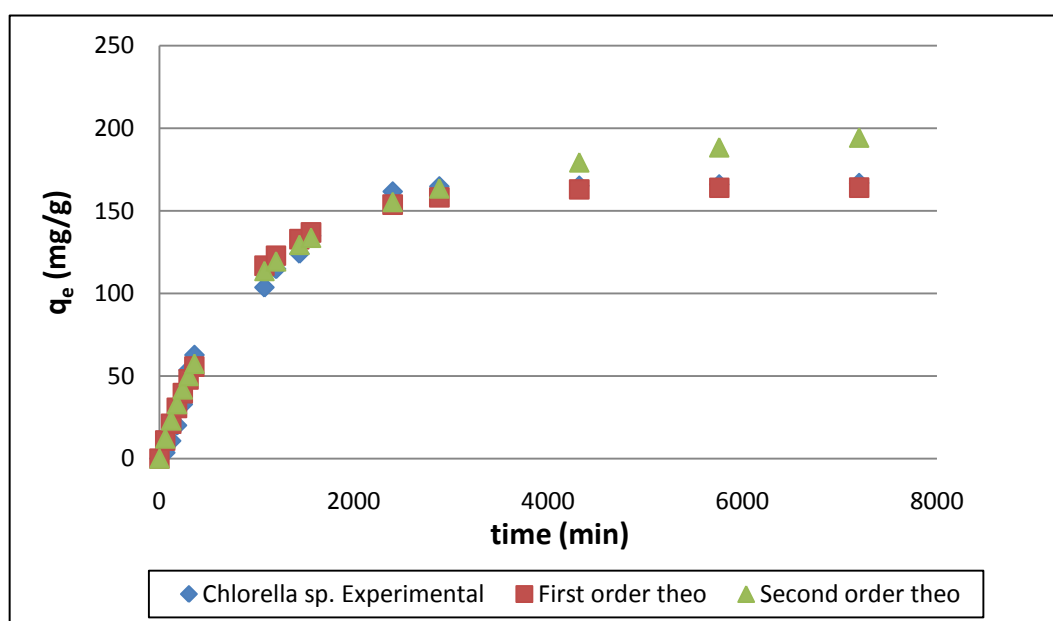
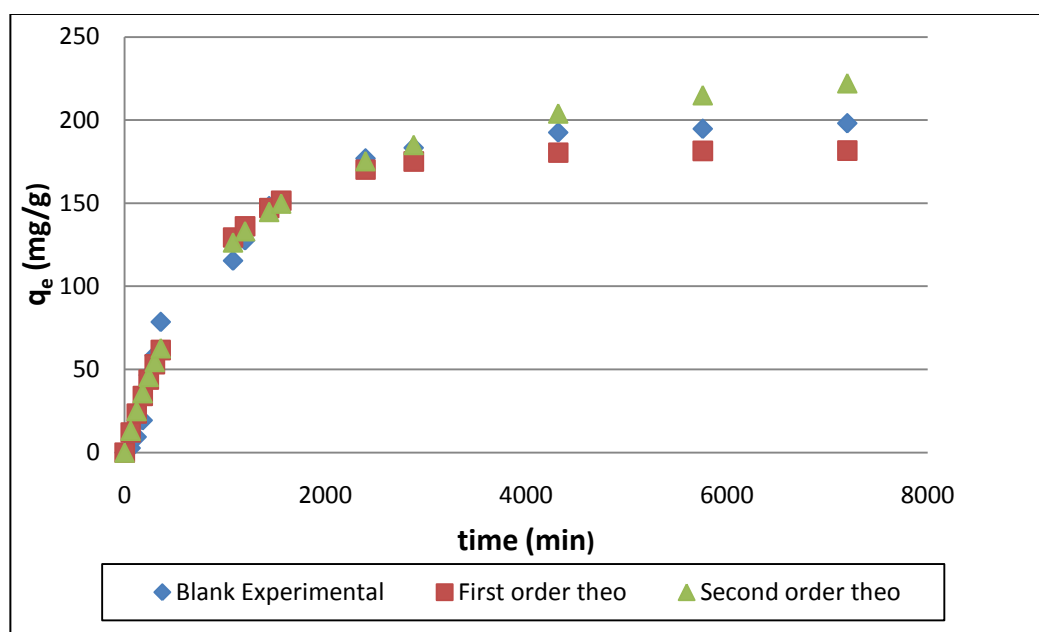


Figure A.4 Comparison of first and second order kinetic model with predicted and experimental q_e values for 2,4-D adsorption on GAC for blank and *Chlorella sp.* conditions at 25°C

CURRICULUM VITAE

Duygu OVA

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**PERSONAL INFORMATION**

Nationality T.C
Date of Birth 23.10.1986
Birth Place İzmir
Marital Status Single
Driving Licence Class B (2005)

EDUCATION

Date	School	Bölümü	Status	GPA
2008-2010	Ege University	Chemical Engineering	Master of Science	
2007-2010	Ege University	Bioengineering	Double Major	3.56 / 4
2004-2008	Ege University	Chemical Eng. (ENG)	Undergraduate	3.48 / 4
1997-2004	American Collagiate Institute	Science		4.99 / 5

EXPERIENCES

07/2004 - 09/2004	: Aytaş A.Ş. A.O.S.B. Çiğli/İZMİR Summer Training- Accounting/Finance Department
19/06/2006 - 14/07/2006	: S.S.Tariş Zeytin ve Zeytinyağı Tarım Satış ve Kooperatifleri Birliği A.O.S.B. Çiğli/İZMİR Manufacturing Department
11/06/2007-06/07/2007	: Pınar Süt Mamülleri San. A.Ş. Kemalpaşa/İZMİR Manufacturing Training

CAREER OBJECTIVES

In the short term, my goal is to be a part of the future of my firm and my team in the direction of both the firm's and my objectives. Also it is to use and improve my background knowledge, gain experience and finally to increase my self-motivation by having the necessary job experience and knowledge.

In the long term, by using the knowledge I got and the experience I gained in the most effective way, to be the director general of the firm.

LANGUAGE SKILLS

ENGLISH **Reading:** Advanced **Writing:** Advanced **Speaking:** Advanced

TOEFL IBT- May 2010: 90/120

ITALIAN **Reading:** Upper-Intermediate **Writing:** Intermediate **Speaking:** Intermediate

Graduated from Il Centro Culturale Italiano

COMPUTER SKILLS

- Basic Programming Languages and Databases: Visual Basic 6.0, MATLAB
- Application Programs: MS Office 2003-2007, ASPEN (Chemical Engineering Computer Application)
- Operating Systems: Windows 98- XP- Vista
- Basic Drawing Programs: Intellicad
- NETSIS

PUBLICATIONS

- **Akbaba, A., Ova, D., Kazar, G., Ulutan, S.,** 2006, Nanotechnology Applications on Food Packaging, *I. Polymeric Composites Symposium and Fair*, Bayraklı, İzmir, 505-512pp.
- **Ova, D., Kagnici, D.,** 2008, Removal of Phenoxy Herbicides by Using Poly- ϵ -Caprolactone Powder, Diploma Project, Ege University Chemical Engineering Department, 63p.

- **Ova, D., Nalcaci, O.O, Boke, N., Ovez, B.,** 2008, Potential of The Bacterial Strain *Acidovorax Avenae Subsp.Aavenae LMG 17238* on Adsorption of 2,4-D, *IV. National Congress of Bioengineering*, Konak, İzmir, 69-70pp.
- **Ova, D., Boke, N., Kocaoglu, İ. S., Ovez, B.,** 2010, The Potential of The Bacterial Strain *Acidovorax Avenae Subsp. Avenae LMG 17238* on The Adsorption of 2,4-D Using Matlab Modelling, *6th Chemical Engineering Conference for Collaborative Research in Eastern Mediterranean Countries (EMCC-6)* , Belek, Antalya.

SEMINARS/TECHNICAL VISITS

Seminars:

- ISO 18000-18001 Environmental Health and Safety Training, *Participation Certificate - İbrahim Mutağ/ Optimal Eğitim ve Danışmanlık*
- ISO 22000:2005 Food Safety Management Systems, *Participation Certificate - Hakan Selçuk/Optimal Eğitim ve Danışmanlık*
- ISO 9001:2000 Quality Management System, *Participation Certificate- Hakan Selçuk/Optimal Eğitim ve Danışmanlık*
- Internal Quality Management ,*Participation Certificate - Hakan Selçuk/Optimal Eğitim ve Danışmanlık*
- Sales Communication and Sales Process Management, *Participation Certificate - İsmail Türkmen/ Etik Yönetim Danışmanlığı*

Technical Visits: Dr.Oetker (Torbalı /İZMİR)

HOBBIES

Piano, Cinema, Sports, Language

MEMBERSHIPS

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