DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

INVESTIGATION OF VEGETABLE OIL CONTAINING WASTEWATER TREATMENT BY BIOLOGICAL METHODS

by

Önder KIZILASLAN

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İZMİR

INVESTIGATION OF VEGETABLE OIL CONTAINING WASTEWATER TREATMENT BY BIOLOGICAL METHODS

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"INVESTIGATION OF VEGETABLE OIL CONTAINING WASTEWATER TREATMENT BY BIOLOGICAL METHODS"** completed by **ÖNDER KIZILASLAN** under supervision of **ASSOC.PROF.DR. İLGİ K. KAPDAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

Assoc.Prof.Dr. İLGİ K. KAPDAN

Supervisor

(Jury Member)

(Jury Member)

Prof.Dr. Cahit HELVACI
Director
Graduate School of Natural and Applied Sciences

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INVESTIGATION OF VEGETABLE OIL CONTAINING WASTEWATER TREATMENT BY BIOLOGICAL METHODS

ABSTRACT

Vegetable oil containing wastewater treatment by biological methods was investigated in this thesis. This study has two parts as batch flask and fed-batch experiments.

In the first part of the thesis, Box-Wilson Statistical Experimental Design method used to investigate the effects co-substrate, vegetable oil and biomass concentrations in batch treatment of v.oil containing synthetic wastewater. The significant effects of these parameters were observed on COD and v.oil removal. Maximum COD and V.Oil removal efficiencies were obtained as 75% and 98%, respectively, at the high concentration of biomass and low concentration of glucose.

In the second part of the thesis, treatment of vegetable oil containing synthetic wastewater by fed-batch operation was studied. The effects of initial V.Oil concentration (1%-6%) and sludge age (15 days-30 days) on COD and V.Oil removal were investigated. The experiments were carried out with and without carbon source. Maximum COD and V.Oil removals were obtained around 3% oil concentrations. Increasing the sludge age significantly affected the COD and V.Oil removal performances. When sludge age was increased, percent COD and V.Oil removal decreased.

Finally the effect of biosurfactant on biodegradability of oil was investigated by fed-batch operation. It was observed biosurfactant that addition did not enhance the COD and vegetable oil removal.

Keywords: Biosurfactant, hydrocarbon, lipids, vegetable oil

BİTKİSEL YAĞ İÇEREN ATIKSULARIN BİYOLOJİK METOTLARLA ARITILMASI

ÖZ

Bu tezde bitkisel yağ içeren atıksuların biyolojik metotlarla arıtımı araştırılmıştır. Bu çalışma kesikli flask ve yarı kesikli deneyleri olmak üzere iki kısımdan oluşmaktadır.

Tezin ilk bölümünde Box-Wilson Experimental Design metotu kullanılarak bitkisel yağ içeren sentetik atıksuyun kesikli arıtımında yardımcı besin(glikoz), bitkisel yağ ve biyokütle konsantrasyonlarının etkisi araştırılmıştır. COD ve yağ giderimi üzerinde bu parametrelerin önemli etkileri gözlemlenmiştir. Yüksek biyokütle konsantrasyonu ve düşük glikoz konsantrasyonunda maksimum COD ve yağ giderme verimleri %75 ve %98 olarak elde edilmiştir.

Tezin ikinci kısmında yarı kesikli işletim ile bitkisel yağ içeren sentetik atık suyun arıtımı çalışılmıştır. COD ve yağ giderimi üzerinde başlangıç yağ konsantrasyonu ve çamur yaşlarının etkileri araştırılmıştır. Deneyler karbon kaynaklı ve karbon kaynaksız olarak uygulanmıştır. Yaklaşık olarak %3 yağ konsantrasyonlarında maksimum COD ve yağ giderimleri elde edilmiştir. Artan çamur yaşı COD ve yağ giderim performansını önemli ölçüde etkilemiştir. Çamur yaşı arttığı zaman COD ve yağ giderimi oranları azalmıştır.

Son olarak yağın biyolojik ayrışımı üzerinde biyosurfaktantın etkisi yarı kesikli işletim ile araştırılmıştır. Biyosurfaktant ilavesinin COD ve yağ giderimini artırmadığı görülmüştür.

Anahtar Kelimeler: Biyosurfaktant, hidrokarbon, yağlar, bitkisel yağlar.

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Wastewater discharged from kitchens, restaurants and the food industry often includes lipid materials such as fat, oil and grease (FOG). FOG has a high chemical oxygen demand (COD) in spite of its small volume ratio. They are responsible for clogging of wastewater pipes and many other problems in the activated sludge process. A lipid overload leads to a decrease in treatment efficiency, the proliferation of filamentous microorganisms and settling problems in the clarifier. Hence, a trap for FOG, such as air flotation, is usually installed before raw wastewater is introduced into the main treatment system. The treatments of trapped and highly concentrated FOG still remain a difficult problem.

Fats and oils are essentially triglycerides consisting of straight-chain fatty acids attached, as esters, to glycerol. The component fatty acids of edible fats and oils vary considerably. They can differ in chain lenght, may be saturated or unsaturated, and may contain an odd or even number of carbon atoms. The term ‘grease’, as commonly used, includes fats, oils, waxes and other related constituents found in wastewater (Wakelin & Forster, 1996).

Lipases can be highly specific (Shimada et al., 1992) and, therefore, attack triglycerides containing specific fatty acids. Alternatively, they can be totally non-specific (Anon, 1993a) and attack triglycerides containing different fatty acids. Tan and Gill (1985; 1987) studied FOG removal by different microorganism in batch-growth studies, and reported that removal could be significantly affected by the substrate specificity of the induced extra-cellular lipases, the physical and chemical characteristics of the substrate, and the pH of the culture medium (Wakelin & Forster, 1997).

Fats, oil and grease called as FOG in the wastewater business can have negative impacts on wastewater collection and treatment systems. Most wastewater collection system blockages can be traced to FOG. Blockages in the wastewater collection system are serious, causing sewage spills, manhole overflows, or sewage backups in home and business. Two types of FOG pollutants are common to wastewater systems. Petroleum-based oil and grease (non-polar concentrations) occur at businesses using oil and grease, and can usually be identified and regulated by municipalities through local limits and associated pretreatment permit conditions. Animal and vegetable-based oil and grease (polar concentrations) are more difficult to regulate due to the large number of restaurants and fast-food outlets in every community. FOG Handbook. (n.d.).

Bioconversion of waste materials is considered to be of prime importance for the near future because of its favorable economics, low capital and energy cost, reduction in environmental pollution, and relative ease of operation. Producing biosurfactants (usable products) from industrial wastewaters is a viable option.

1.2 Fats

Fats form a category of lipid, distinguished from other lipids by their chemical structure. This category of molecules is important for many forms of life, serving both structural and metabolic functions. They are an important part of the diet of most heterotrophs (including humans). There are many different kinds of fat, but each kind is a variation on the same chemical structure. All fats consist of three fatty acids (chains of carbon and hydrogen atoms, with an oxygen atom at one end) bonded to glycerol (a "backbone" of carbon, hydrogen, and oxygen). These fatty acids would each be a horizontal line; the glycerol "backbone" would be the vertical line that joins the horizontal lines. Fat. (n.d.).

Different fatty acids are comprised of different numbers of carbon and hydrogen atoms. The carbon atoms, each bonded to two neighboring carbon atoms, form a zigzagging chain; the more carbon atoms there are in any fatty acid, the longer its

chain will be. Fatty acids with long chains make the fat they are a part of more massive, raising its melting point and yielding more energy per molecule when metabolized. Fat. (n.d.).

A fat's constituent fatty acids may also differ in the number of hydrogen atoms that branch off of the chain of carbon atoms. Each carbon atom is typically bonded to two hydrogen atoms. When a fatty acid has this typical arrangement, it is called "saturated", because the carbon atoms are saturated with hydrogen. They are bonded to as much hydrogen as they possibly could be. Occasionally, though, a carbon atom may instead bond to only one other hydrogen atom, and have a double bond with a neighboring carbon atom. This results in an "unsaturated" fatty acid. A fat containing only saturated fatty acids is itself called saturated. A fat containing at least one unsaturated fatty acid is called unsaturated. Fat. (n.d.).

Saturated and unsaturated fats differ in their energy content and melting point. Since an unsaturated fat contains fewer carbon-hydrogen bonds than a saturated fat with the same number of carbon atoms, unsaturated fats will yield slightly less energy during metabolism than saturated fats with the same number of carbon atoms. Fat. (n.d.).

1.3 Lipids

The importance of lipids as one of the fundamental classes of biological compounds is well established. The application of our knowledge of the biochemistry, chemistry and physiology of lipids to biotechnology, the fats and oils industry and medicine have continued to expand space. Lipid research. (n.d.).

“Lipids are hydrophobic biological compounds that are insoluble in water, but soluble in nonpolar solvents such as benzene, chloroform, and ether” (Shuler, & Kargi, 2002, p.39). Oil is a very complex mixture of indefinite number of compounds each of which has its own structure, characteristics and properties. These compounds can be classified in different classes or groups of compounds according to their chemical composition and structure (Türkman, 2005).

1.3.1 Effects of Lipid or Lipid-rich Wastewater

- They are responsible for clogging of wastewater pipes,
- A lipid overload leads to a decrease in treatment efficiency,
- the proliferation of filamentous microorganisms,
- settling problems in the clarifier,
- can form oil films on water surfaces,
- preventing the diffusion of oxygen from air into water,
- leading to the death of many forms of aquatic-life (Mongkolthanaruk & Dharmsthiti, 2002).

1.4 Vegetable oil

1.4.1 Production of Vegetable Oil

Crude oil, straight from the crushing operation, is not considered edible in the case of most oilseeds. The same is true for the remaining meal. The processing of soy oil is typical of that used with most vegetable oils. Crude soy oil is first mixed with caustic soda. Saponification turns free fatty acids into soap. The soap is removed with a centrifuge. The remaining oil is deodorized by heating under a near-perfect vacuum and sparged with water. The condensate is further processed to become vitamin E food supplement, while the oil can be sold to manufacturers and consumers at this point. Fat. (n.d.).

Some of the oil is further processed. By carefully filtering the oil at near-freezing temperatures, "winter oil" is produced. This oil is sold to manufacturers of salad dressings, so that the dressings do not turn cloudy when refrigerated. The oil may be partially hydrogenated to produce various ingredient oils. Lightly hydrogenated oils have very similar physical characteristics to regular soy oil, but are more resistant to becoming rancid. Margarine oils need to be mostly solid at 90 degrees F so that the margarine does not melt in warm rooms, yet it needs to be completely liquid at 98 degrees F, so that it doesn't leave a "lardy" taste in the mouth. Fat. (n.d.).

1.4.2 Sources of Vegetable Oils

Vegetable oil or vegoil is fat extracted from plant sources, known as oil plants. Although in principle other parts of plants may yield oil, in practice seeds form the almost exclusive source. Vegetable oils are used as cooking oils and for industrial uses. Some types, such as cottonseed oil, castor oil and some types of rapeseed oil, are not fit for human consumption without further processing. Like all fats, vegetable oils are esters of glycerin and a varying blend of fatty acids, and are insoluble in water but soluble in organic solvents.

Oils can be classified in several ways, for example:

- By source - most, but not all vegetable oils are extracted from the fruits or seeds of plants. One classification might group oils from similar plants, e.g. "Nut oils".
- By use - oils from plants are used in cooking, for fuel, for cosmetic and medical purposes, and for other industrial purposes

1.4.3 Vegetable Oil Used as Fuel

Use of vegetable oil directly as a fuel is one of the most environmentally friendly sources of power. The use of waste vegetable oils is obviously greener, but requires filtering and settling. Some waste may not be suitable.

Sunflower oils are also used for this purpose and with a seven degree Celsius (13 °F) lower freezing point can provide slightly better cold weather starting. It is worth mentioning some indirect injection diesel engines will run happily without alteration, especially in warmer climates or summer. Petrol may be added as a thinner to the fuel, as most manufacturers recommend this for winter up to ten percent. In practice many people may add slightly more, as the problems of vapour locks are less likely at low temperatures. However, excessive thinning can cause a lack of lubrication in injectors, resulting in their overheating. Fat. (n.d.)

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1.4.4 Industrial Uses of Vegetable Oil

- Vegetable oils are increasingly being used in the electrical industry as insulators as vegetable oils are non-toxic to the environment, biodegradable if spilled and have high flash and fire points. However, vegetable oils have issues with chemical stability (there has to be a tradeoff with biodegradability), so they are generally used in systems where they are not exposed to oxygen and are more expensive than crude oil distillate. Three examples are Midel 7131 by M & I materials, FR3 by Cooper Power and Biotemp by ABB. Midel 7131 is a synthetic oil, manufactured by an alcohol + acid reaction.
- Common vegetable oil has also been used experimentally as a cooling agent in PCs. Fat. (n.d.).

CHAPTER TWO
BIOSURFACTANTS

2.1 Surfactants

2.1.1 Background on Surfactants

Surfactants, which are amphipathic molecules with both hydrophilic (water-soluble) and hydrophobic (water-insoluble) functional groups, act at the surface, or interface, between polar and nonpolar phases to modify the surface properties of both phases due to presence of the hydrophobic group (Bognolo, 1999).

Surfactants, which constitute an important class of industrial chemical widely used in almost every sector of modern industry, are surface active compounds capable of reducing surface and interfacial tension between liquids, solids and gases (Desai & Banat, 1997). Most of the surface active compounds currently in use are chemically synthesized. However, increasing environmental awareness has led to serious consideration of biological surfactants as possible alternatives to existing products (Kim et al., 1999). Cationic, anionic and nonionic surfactants can be used for soil washing or flushing. The surfactants must be recovered and reused for the process to be economic. Surfactants are amphiphilic compounds (containing hydrophobic and hydrophilic portions) that reduce the free energy of the system by replacing the bulk molecules of higher energy at an interface (Benincasa, Contiero, Manresa, & Moraes, 2002).

Surfactants are used in various industries like textile, paper, polymer, plastic, cosmetics, pharmaceuticals, food and machinery manufacture. They are also one of the most frequently used chemicals in our daily lives. For instance, they play an indispensable role even in ballpoint pens, and in canned coffee. Among natural surfactants, ones of microbial origin are especially classified into “biosurfactants” (BS) (Kitamoto, Isoda, & Nakahara, 2002).

2.2 Biosurfactants

2.2.1 Background on Biosurfactants

Biosurfactant was first discovered as extracellular amphiphilic compounds in the research on hydrocarbon fermentation, which is started in the late 1960s. At the beginning when BS was discovered, they were attracted attention as “alternative surfactants” due to their high biodegradability and safety. During the last decade, unique properties of BS, like biological activities, which are not observed at all in conventional chemical surfactants, have one after another been found. Therefore, BS has been increasingly attracting attention in various fields as multifunctional materials for the new century (Kitamoto, Isoda, & Nakahara, 2002).

Biological surfactants, namely `biosurfactants', are biomolecules containing both a lipophilic and hydrophilic moiety. The lipophilic part is the hydrocarbon chain of a fatty acid or sterol ring. The polar or hydrophilic part is the carboxyl group of fatty acids or amino acids, the phosphoric group of phospholipids, hydroxyl group of saccharides, and peptides. Most of the biosurfactants are produced by bacteria, yeasts, and fungi during cultivation on various carbon sources. Biosurfactants are biodegradable and can be produced from renewable substrates (Fiechter, 1992). At present, biosurfactants are mainly used in the petrochemical industry to enhance oil recovery and for hydrocarbon remediation (Morikawa, Hirata, & Imanaka, 2000).

2.2.2 Main Classes of Biosurfactants

Biosurfactants are usually classified based on their biochemical nature and the microbial species producing them. Biosurfactants may be classified into five groups.

- Glycolipids, e.g. threalose, sophorose and rhamnose lipids and mannosylerithritol lipids. They are involved in the uptake of low polarity hydrocarbons by micro-organisms.

- Liposaccharides, e.g. the high molecular weight, water soluble extracellular emulsifiers produced by hydrocarbon degrading bacteria like *Acinetobacter calcoaceticus* (emulsans).
- Lipopeptides, e.g. ornithine lipids and the subtilysin produced by *Bacillus subtilis*, claimed to be the most effective biosurfactant reported to date.
- Phospholipids: although they are present in every micro-organism, there are very few examples of extracellular production, the most notable one being the produced biosurfactants by *Corynebacterium lepus*.
- Fatty acids and neutral lipids, e.g. ustilagic acid, the corynomycolic acids, the lipotheichoic acids (sometimes classified as glycolipids) and the hydrophobic proteins (Bognolo, 1999)

2.2.3 Uptake of Water-insoluble Substrate

The physiological function of BS in a producing microbial cell is not fully understood. However, there has been speculation about their involvement in emulsification of water-insoluble substrates. When microorganisms are cultivated on n-alkanes or vegetable oils, growth-stimulating compounds are often accumulated in the culture medium. These compounds have roles emulsifying the substrate, extending the interfacial area between the microorganism and the substrate, and facilitating mass transfer on the surface of microorganism. On the other hand, trehalose lipids (9), which are cell wall-associated BS, are certainly involved in cellular adaptation to the presence of n-alkanes. Trehalose lipids render the cell surface hydrophobic, which may then facilitate the attachment and subsequent passive transport of the substrates into the cell.

2.2.4 Storage of Carbon And Energy Sources of Biosurfactants

BS which produced by microorganisms are storaged on microorganisms cell wall to resist the osmotic pressure and to be used as an extra carbon source. Sophorolipids produced by the osomophilic yeast are considered to act as extracellular carbon

storage materials and to lead to a way of adaptation to high osmotic strength due to high sugar concentrations (Kitamoto, Isoda, & Nakahara, 2002).

2.3 Microbial Production of Biosurfactants

2.3.1 Biosynthesis of Biosurfactant

Microbial processes are mostly used for the production of glycolipid BS from the view point of economical efficiency. Most known BS is of bacterial origin, and only a few BS come from yeasts and fungi. Structures of the hydrophilic and hydrophobic groups in glycolipid BS can be arranged to some extent by substrates or the reaction conditions employed. The following four cases are considered for the microbial production of glycolipid biosurfactant (Kitamoto, Isoda, & Nakahara, 2002).

- Neither hydrophilic group nor hydrophobic group depends on the structure of the substrate.
- The structure of the hydrophilic group is fixed, while the hydrophobic group depends on the structure of the substrate.
- The structure of hydrophobic group is fixed, while the hydrophilic group depends on the structure of the substrate.
- Both hydrophilic and hydrophobic groups depend on the structure of water-soluble and water-insoluble substrates, respectively.

2.3.2 Production of Glycolipid BS by Microorganisms

Table 2.1 summarizes recent studies on microbial production of glycolipid BS. In such processes, not only growing cells but also resting or immobilized cells are also used. Except lipopeptide BS, biosyntheses of glycolipid BS are very complicated and are hardly characterized: many BS cannot easily be synthesized by chemical processes (Kitamoto, Isoda, & Nakahara, 2002).

Tablo 2.1 Production of glycolipid biosurfactants by microorganisms

Biosurfactant	Microorganism	Yield ($g \cdot l^{-1}$)	Carbon source	Reference
Mannosylerythritol lipid				
MEL-A, -B and -C	*Candida antarctica* T-34	140	*n*-Octadecane	23
		47	Soybean oil	21
MEL-SY16	*C. antarctica* KCTC 7804	41	Glycerol, oleic acid	24
MEL-A, -AB and -B	*Ustilago maydis* DSM 4500	30	Sunflower oil	25
Rhamnolipid				
RL-1, -2, -3 and -4	*Pseudomonas* sp. DSM 2874	15	*n*-Tetradecane	8
RL-1 and -2	*P. aeruginosa* DSM 7107	112	Soybean oil	8
RL-1 and -2	*P. aeruginosa* UI 29791	46	Corn oil	8
RL-1 and -2	*P. aeruginosa* IFO 3924	32	Ethanol	8
RL-A and -B	*P. aeruginosa* BOP 101	14	*n*-Paraffin	38
Trehalose lipid				
TL-1 and -2	*Rhodococcus erythropolis* DSM 43215	2	*n*-Alkane	9
Trehalose-tetraester	*R. erythropolis* DSM 43215	32	*n*-Decane	9
STL-1 and -2	*R. erythropolis* SD-74	40	*n*-Hexadecane	32
Cellobiose lipid				
CL-A, -B and -C	*Ustilago maydis* ATCC 14826	16	Coconut oil	34
Sophorose lipid				
SL mixture	*Candida bombicola* ATCC 22214	422	Whey, rapeseed oil	12
SL mixture	*C. bombicola* CBS 6009	317	Glucose, rapeseed esters	33
SL mixture (SL-1: 73%)	*C. bombicola* ATCC 22214	160	Glucose, canola oil	39
SL (lactone)	*C. apicola* IMET 43747	90	Glucose, sunflower oil	15
Oligosaccharide lipid				
GL-1, -2 and -3	*Tsukumurella* sp. DSM 44370	30	Sunflower oil	37

The most useful biosurfactants are MEL, trehalose lipids, sophorolipids and rhamnolipids; these are given in Figure 2.1 (Kitamoto, Isoda, & Nakahara, 2002).

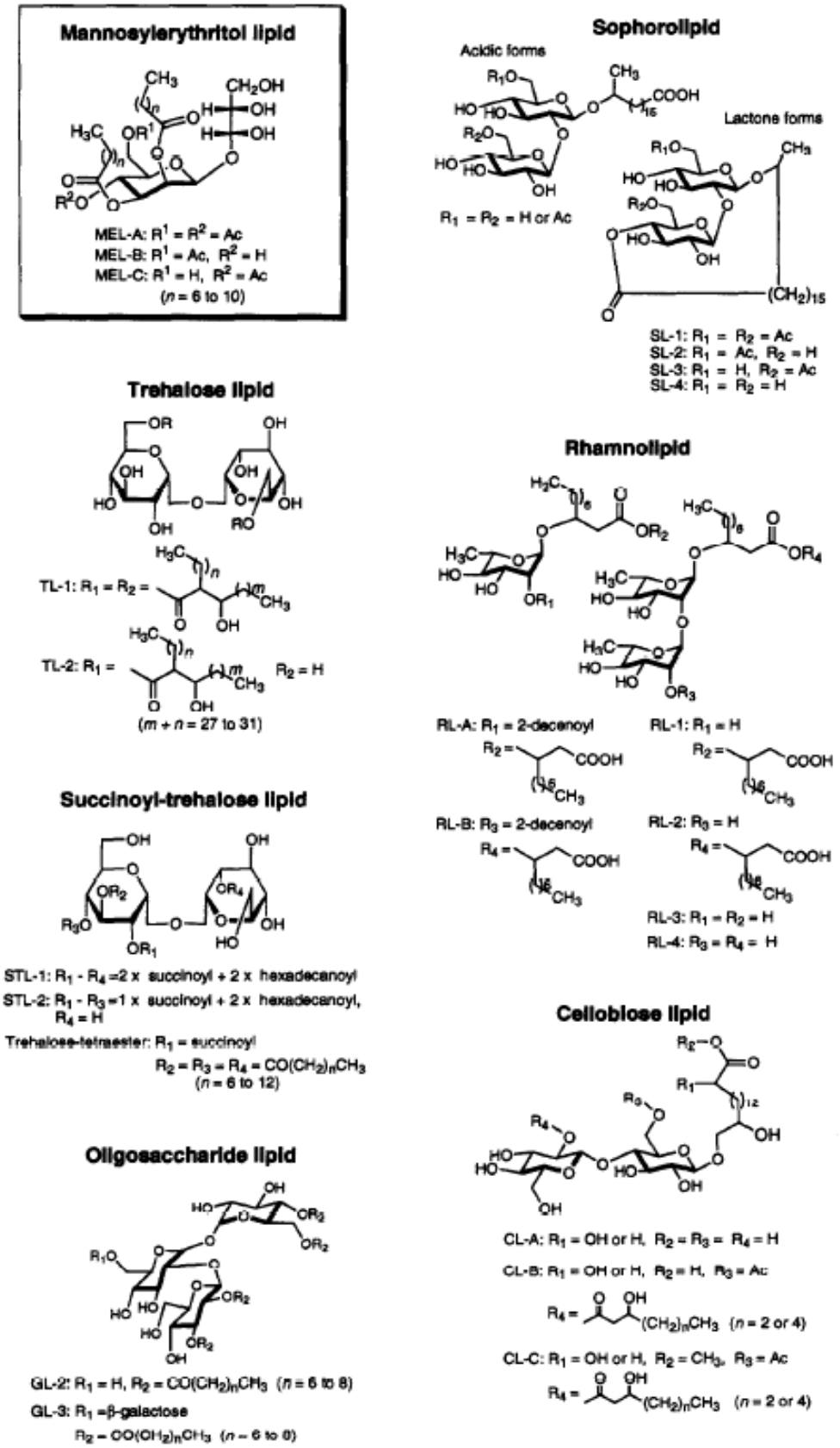

Figure 2.1 Typical structures of biosurfactants

2.4 Types of Biosurfactant

2.4.1 Mannosylerhtritol Lipids (MEL)

MEL(yeast glycolipids) are one of the most promising biosurfactants known and are abundantly produced from vegetable oils by *Pseudozyma*(previously *Candida*) *antarctica*. MEL have an excellent the superior balance between hydrophilic and hydrophobic groups. MEL can thus be applied to various kinds of drog- and gene-delivery systems, coupling with other carrier materials like phospholipids and polymers (Kitamoto, Isoda, & Nakahara, 2002).

2.4.2 Rhamnolipid

Rhamnolipids are currently produced commercially by "*Pseudomonas aeroinosa"*. Rhamnolipids have a lower CMC despite being an anionic surfactant. They show highly emulsifying, dispersing, foaming and penetrating actions. The lipid production is growth associated and enhanced under the nitrogen-limiting conditions. When rhamnolipids were discovered in the culture medium of the bacteria in 1949 as an antibiotic against *Mycobacterium tuberculosis,* the yield was only 2.5 g/l. Recently, however, the yield of rhamnolipids, has improved and reached more than 100 g/l (Kitamoto, Isoda, & Nakahara, 2002).

2.4.3 Trehalose Lipid

Trehalose lipids are chemically stable and their surface activities are independent over a wide range of temperature, pH values and salt concentrations. They were discovered as a code factor on the cell surface of Mycobacteria (Corynebacterium, Nocardia and Rhodococcus). The productivity of trehalose lipid is not larger than that of other glycolipid BS, because the lipids are mostly cell wall-associated. They are involved in cellular adaptation to the presence of n-alkanes.Trehalose lipids render the cell surface hydrophobic, which may then facilitate the attachment and subsequent passive transport of the substrates (Kitamoto, Isoda, & Nakahara, 2002).

2.4.4 Sophorose Lipid

Sophorolipids are one of the most promising biosurfactant known, due to their high productivity and ease of recovery. Several yeasts of *C. Bombicola* (formerly *Torulopsis bombicola)* and *C. Apicola* are known to produce sophorolipids in large amounts from various substrates such as carbohydrates (glucose, fructose, sucrose, lactose), vegetable oils, animal fats, and n-alkanes. Sophorolipids produced by the osomophilic yeast are considered to act as extracellular carbon storage materials and lead to a way of adaptation to high osmotic strength due to high sugar concentration. Their derivatives which have a different hydrophilic-hydrophobic balance, show a wide range of surface activities such as emulsifying, wetting, cleaning, and solubilizing (Kitamoto, Isoda, & Nakahara, 2002).

2.5 Charactheristics of Biosurfactants

- *Surface and interface activity*: Biosurfactants reduce to surface and interface tension compared to chemical surfactants very much. They are more effective and efficient than chemical surfactants.
- *Temperature tolerance*: Some biosurfactants and their surface activity are unaffected by temperatures as high as 90°C.
- *Ionic strength tolerance*: Biosurfactants are not precipitated or salted-out in up to 10% saline solutions, whilst 2–3% salt is sufficient to deactivate chemical surfactants.
- *Biodegradability*: Biosurfactants are readily degraded in water and soil.
- *Emulsion breaking*: Emulsions made with biosurfactants can be easily split by addition of enzymes (Bognolo, 1999).
- Biosurfactants are biodegradable and pose no additional pollution threat.
- Most studies indicate that they are non toxic to microorganisms.
- Biosurfactant production is less expensive, can be easily achieved exsitu at the contaminated site, and has the potential of occurring in situ (Kitamoto, Isoda, & Nakahara, 2002).

2.6 Industrial Applications of Biosurfactant

Biosurfactants also have potential applications in agriculture, petrochemical industries, cosmetics, pharmaceuticals, detergents, food processing, laundry supplies, paint industries and others. (Bognolo, 1999).

2.6.1 Oil Industry

Because of their physico-chemical properties, biosurfactants are more suitable than many synthetic surfacants for applications in the oil industry, which explains why the large majority of the biosurfactants produced (estimated to be of the order of 400–500 tons $year^{-1}$, including captive use for tertiary oil recovery or tank cleaning) are used in petroleum-related applications (Bognolo, 1999).

2.6.1.1 Tank Oil Cleaning

Sludge and heavy oil factions that settle at the bottom of oil storage tanks are highly viscous or even solid deposits that cannot be lifted by conventional pumps. Their removal usually requires solvent washing or manual cleaning, both being hazardous, time consuming and expensive processes. Further, they leave large volumes of oil contaminated solids to be disposed of. One of the most important applications was made in Kuwait. Circulation in the tank was initiated by suction at the water oil interface and reinjection through the tank bottom and continued uninterrupted for 5 days. By this time the sludge had been dispersed in small droplets and taken in the tank. At the end of the operation 90 % of the sludge originally present was recovered and leaving about 75 m^3 of easily disposable, non-hydrocarbon materials (Bognolo, 1999).

2.6.1.2 Bitumen Recovery from Tar Sand

The process operated until then involved treating the tar sand with hot water (80 ^{0}C) at high pH=8.5-10, which was both expensive and problematic environmentally. The alternative, low-temperature, low-pH processes benefited from the use of synthetic surface active agents at the rate of 0.006–0.012%, and the production of surface-active materials by hydrocarbon-degrading micro-organisms was an attractive target because of the reduced environmental impact and the potential economic advantages. Promising results were obtained with cultures from *Arthobacter*, *Pseudomonas* and *Corynebacterium* genus and *Bacillus subtilis* (Bognolo, 1999).

Other applications include stabilization of coal in water slurries by biosurfactants; wastewater treatment, e.g. for the treatment of waste water from nuclear fuel processing plants ; paints, crop protection formulations, corrosion inhibition, textile detergents and cleaning agents (Bognolo, 1999).

2.7 Economics of Biosurfactant

Many of the potential applications that have been considered for biosurfactants, as well as an expansion of the few already established depend on whether these can be produced economically in commercial quantities. Much work is still needed reduction. For process optimization at the biological and engineering level. Whilst it is acknowledged that the improvent in the production technology of biosurfactants has already enabled a 10–20 fold improvement in productivity, it is likely that a further significant improvement (albeit possibly of a smaller order of magnitude) is required to make the technology fully commercially viable. The parameters that can be affected to improve the economics of biosurfactants manufacture include (Bognolo, 1999).

Raw materials, i.e. choice of nutrients, this can take the form of:

- Maximizing biosurfactant yield for any given micro-organism strain. This requires an economic assessment of the yield increase versus the additional cost of nutrients.
- Optimize the nutrient medium, i.e. provide the right balance of C, N, P and other oligoelements.
- Take any given waste by-product and develop/optimize the micro-organism strain for its metabolization.
- Yield: choice of strain, biosynthesis control and alteration of the genetic of the producer are parameters that can significantly affect biosurfactant yields and economics.
- Bioprocess: this can be optimized through reactor design operating conditions and recycling of spent medium.
- Product isolation/recovery: most of the biosurfactants technologies originally proposed involved more or less elaborated forms of puribeen considered for biosurfactants, as well as a fication and isolation. The possibility of in-situ growth or the use of non-refined fermentation broths can undoubtedly lead to substantial cost reduction.

2.8 Literature on Physical and Biological Treatment of Lipid Rich Wastewater

Dissolved Air Flotation (DAF) is an efficient flotation method for water clarification. The term refers to the method of producing flotation by dissolving air in the water under pressure and then releasing the pressure. When the pressure is released the solution becomes supersaturated with air as millions of small bubbles form. The bubbles attach to any particles in the water causing their density to become less than that of water. The particles then rapidly float to the surface for collection and removal, leaving the clarified water behind. The most reliable and positive method of producing bubbles of the proper size is to dissolve air into water under pressure and to then reduce the pressure of the solution. As the pressure is reduced,

the air comes out of solution in the form of microbubbles. Retrieved September 13, 2007, from http://www.dissolvedairflotation.com/introductiontodaf.html.

In water treatment, the DAF is used as primary clarification and in wastewater treatment, the DAF process is applied for sludge thickening, secondary clarification and as polishing after secondary clarification. The mechanism of dissolved air flotation is to allow microbubbles of air to attach to the suspended particles in the water. The idea is to develop agglomerates with lower density than water, causing the floc to rise through the water and accumulate at the surface where they can be removed as sludge. The micro-bubbles are generated when pressurized and air-saturated recycle water is released in the contact zone. When the pressure is instantaneously reduced, the dissolved air precipitates into microscopic air-bubbles, which are mixed with the main water (Lundh, Jönsson , & Dahlquist, 1999).

The Pilot Plant consists of a tank and the equipment for the production of the dispersion water (Figure 2.2). The flow entered the flotation basin at the inlet (1) and is transported downwards to the inlet slot (2). The dispersion is added in the contact zone (3) via a bank of three needle valves mantled in the inlet slot. The water is brought upwards by the baffle (4) and entered the separation zone (5). The water left the tank through the outlet on the bottom of the tank (6). Recycled water (Qr) for the production of dispersion was taken from the outlet and supplied the recycle pump (7), which provided the pressure tank (9) with water. An enjector (8) injected air into the re-cycle water. The enjector and the pressure tank were pressurised with 5-bar compressed air (11). A flow-meter (10) and valves before and after the pressure tank controlled the recycle flow (Lundh, Jönsson , & Dahlquist, 1999).

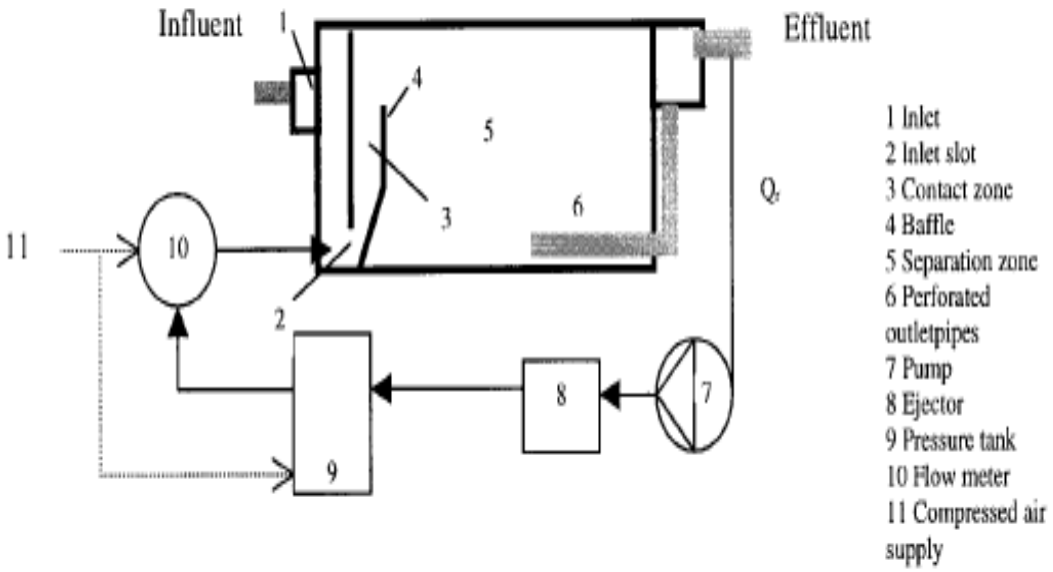

Figure 2.2 Schematic diagram of DAF

Tansel & Pascual (2004) investigated the factorial evaluation of operational variables of a DAF (dissolved air flotation) process to improve PHCs (Volatilization of petroleum hydrocarbons) removal efficiency. In this study, two types of source water were used during the experiments: brackish and pond. The brackish water at 10,000 ppm salt concentration was prepared using artificial sea salt manufactured by Aquarientechnik (Wartenberg, Germany) and distilled water. The pond water was collected from a stormwater pond located at the Florida International University campus in Miami, Florida. The pond water was selected to study the effects of naturally occurring organics in surface waters on the performance of the UF system. Pilot-scale experimental DAF system was used. A serious of batch and continuous experiments (utilizing full pressurization and effluent recirculation) were conducted using a 60-L DAF system which could be operated either in batch or continuous modes. Batch runs were conducted as a 24 factorial design to study the effects of detention time, influent PHC concentration, coagulant use, and source water type on PHC removal efficiency. The continuous flow experiments were conducted as a 25 factorial design. The operational variables studied included run time, influent PHC concentration, coagulant use, source water type as well as mode of continuous flow operation (i.e., full pressurization and effluent recirculation). The experiments were conducted as a factorial design to evaluate both the individual effects and the

interactions of the operational variables which included oil concentration, detention time, water type (brackish and pond), coagulant use, and operational mode. At the end of study the factorial analysis showed that for the batch mode of operation, oil concentration, detention time, coagulant use, and water type had a significant effect on PHC removal. However, for the continuous DAF runs, the only variable that was significant at the 95% confidence level was detention time. Coagulant use did not have a significant effect on PHC removal efficiency for the continuous runs due to shearing of the flocs. The average PHC removal efficiency for the batch runs was 76.69+2.02%; for the continuous runs with full pressurization it was 86.00+2.38%; for continuous runs with effluent recycle it was 81.83±3.42%.

Wastewater treatment of a vegetable oil factory by a hybrid ultrafiltration-activated carbon process is studied by Mohammadi & Esmaeelifar (2005). In the experiments, a UF membrane (UFPHT20-6338) was used. Also, treatment of the wastewater by UF-powdered activated carbon (PAC) was studied. They focus on treatment of wastewater of a vegetable oil factory by UF and UF-PAC with emphasis on process fundamentals and operating conditions. During the experiments, samples of Behshahr Ind. Co. Wastewater as feed were used. Contaminants of the feed can be categorized into two parts: (1) Organic components such as vegetable fats and oils; fatty acids; glycerine; soap; colored components; gums and detergents. (2) Mineral components such as sodium polyphosphate; sodium silicate and sulphonate; calcium, magnesium and sodium carbonates and chlorides. The feed was collected daily and used immediately. Its pH was 10.5. They have been studied effect of operating conditions such as pressure difference, cross flow velocity, temperature, concentration of organic compounds and pH on permeation flux, flux decline and fouling resistance. UF-powdered activated carbon (PAC) and membrane bioreactor showed great promise in water and wastewater treatment. In UF-PAC experiments, PAC was added to feed tank at different concentrations. According to the results, effect of pressure, temperature, concentration, velocity, and pH have significantly been seen. A pressure difference more than 3×10^5 Pa (3 bar), a high cross flow velocity (depending on economic considerations), a temperature of 30 ◦C and a pH of 9 are the best operating conditions. Analysis of the wastewater treated by UF

represents 91, 87, 100, 85 and 40% reduction in COD, TOC, TSS, [PO_4^{3-}] and [Cl^-], respectively. Analysis of the wastewater treated by UF-PAC represents 94, 93, 100, 99 and 43% reduction in COD, TOC, TSS, [PO_4^{-3}] and [Cl^-], respectively. A comparison between the results shows that UF is better than conventional biological method and UF-PAC is better than UF.

Mongkolthanaruk & Dharmsthiti (2002) studied that biodegradation of lipid-rich wastewater by a mixed bacterial consortium. A mixed bacterial culture comprising *Pseudomonas aeruginosa* (LP_{602}), *Bacillus sp.* (B_{304}), *and Acinetobacter calcoaceticus* (LP_{009}) for use in tretment of lipid-rich wastewater was formulated. In the study, aerobic-batch reactor system was used during the experiments, they used domestic wastewaters (lipid-rich wastewater). Average lipid content of wastewater is 21 g/lt. LP_{602} and B_{304} were cultivated at 30 ^{0}C and LP_{009} at 15 ^{0}C. Incubation was carried out with shaking at 200 rpm for 48 h. External nitrogen, phoshorus, and mineral were added. Bacterial growth was determined by measuring the optical density at 600 nm. At the end of the study when *P.aeruginosa* was added to the wastewater, the BOD was reduced by 73% within 15 days of incubation. At the same form removal of lipid without BSS was reduced by 73% within 15 days of incubation. However, 95% lipid and BOD removal with nutrient supplement within 10 days. Removal of lipid and BOD provides with addition $CaCl_2$ and BSS within 5 days. When *P.aeruginosa* and *Bacillius sp.* were added to the wastewater, did enhance the wastewater treatment ability of *P.aeruginosa*. It was found no negative effect in waste treatment by the mixed culture and BOD and Lipid content of the effluent was higher than 20 mg/L which are the discharge limit. When a mixed culture of *P.aeruginosa, Bacillius sp.* and *Acinetobacter calcoaceticus* were added to the wastewater, Use of the combined culture of LP_{602} and B_{304} could not reduce the BOD and the lipid content in the wastewater to an acceptable level for environmental release. Thus, addition of a third strain of lipase-producing bacteria, *Acinetobacter calcoaceticus* LP_{009} (Pratuangdejkul and Dharmsthiti, 2000) was tried to facilitate the bioremediation process. Results showed that this consortium of 3 bacterial cultures could be used for lipid-rich wastewater treatment. The BOD and the lipid content were reduced from ~3600 mg/ml and 21,000 mg/ml, respectively, at day 0 to <20

mg/lt by day 12. Such levels of BOD and lipid content were acceptable for wastewater discharge into the environment. In addition to, When 1% Brij58 and $CaCl_2$ were added to the wastewater, no significant effect of biosurfactant on lipid removal. The main mechanism was the removal of BOD and lipid by the mixed bacterial culture and the highest lipase activity was observed in *P.aeruginosa*.

El-Masry, El-Bestawy & El-Adl (2004) studied on Bioremediation of vegetable oil and grease from polluted wastewater. In this study, a bench scale sand-biofilm system was used in order to investigate its capacity decontaminate vegetable oil and fat-containing wastewater. They have been used combination of *Pseudomonas sp.* (L1) and *P.diminuta* (L2) microorganisms for biofilm formation on sand particles. These microorganisms were investigated for oil and grease degradation either individually or in combinations. Since the combination (*Pseudomonas sp.* and *P.diminuta)* produced the highest degradative activity, it was used in this study in a biofilm sand filter system for vegetable oil and grease removal. This system was tested either as one unit or two units in sequence where different flow rates (30, 50, 100 ml/h) were applied compared to a control unit. According to the results, both biofilm systems reduced oily wastewater, even in cases of high degree of pollution (fat, oil and grease (FOG), 7535 ppm; biochemical oxygen demand (BOD_5), 525 ppm; chemical oxygen demand (COD), 1660 ppm). Results also showed a removal of FOG with efficiency at 100 %; BOD_5 at 95,9 % and COD at 96 %, at 50 ml/h flow rate using one unit of biofilm system. On using two units in sequence, a complete removal of FOG, BOD_5 and COD with efficiency 100 %, at flow rate 100 ml/h was achieved.

Nakano & Matsumura (2002) investigated the improvement of treatment efficiency of thermophilic oxic process for highly concentrated lipid wastes by nutrient supplementation. In the study, tests were carried out under the thermophilic oxic conditions using 15 g of three kinds of actual HCLW (High Concentration Lipid Waste), namely, HCLW 1 (Cooking Oil and lipid content: 98.3% w/v), HCLW2 (Trapped waste from food oil company and lipid content 92.4% w/v) and HCLW3 (meat factory and lipid content 55.3% w/v). The degradation efficiency after 120-h

treatment was compared. This process was batch aerobic solid state fermentation and temperature was 60 ^{0}C. In the absence of any supplements, minimal degradation after treatment for 120 h was observed for all kinds of HCLW although HCLW1 showed somewhat high degradation efficiency. About 50 to 60 % degradation efficiency could be attained for all kinds of HCLW. The addition of some other components (such as U (urea), TE (trace elements) and PO_4 was carried out to examine the achievable treatment efficiency of the process. Addition of YE (yeast extract) improved the degradation efficiency of HCLW1 (70.1%). When the PO_4 content in TE was increased, a negative effect on degradation was shown for HCLW2. However different results were obtained for HCLW1 and HCLW3. The addition of PO_4 gave a significant degradation efficiency for HCLW3. The best degradation efficiency of 81.9% was obtained for HCLW3 when PO_4 was added into nutrient supplement. Only minimal (8.6%) degradation efficiency of oil was observed when no supplements (only 60 ml of water) were added and 91.4% of oil still remained even after 120 h of treatment. However 71.7% degradation efficiency when YE, U, TE and PO_4 were provided.

At the similar study (Nakano & Matsumura, 2002), tests were carried out under the same thermophilic oxic conditions using 15 g of fresh salad oil and lard. In this study, therefore, commercial dried baker's yeast (DBY) and SW (sludge waste) were examined as substitutes. Sewage sludge was used as nutrient supplement. To evaluate the stimulative effect of the substitutes on TOP, the degradation efficiency based on TRL (total residual lipid) obtained after a 120-h treatment was compared. As a result of minimal degradation of lipids was observed when no supplements (only 60 ml of water) were added with more than 90% of lipids remaining even after 120-h treatment. Added YE 1.5 gr + U 1.0 gr + TE, a degradation efficiency of 68.3% and 76.9% was attained for salad oil and lard, respectively. On the other hand, with 1.5 g of commercial DBY, a similar degradation efficiency for salad oil (67.6%) was obtained when added in combination with 1 g of urea and TE. Thus, dried yeast was confirmed to have a similar stimulative effect as YE. A further improvement in the degradation efficiency was obtained by using another substitute, SW. With a combination of 1.5 g of SW, a degradation efficiency of 82.9% after 120-h treatment

was attained for salad oil. In conclusion, it was confirmed that the degradation efficiency for fresh lipid materials obtained with a readily free and abundant material like SW was high compared to that obtained with the expensive nutrient material, YE. Experiments showed that there could be obtained high oil removal efficienciency by the addition of yeast extract or sewage sludge to the thermophilic oxic process. Sewage sludge had been applied alternatively, because of the high costs of yeast extract. In the result of experiments it has been understood that using of sewage sludge for oil removal provides higher dispersion efficiency than yeast extract. Besides that because of inorganic nitrogen and high mineral composite of SW, there is no need to add marginal substrate.

In a similar study, the potentiality of free Gram-negative bacteria for removing oil and grease from contaminated industrial effluents was investigated by El-Masry, El-Bestawy & El-Adl (2004). In this study, *Pseudomonas sp.* (L1) and *P.diminuta* (L2), *P. pseudoalcaligenes* (L3), and *Escherichia sp.* (L4) were investigated under different pH levels (6,5-7,0-7,5 and 8,0), different temperatures (30 and 37 ^{0}C) and different concentrations (1-1,5 and 2 %). Bacterial cultures were incubated under aerobic conditions at 30 ^{0}C and agitated at 150 rev/min for 13 days. After incubation for 48 h under the optimum pH and incubation temperature, the % of free fatty acids (FFA %) was determined, as indication of palm oil degradation by the tested bacterial isolates. The degradation after 13 days was 98,2 %, 99 %, and 86,3 % for *P.diminuta* and *Pseudomonas sp.,* the combination of *P.diminuta, P. pseudoalcaligenes and Pseudomonas sp. ,* finally the combination of *P.diminuta, Escherichia sp.*, *Pseudomonas sp. and P. pseudoalcaligenes,* respectively compared to the shorter incubation period (6-days) recording 66,8-76,8-49,8 respectively. Results revealed differences in their optimum conditions for maximum degradation of vegetable oil. This bacterial species were tested individually or in combinations using synthetic aqueous medium supplemented with 1 % palm oil, incubated at 30 ^{0}C, and agitated at 150 rev/min for 13 days. All the tested bacteria were able to degrade the palm oil completely and utilized the free fatty acids (FFA) as a carbon source. The combination *Pseudomonas sp.* and *P.diminuta* produced the highest degradative activity, followed by *Pseudomonas sp., P.diminuta* and *P.*

pseudoalcaligenes. Also *Pseudomonas sp.* and *P.diminuta* produced the highest activity in reducing COD (93 %) and BOD_5 (100 %).

2.9 Objective and Scope

In the first part of the thesis, lipid and COD removal was studied with using of lipids break into pieces bacterium culture. Effect of significant operating parameters on system performance is determined. After then lipid removal is supported with addition of biosurfactant.

Based on this approach, major objectives of this thesis can be summarized as follows:

- to investigate effect of glucose concentration, biomass concentration, and lipid concentration on COD and lipid removal
- to enhance COD and lipid removal with addition co-substrate as feed source.
- to enhance COD and lipid removal without carbon source.
- to determine effect of sludge age on COD and lipid removal .
- to support lipid removal with addition of biosurfactant.
- to determine effect of biosurfactant on COD and lipid removal (with/without carbon source).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Organisms

Aerobic sludge culture was obtained from the wastewater treatment plant of PAKMAYA Bakers Yeast Company (Izmir, TURKEY). The activated sludge culture was cultivated in batch aeration tank for 15 days. Then the culture was acclimated to vegetable oil by batch aeration for 15 days. The stock cultures were preserved in the freezer in frozen form.

3.2 Media Composition

Wastewater used throughout the studies was composed of glucose as carbon source, urea as nitrogen source, KH_2PO_4 as phosphorus source, $MgSO_4.7H_2O$ (50 mg/L) and various concentrations of vegetable oil (1-6%). The concentrations of nitrogen, phosphorus and glucose were adjusted to maintain COD/N/P ratio in the feed as 100/5/1 in the experiments.

3.3 Biosurfactants

The rhamnolipid (designated JBR 210) was kindly donated by Jeneil Biosurfactant Company, Saukville, WI, USA as a mixture of R1 and R2. R1 has the chemical formula $C_{26}H_{48}O_9$, and R2, $C_{32}H_{58}O_{13}$. This product was named as JBR 425, which is an aqueous solution of rhamnolipids at 25% concentration. Critical micelle concentration (CMC) of JBR 425 is 15 mg/l. Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and β-hydroxydecanoic acid.

3.4 Experimental Procedure

The experiments contain two parts as batch flask and fed batch experiments. Batch flask experiments conducted by using Box-Wilson Statistical Experimental Desing Method. Fed batch were designed based on investigation of important experimental parameters on COD and oil removal.

3.4.1 Box-Wilson Experiment Design

The Box-Wilson design is a response surface methodology, which is an empirical modeling technique, devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results. Basically this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model (Çatalkaya&Şengül, 2005).

The Box-Wilson statistical experimental design was employed to determine the effects of operating variables on COD (chemical oxygen demand) and V.Oil removal efficiency and to find the combination of variables resulting in maximum COD and V.Oil removal efficiency. The independent parameters were biomass, vegetable oil and glucose concentration. The dependent variables were COD and vegetable oil removal efficiencies. The axial and factorial experimental points were given in Table 4.1. The center points were repeated 3 times.

Experiments were carried out on an incubator shaker in 250 mL flasks with 200 mL reaction volume. The biomass concentration varied between X= 1200 and 2400 mg/L, glucose concentration was between G=400 and 2000 mg/L and vegetable oil concentration was between 2%-8%. The flasks were incubated in the shaker for 12 days at T=25±1 ^{0}C and rotational speed of 100 rpm. At the end of 12 days, samples were removed and centrifuged at 5000 rpm for 30 minutes for analysis COD, biomass and oil.

3.4.2 Experiments with Fed-Batch Operation

3.4.2.1. Experimental Setup

Fed-batch operation of an aeration tank involves addition of nutrient media into aeration tank from feeding tank until the tank is full. Aeration tank contains highly active and dense organisms at the beginning of operation. Feed substance is added intermittently into the aeration tank without effluent removal in fed-batch operation. Certain amount of sludge was removed from the reactor everyday to adjust the sludge age. As the feed substance is added slowly, the liquid volume in the reactor increases with time linearly according to the following equation since no effluent is removed.

$$V=V_0+ Qt$$

where V is the total volume (L), and V_0 is the initial volume (L), and Q is the flow rate (m^3/h), and t is the time (hours).

Schematic diagram of the experimental setup is depicted Figure 3.1. The system consists of a fed-batch aeration tank, pipes, air pumps, wastewater, diffusers, wastewater feeding pump (dosage pump), hot plate and feeding tank. The total liquid volume in the aeration tank and feeding tank was 5000 ml. Feeding tank was placed on hot plate and mixed to supply a homogeneous feed and to keep its temperature between 20 ^{0}C-25 ^{0}C.

The initial liquid volume in the tank was 1 liter containing dens activated sludge culture. V.Oil and nutrient containing synthetic wastewater was fed to the reactor continuously without withdrawing liquid form the system. The liquid phase was continuously aerated and mixed with a mixer. The synthetic wastewater feeding rate was 0.5 L/h. The total feeding and aeration period was 8 hours. The aeration was stopped when 5 liters of reactor volume was occupied with the synthetic wastewater. As the reaction was completed the aeration was stopped to let the sludge settle. After 1 hours of sedimentation, about 4 L of supernatant was withdrawn from the system.

Sludge age (θc) was adjusted to 15-30 days depending on the experimental conditions. The sludge age was adjusted by discarding certain volume of activated sludge from aeration tank every day. The effect of glucose concentration, vegatable oil concentration, sludge age and biosurfactant addition on COD and V.Oil removal were investigated. COD, biomass and fat/grease analysis were carried out on the samples. Temperature, pH and dissolved oxygen (DO) of the medium during operation were T=25±5 ^{0}C, pH=7.5±0.5 and DO=2.5±0.5 mg/L, respectively

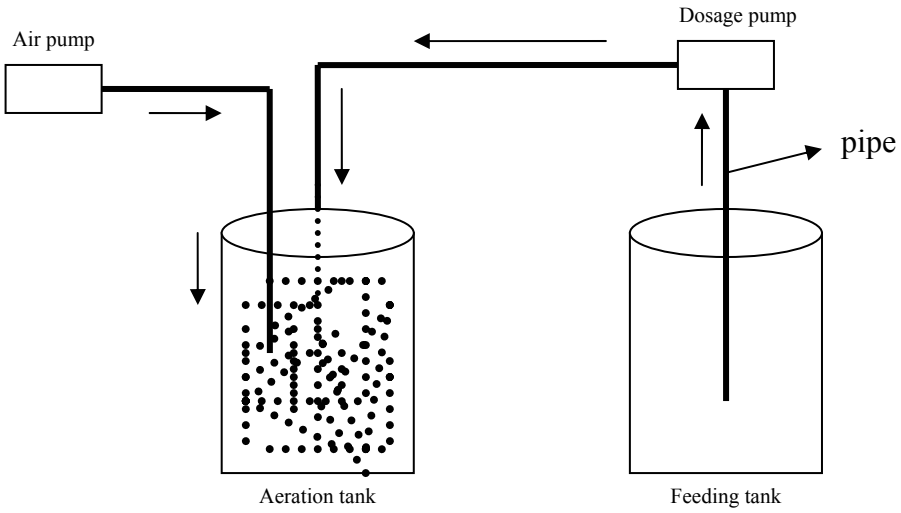

Figure 3.1 Schematic diagram of experimental setup

3.5 Analytical Methods

3.5.1 Sampling

Daily samples withdrawn from reactor were centrifuged 5000 rpm for 30 minutes until clear supernatants was obtained. COD, biomass and lipid concentrations were measured on the samples.

3.5.2 Chemical Oxygen Demand (COD) Analysis

COD measurements were carried out by Closed Reflux Colorimetric methods according to Standard methods (APHA, 1989). In closed reflux colorimetric method, borisilicate culture tubes with 10 ml capacity were used. Digestion solution was prepared by adding 10.216 g $K_2Cr_2O_7$, 167 ml conc. H_2SO_4 and 33.3 g $HgSO_4$ into distilled water to be 1000 ml and the solution was cooled to room temperature. Sulfuric acid reagent was prepared as in open reflux method. Potassium hydrogen phthalate (KHP) standard was used to obtain COD concentration-absorbance calibration curve. KHP was lightly crushed and then dried to constant weight at 120 ^{0}C. Then different initial KHP concentrations were dissolved in distilled water to obtain different COD concentrations. KHP solution had a theoretical COD of 900 mg/L for 0.765 g KHP/L. At least five or more standards of KHP were prepared to obtain COD concentrations of between 50 to 900 mg COD/L. Novaspec II, (Pharmacia Biotech) visible spectrophotometer was used to measure the absorbance of the color developed at 600 nm after 2 hours of reaction at 148 ^{0}C was completed. COD content of the samples were determined by using absorbance vs. concentration calibration curve. The samples were diluted prior to measurements to reduce the concentration between 50- 900 mg/L if necessary.

3.5.3 Biomass Measurement

Biomass concentrations were determinated by filtering the samples through milipore filters (0.45 μm) and drying until constant weight in an oven at 103 ^{0}C. The calculations were made by using the following equation (Greenberg A.E, 1989, pp.5, 9-10).

$$M = (A - B) \times 1000/V$$

Where, (M) is the biomass concentration (mg/L), (A) is the weight of filter and residue after drying; (B) is the weight of filter after drying and (V) is the volume (ml) of sample.

3.5.4 Lipid Determination Method

The oil content in the liquid phases of the system was determined by using Soxhlet Extraction Method. This method consists of a hot plate, COD bottle (500 ml), an extraction Chamber and a condenser. The pH of the sample was adjusted to pH 2 or lower with 1:1 HCI and the samples were filtered in the glass funnel. The filtrate was placed into the extraction chamber. This extraction chamber was connected to COD bottle containing 400 ml n-hexane and a condenser. When COD bottle is heated by a hot plate the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. When the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling COD bottle. Extraction period was about four hours. At the end of the extraction, COD bottle containing the solvent and lipid was removed. And then the solvent in the COD bottle was evaporated on hot plate and solvent in the COD bottle was dried in an oven at 103 ^{0}C for 48 hours until solvent is completely evaporated. The amount of the remaining lipid was determinated by using the following equation.

$$\text{Lipid (g/L)} = (M1-M2)/V*1000$$

Where, (M1) is the weight of COD bottle and residue amount after drying; (M2) is the weight of COD bottle and (V) is the volume (ml) of sample (Cyberlipid Center, n.d.)

3.5.5 Dissolved Oxygen and pH Measurements

Dissolved oxygen (DO) measurement was evaluated with Oxi 330/SET Best-Nr. 200232.

The analyzer was calibrated properly before use. pH values were measured by using 890 MD pH meter. Calibration of pH meter was carried out with standard solutions.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Box-Wilson Experimental Design

The Box-Wilson experimental design was used in developing a statistical model for COD and Vegetable Oil removal. The significant variables like glucose, vegetable oil and, biomass concentration were chosen as the independent variables and designated as X1, X2,X3, respectively. Glucose concentration (X1) was ranged from 421 to 2000 mg/L, V.Oil concentration (X2) was ranged from 1% to 4% (w/v), and biomass concentration (X3) was ranged from 1198 to 3594 mg/L.

The experimental conditions determined by the Box-Wilson statistical design method are presented in Table 4.1. Computation was carried out using multiple regression analysis that uses the least squares method. The following response function was utilized in the correlating of the COD and V.Oil removal efficiency (Y) with independent parameters. A StatEast computer program was employed for the determination of the coefficients of Eq. (1) by regression analysis of the experimental data:

$$Y=b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

Where Y is the predict yield, b_0 is the constant, b_1, b_2, and b_3 are the linear coefficients, b_{12}, b_{13}, and b_{23} are the coefficients of interactions between factors, and b_{11}, b_{22}, and b_{33} are the quadratic coefficients.

Tablo 4.1 Experimental conditions according to a Box-Wilson statistical design for COD and V.Oil removal

Experimental No	Glucose Conc. (mg/L)	V.Oil Conc. (%)	Biomass Conc. (mg/L)
1	1578	2.5	2396
2	421	2.5	2396
3	1000	1.6	2396
4	1000	3.4	2396
5	1000	2.5	3091
6	1000	2.5	1701
7	2000	4	3594
8	0	4	3594
9	2000	1	3594
10	2000	4	1198
11	0	1	3594
12	2000	1	1198
13	0	4	1198
14	0	1	1198
15	1000	2.5	2396
16	1000	2.5	2396
17	1000	2.5	2396

COD and V.Oil removal efficiens obtained from the experiments are summarized in Table 4.2. The observed COD and V.Oil removal efficiencies were compared with the predicted values. The observed COD and V.Oil removal efficiencies varied between 35% and 95%, 45% and 96% for COD and V.Oil, respectively. Experimental results were evaluated according to Eq.(1).

Tablo 4.2 Observed and predicted values for COD and V.Oil removal efficiency

	Observed COD and V. Oil Removal Efficiency (%)		Predicted COD and V. Oil Removal Efficiency (%)	
Exp. No	**COD**	**Vegetable Oil Removal**	**COD**	**Vegetable Oil Removal**
1	92	74	96	73
2	86	69	81	70
3	87	69	83	74
4	74	68	77	62
5	50	77	48	76
6	35	65	36	66
7	95	80	92	81
8	62	77	64	78
9	76	86	78	86
10	81	61	82	62
11	91	96	90	95
12	80	89	78	87
13	23	45	21	45
14	52	83	56	82
15	67	72	70	69
16	72	67	70	69
17	70	69	70	69

The calculated coefficients given in Table 4.3 were used for the calculating of predicted values of COD and V.Oil removal efficiencies.

Tablo 4.3 Coefficient of the response function

	Values	
Coefficients	**COD**	**V. Oil**
b_0	-1.48152	0.99655
b_1	-0.00096	-7.5×10^{-5}
b_2	-0.77859	-0.08966
b_3	0.00293	-0.00013
b_{11}	5.45×10^{-7}	6×10^{-8}
b_{22}	0.12929	-0.01351
b_{33}	-5.8×10^{-7}	3.24×10^{-8}
b_{12}	$6{,}49\times10^{-5}$	1.98×10^{-5}
b_{13}	-7×10^{-8}	-3.1×10^{-8}
b_{23}	1.34×10^{-5}	2.82×10^{-5}
R^2	0.99	0.98

The correlation coefficients (R^2) between the observed and predicted values were 0.99 and 0.98 for COD and V.Oil, respectively.

Table 4.4 (ANOVA) Analysis of variance table for response COD

Sources	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob>F**
Model	0.41	9	0.046	31.14	0.0002
* A-glucose	0.098	1	0.098	66.73	0.0002
* B-v.oil	0.024	1	0.024	16.35	0.0068
* C-biomass	0.080	1	0.080	54.42	0.0003
* AB	0.059	1	0.059	40.10	0.0007
* AC	0.046	1	0.046	31.57	0.0014
BC	7.833E-003	1	7.833E-003	5.34	0.0602
* A^2	0.096	1	0.096	65.45	0.0002
* B^2	0.027	1	0.027	18.09	0.0054
* C^2	0.24	1	0.24	162.02	<0.0001
Residual	8.800E-003	6	1.467E-003		
Lack of Fit	7.533E-003	4	1.833E-003	2.97	0.2672
Pure Error	1.267E-003	2	6.333E-004		
Cor Total	0.42	15			

(* =significant)

(ANOVA) Analysis of variance table for response COD are summarized in Table 4.4. Values of "Prob>F" less than 0.0500 indicate model terms are significant. In the case according to ANOVA table, A (Glucose), B (V.Oil), C (Biomass), AB, AC, A^2, B^2, C^2 parameters are significant parameters.

Table 4.5 (ANOVA) Analysis of variance table for response vegetable oil

Sources	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob>F**
Model	0.13	9	0.014	8.87	0.0076
A-glucose	2.073E-003	1	2.073E-003	1.29	0.3000
* B-v.oil	0.055	1	0.055	34.28	0.0011
* C-biomass	0.032	1	0.032	19.59	0.0044
AB	4.083E-003	1	4.083E-003	2.53	0.1625
AC	6.393E-003	1	6.393E-003	3.97	0.0935
* BC	0.012	1	0.012	7.24	0.0360
A^2	1.184E-003	1	1.184E-003	0.73	0.4243
B^2	3.094E-004	1	3.094E-004	0.19	0.6766
C^2	7.136E-004	1	7.136E-004	0.44	0.5305
Residual	9.668E-003	6	1.611E-003		
Lack of Fit	8.401E-003	4	2.100E-003	3.32	0.2449
Pure Error	1.267E-003	2	6.333E-004		
Cor Total	0.14	15			

(* =significant)

(ANOVA) Analysis of variance table for response vegetable oil are summarized in Table 4.4. Values of "Prob>F" less than 0.0500 indicate model terms are significant. In the case according to ANOVA table, B (V.Oil), C (Biomass), BC parameters are significant parameters.

4.1.1 Evaluation of COD Removal

4.1.1.1 The Effect of Glucose and V.Oil Concentration at Constant Biomass Concentration

Variation of percent COD removal with glucose concentration at different V.Oil concentration is depicted Figure 4.1. Biomass concentration was kept constant at 1500 mg/L. As seen from the figure, percent COD removal decreased with increasing vegetable oil concentration. It could be because of the inhibition effect of

high vegetable oil concentration in the synthetic wastewater. Similarly, the efficiency decreased with increasing glucose concentration up to 1000 mg/L. However, it showed an increasing trend for all v. oil concentrations and higher glucose concentrations. COD removal efficiency increased from about 50% to 95% when V.Oil concentration decreased from 3% to 1% at 2000 mg/L glucose concentration This result can be explained as the low glucose concentrations do not sustain the growth of the microorganisms and therefore the organisms obtain their energy partially form V. Oil as a result low COD removal efficiencies were obtained. But at high glucose concentrations, availability of easily biodegradable substances enhanced the growth of microorganisms and provided better substrate consumption and hence COD removal. Microorganisms consumed carbon and so removed the COD in wastewater because of decreasing in vegetable oil concentration. Increasing glucose concentration and decreasing V.Oil concentration resulted in increases in percent COD removals at a constant biomass concentration because of high glucose concentration and low V.Oil concentration. As a result, the maximum COD removal efficiency of over 90% was observed at 1% V.Oil, 2000 mg/L glucose and 1500 mg/L biomass concentration.

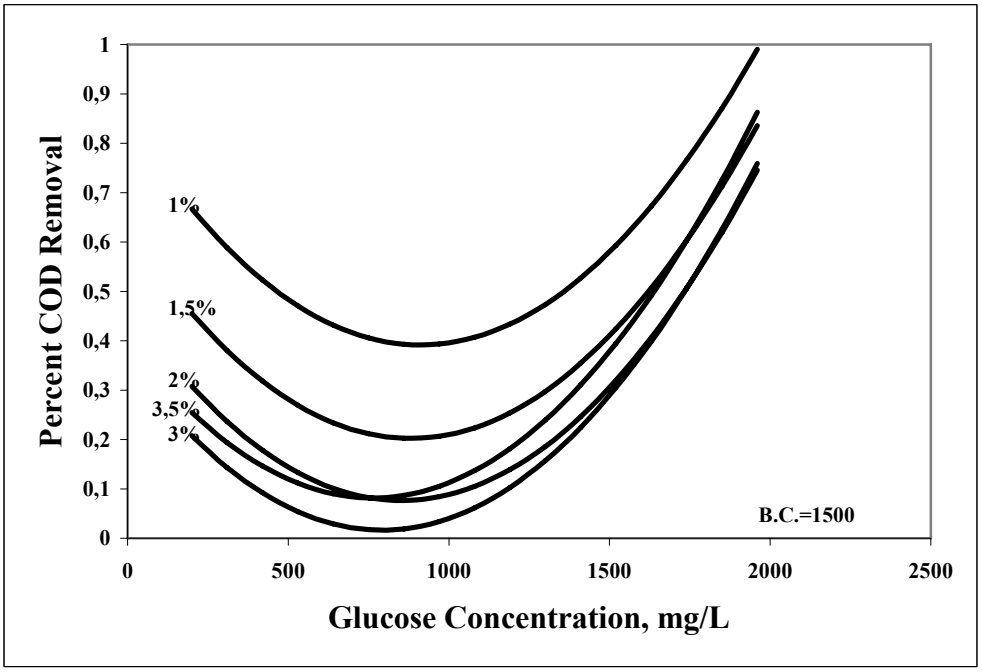

Figure 4.1 Variation of percent COD removal with glucose and V.Oil conc. at B.C= 1500 mg/L, B.C. (Biomass concentration)

Variation of percent COD removal with vegetable oil concentration (X1) at different glucose concentration (X2) is shown in Figure 4.2. Biomass concentration was kept constant at 1500 mg/L. As seen from the figure, when vegetable oil concentration increased from 1% to around 2.5%, COD removal efficiency decreased from 90% to 80% for 1800 mg/L glucose concentration. However, the efficiency increased to 90 % again when v. oil concentration increased to 4%. Microorganisms could not consume the high concentration of v. oil when there are not enough carbon sources. As glucose concentration was increased, microorganisms had enough food to be active and so removed both glucose and v. oil and hence better COD removal was obtained. The most important result was observed when there was no glucose in the synthetic media. At 1% v. oil concentration and without external carbon source, the COD removal efficiency reached to maximum level of 95%. But as the v. oil concentration increased, the efficiency decreased to 60%. Since the V.Oil was the only COD source in the synthetic media, COD removal can be attributed to V.Oil removal. Decreased COD removal is an indication of decreasing V.Oil removal. So this result indicates that external carbon source addition enhances the biological V.Oil degradation.

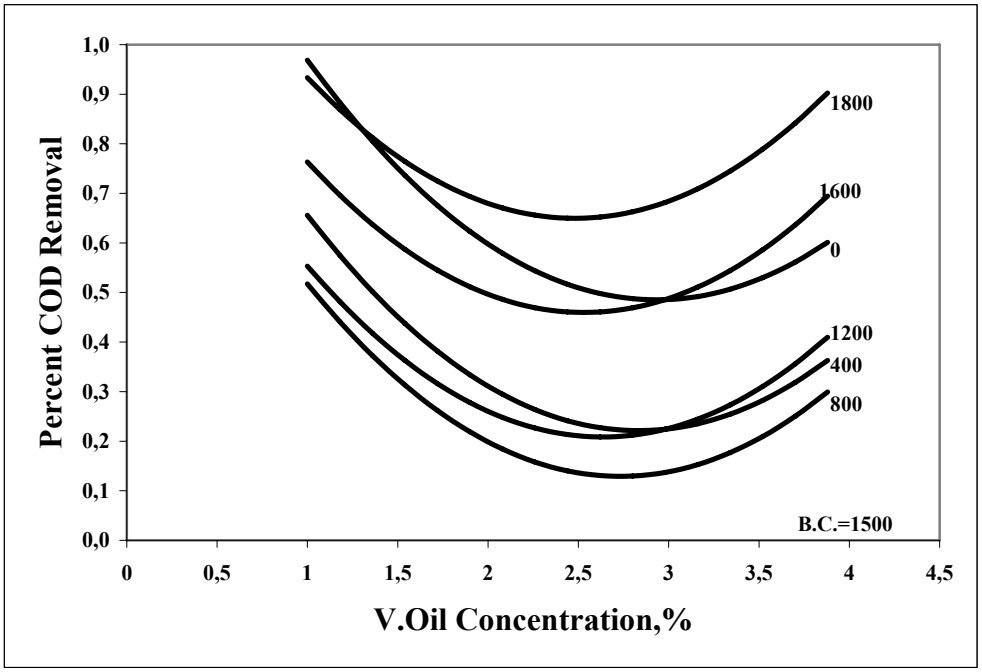

Figure 4.2 Variation of percent COD removal with V.Oil conc. and glucose concentration at B.C. = 1500 mg/L, B.C. (Biomass concentration)

4.1.1.2 The Effect of Biomass and V.Oil Concentration at Constant Glucose Concentration

Figure 4.3 depicts the variation of percent COD removal with V.Oil concentration at different biomass concentration at constant glucose concentration of 1600 mg/L. Maximum removal efficiency was obtained as 99% in 1% vegetable oil at 3200 mg/L biomass concentration. However, increasing v. oil concentration to around 2.5% resulted in decreasing COD removal efficiency to around 75%. The increasing biomass concentration significantly affected the COD removal. The efficiency increased from 30% to over 95% at 1 % v. oil concentration when biomass concentration was raised from 1100 mg/L to 1650 mg/L. Further increase in the biomass concentration did not result in significant improvement in the COD removal. So these results indicated that, biomass concentration is a significant factor in treatment wastewater of V.Oil containing. Better COD removal can be observed at high biomass concentrations. But the maximum required biomass concentration is around 1600 mg/L.

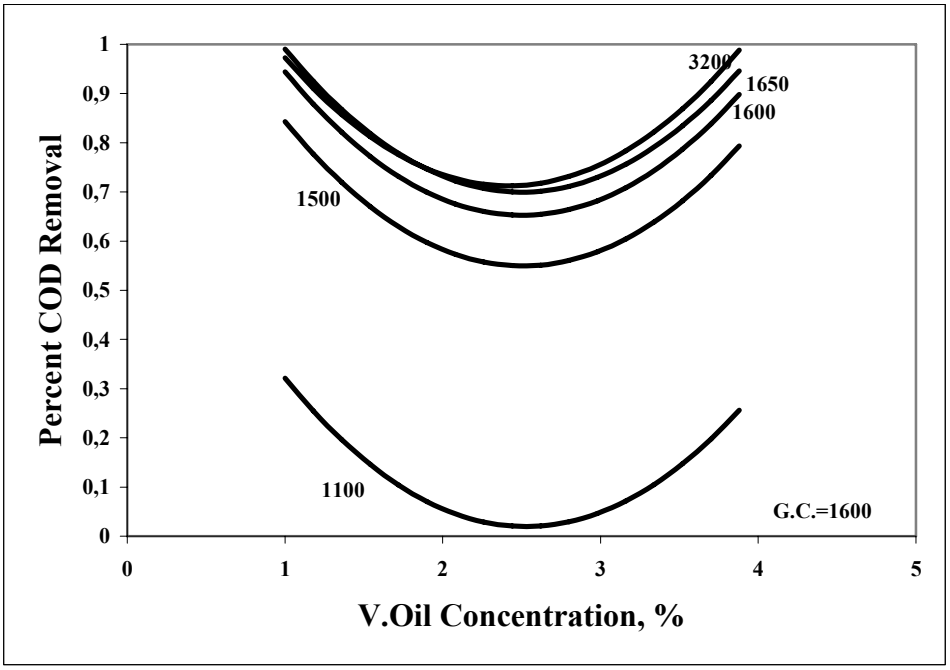

Figure 4.3 Variation of percent COD removal with V.Oil and biomass concentration at G.C. =1600 mg/L, G.C. (Glucose concentration)

Variation of percent COD removal with biomass concentration at a constant glucose concentration of 1600 mg/L at different vegetable oil concentration is shown Figure 4.4. Apparently, percent COD removal increased with increasing biomass concentration. That is percent COD removal increased from 40% to 99% when biomass concentration increased from 1500 mg/L to 2500 mg/L at a vegetable oil concentration of 2.9%. In other words, when biomass concentration is high and vegetable oil concentration is low, microorganisms consumed glucose and so COD was removed. In that case, it could be said that the system should be operated at 2500 mg/L and 1600 mg/L for biomass and glucose concentration, respectively in order to obtain maximum COD removal efficiency at 3% V.Oil concentration.

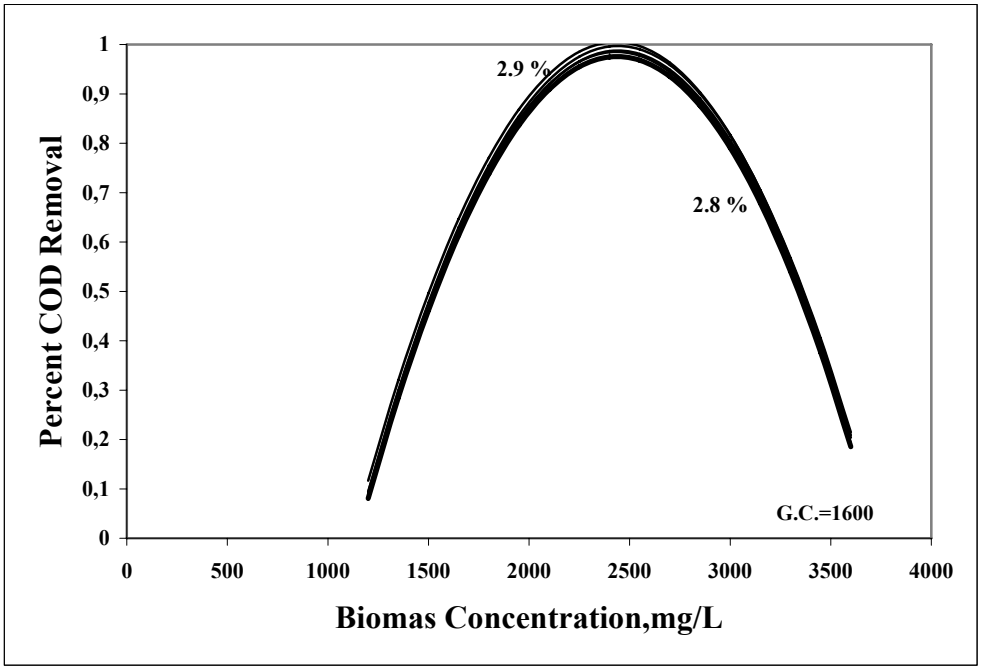

Figure 4.4 Variation of percent COD removal with biomass and V.Oil concentration at G.C. =1600 mg/L, G.C. (Glucose concentration)

4.1.1.3 The Effect of Glucose and Biomass Concentration at Constant V.Oil Concentration

Variation of percent COD removal with glucose concentration at a constant vegetable oil concentration of 2.5% at different biomass concentration is depicted Figure 4.5. At low biomass (1500 mg/L) and glucose concentrations (G.C<1500 mg/L), lower COD removal efficiencies were observed compared to higher biomass and glucose concentrations. The COD removal was around 50% when the biomass concentration B.C=1650 mg/L and glucose concentration was around G.C= 250 mg/L at 2.5% V.Oil concentration. Under this condition, there was limited amount of easily biodegradable co-substrate which is glucose but excess amount of V.Oil which biodegradability is lower compared to glucose. So, there was limited amount of V.Oil degradation which resulted in lower COD removal. In addition, low glucose concentrations did not sustained the growth of microorganisms. This result caused lower V.Oil and COD removal compared to 2000 mg/L glucose concentration. However in the presence of excess amount carbon sources (G.C >1000 mg/L), the COD removal efficiency increased form 35% to over 90% at 1650 mg/L biomass

concentration. This result can be explained as growth of biomass was enhanced which resulted in better vegetable oil removal. So the optimum conditions at 2.5% V.Oil concentration can be defined as 1650 mg/L biomass concentration and 2000 mg/L glucose concentration to obtain more that 90% COD removal efficiency.

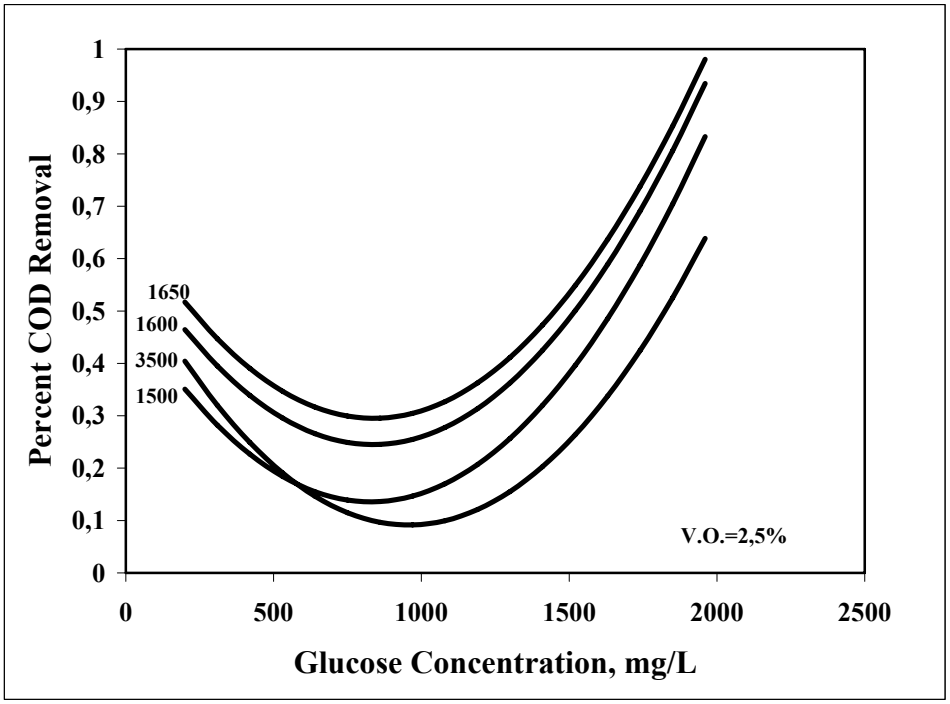

Figure 4.5 Variation of percent COD removal with glucose and biomass concentration at V.O. = 2.5%, V.O.(Vegetable oil concentration)

Figure 4.6 shows the variation of percent COD removal with biomass concentration at a vegetable oil concentration of 2.5% at different glucose concentration. Apparently, COD efficiency increased with increasing biomass concentration and COD yield reaches the optimum at biomass concentration of 2500 mg/L and glucose concentration of 150 mg/L. COD removal efficiency increased about from 30% to 98%. We are said that, the system should be operated at vegetable oil concentration of 2.5%, biomass concentration of 2500 mg/L and glucose concentration of 150 mg/L in order to obtain maximum percent COD removal.

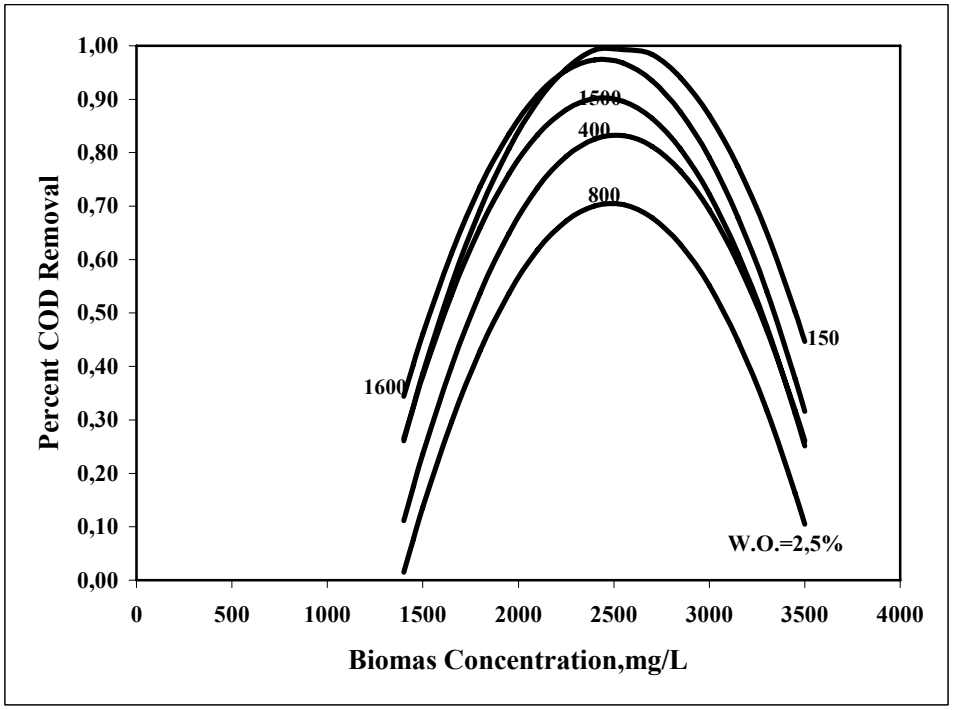

Figure 4.6 Variation of percent COD removal with biomass and glucose concentration at VO= 2.5%, V.O.(Vegetable oil concentration)

4.1.2 Evaluation of V.Oil Removal

4.1.2.1 The Effect of Glucose and V.Oil Concentration at Constant Biomass Concentration

Figure 4.7 depicts the variation of percent vegetable oil removal with glucose concentration at a constant biomass but different vegetable oil concentration. As seen from the figure, vegetable oil removal efficiency increased with decreasing vegetable oil concentration and with increasing glucose concentration. When oil concentration was increased at low glucose concentrations, microorganisms can not get enough carbon source to be active and hence to biodegradability the V.Oil. As a result, vegetable oil removal efficiency decreases from 80% to 55% at low glucose concentrations of 500 mg/L. Increasing glucose concentration slightly improve the V.Oil removal efficiency especially at high concentrations of V.Oil. Percent removal remains almost constant at 1% V.Oil concentrations when glucose was increased from 500 mg/L to 2000 mg/L. However, removal raised from 53% to 60% at 4% V.Oil concentration as the glucose concentration reached to its maximum value.

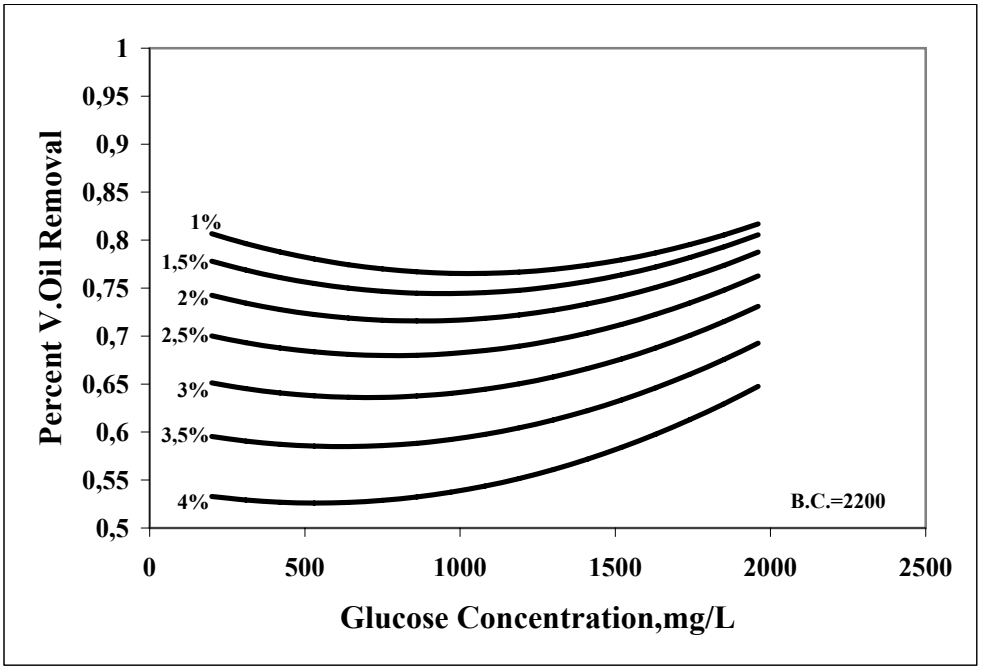

Figure 4.7 Variation of percent V.Oil removal with glucose and V.Oil concentration at 2200 mg/L biomass concentration (B.C.)

Variation of percent V.Oil removal with V.Oil concentration at a constant biomass concentration at different glucose concentration is shown Figure 4.8. As seen from the figure percent vegetable oil removal decreased with increasing V.Oil concentration. Similarly, decreasing glucose concentration resulted in decreases in percent V.Oil removal. Microorganisms could not completely consume the oil at high concentrations. But, glucose was preferred as carbon source rather than V.Oil. When V.Oil concentration increased, percent V.Oil removal decreased about from 85% to 75% at 2000 mg/L glucose concentration.

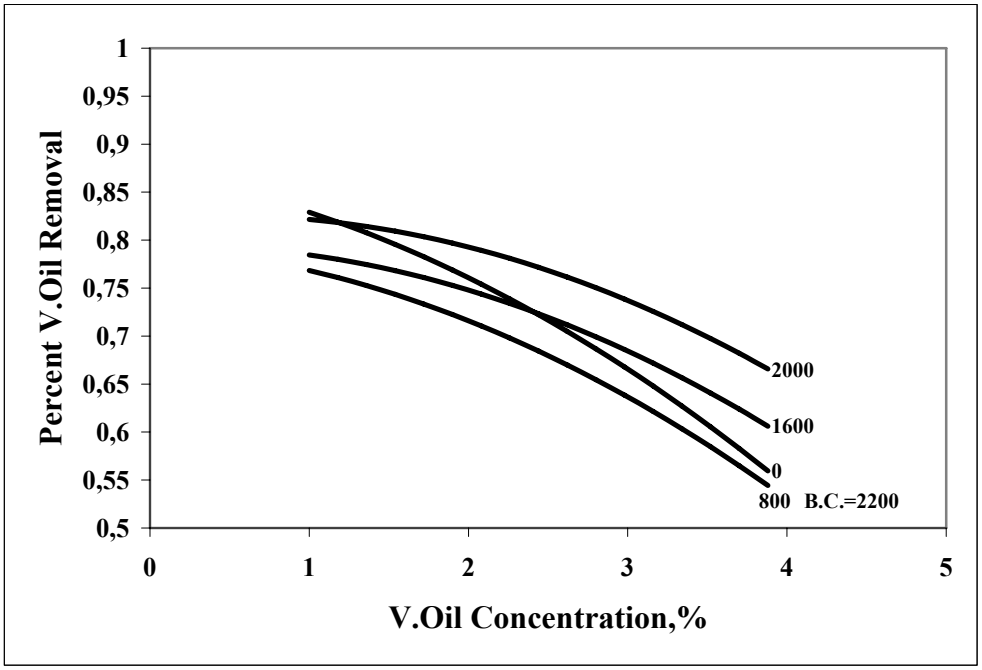

Figure 4.8 Variation of percent V.Oil removal with V.Oil and glucose concentration at B.C. =2200 mg/L, B.C. (Biomass Concentration)

4.1.2.2 The Effect of Biomass and V.Oil Concentration at Constant Glucose Concentration

Figure 4.9 shows the variation of percent vegetable oil removal with different biomass and V.Oil concentration at constant glucose concentration of 1500 mg/L. Percent vegetable oil removal increased with decreasing oil concentration and increasing biomass concentration when oil concentration increased from 1 % to 4% at 1000 mg/L biomass concentration, the efficiency decreased from 85% to 53%. The low biomass concentration could not efficiently remove the high V.Oil concentrations. Fortunately, the increasing biomass concentration from 1000 mg/L to 3500 mg/L improved the V.Oil removal at 1600 mg/L glucose concentration. The efficiency increased from 53% to 70% at 4 % V.Oil. This result indicates that the biomass concentration significantly affect the V.Oil removal. The higher the biomass concentration, the better the V.Oil removal even at high concentrations of V.Oil. In addition, the significance of the biomass concentration is more apparent at high V.Oil concentrations compared to lower ones. The percent increase in the V.Oil removal at 4% V.Oil as the biomass concentration was increased lower in comparison to that of 1% V.Oil. In summary, it can be concluded that about 90%

V.Oil removal can be obtained at 1% V.Oil, 3500 mg/L biomass and 1500 mg/L glucose concentration.

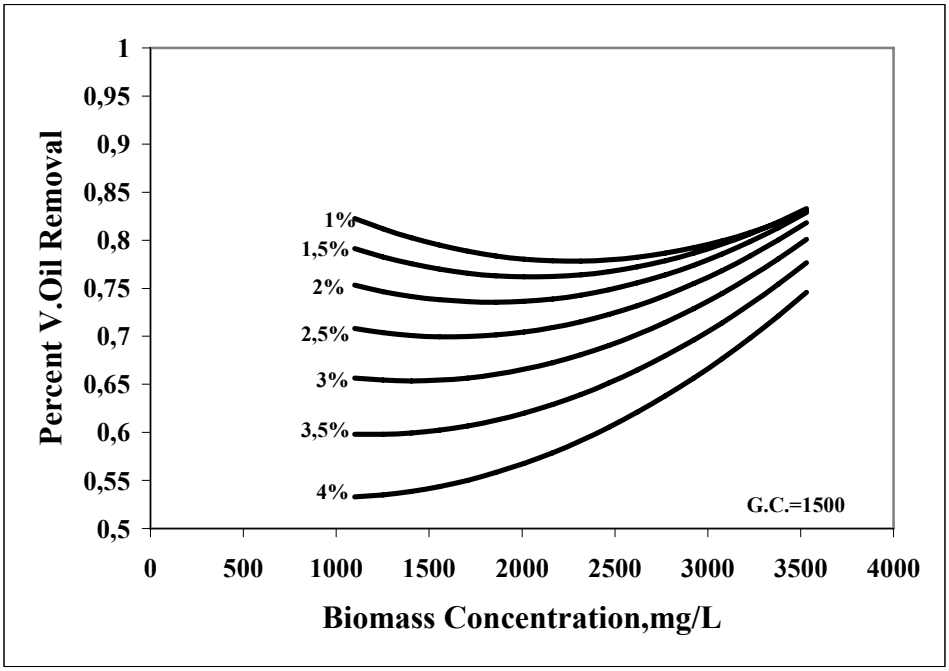

Figure 4.9 Variation of percent V.Oil removal with biomass and V.Oil concentration at G.C. =1500 mg/L, G.C. (Glucose Concentration)

Figure 4.10 indicates the variation of percent vegetable oil removal with different vegetable oil concentration and biomass concentration at constant glucose concentration. When vegetable oil concentration increased and biomass concentration decreased, percent vegetable oil removal decreased. Increasing oil concentration might have increased the surface tension which caused limitation in consumption of vegetable oil in the wastewater. As seen from the Figure the effect of biomass concentration on the V.Oil removal is more significant at high concentration. The percent removal at low V.Oil concentrations (1%) for all biomass concentrations were almost the same and around 80% in average. However, at 4% V.Oil concentration, the efficiency was substantially different for each biomass concentrations. It was around 77% at 3500 mg/L but decreased to 55 % form 1000 mg/L. This result indicated that high concentrations of biomass are required for efficient V.Oil removal from wastewater for over 3% V.Oil concentrations.

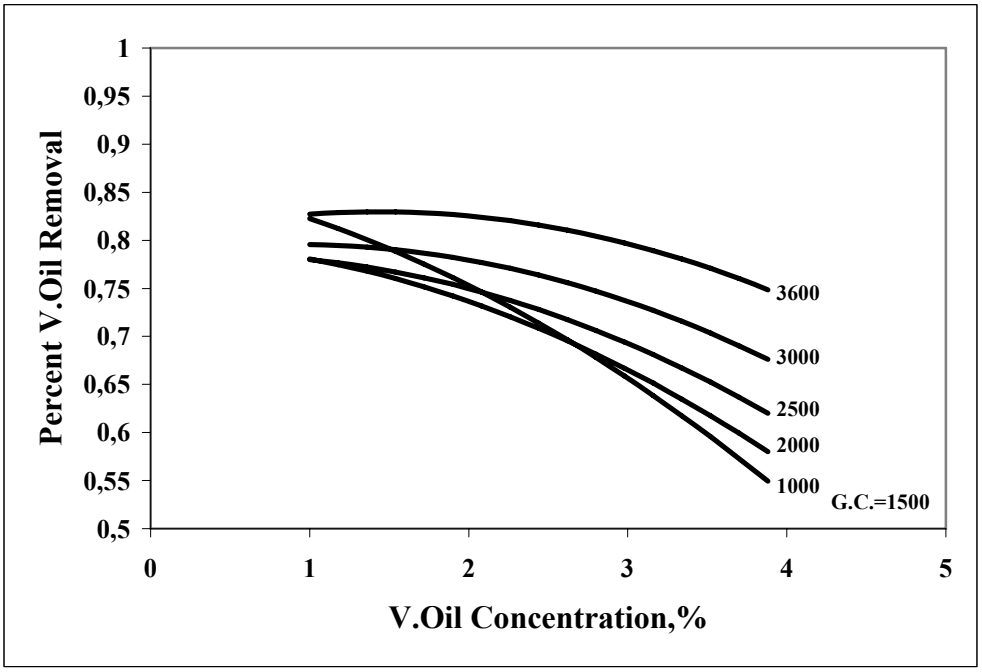

Figure 4.10 Variation of percent V.Oil removal with V.Oil and biomass concentration at G.C. =1500 mg/L, G.C. (Glucose concentration)

4.1.2.3 The Effect of Glucose and Biomass Concentration at Constant V.Oil Concentration

Figure 4.11 depicts the variation of percent vegetable oil removal with glucose concentration and biomass concentration at constant V.Oil concentration. When oil concentration is 2.6%, V.Oil efficiency increased from 65% to 85% at <500 mg /L glucose concentration as biomass concentration was increased from 1100mg/L to 3500 mg/L. Increasing glucose concentration at high biomass concentrations did not substantially affect the V.Oil removal. The efficiency was around 85% at 3500 mg/L biomass concentration for both 500 mg/L and 2000 mg/L glucose concentrations. However at low biomass concentrations such as 1100 mg/L, the removal increased form 63% to 73% as glucose concentration was increased from 500 mg/L to 2000 mg/L. This result means that biomass concentration is more important parameter compared to glucose concentration in treatment of V.Oil. Controlling biomass concentrations rather than co-substrate addition could be more practical in V.Oil removal from wastewater. However, if biomass concentration can not be kept high in the system, the co-substrate addition could help in improving the system performance in terms of V.Oil removal.

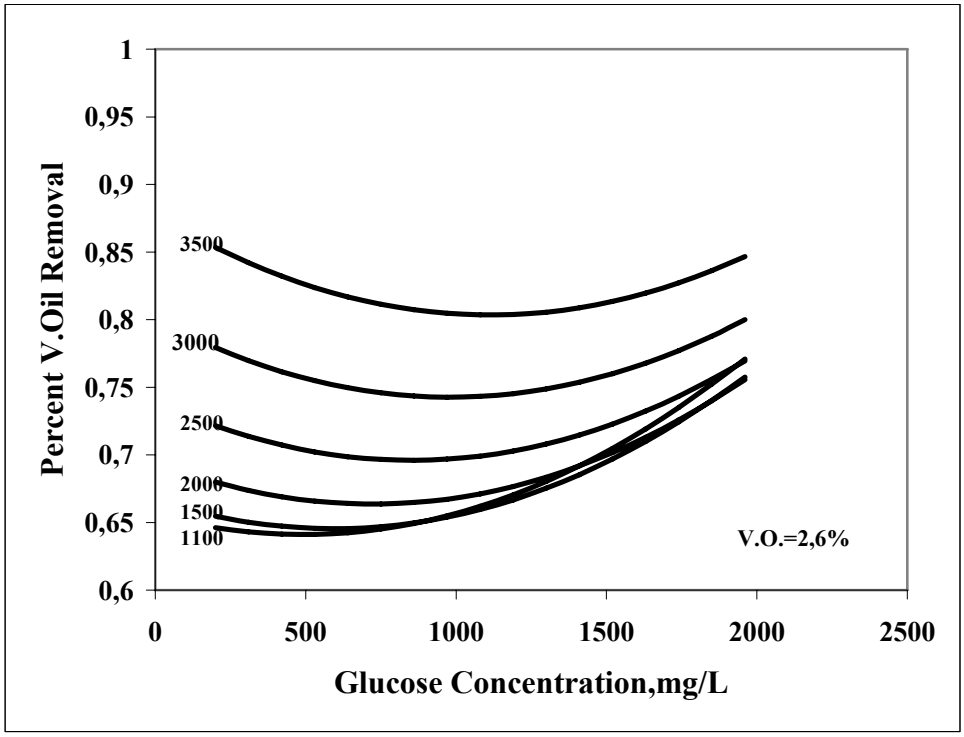

Figure 4.11 Variation of percent V.Oil removal with glucose and biomass concentration at V.O.=2.6%, V.O.(Vegetable oil concentration)

Variation of percent vegetable oil removal with biomass concentration and glucose concentration at constant vegetable oil concentration of 2.6% is depicted in Figure 4.12. Percent vegetable oil removal increased with increasing biomass concentration. On the other hand, vegetable oil removal increased with decreasing glucose concentration. When glucose concentration was at its low level (500 mg/L) , microorganisms consumed all vegetable oil in wastewater. Vegetable oil removal efficiency increased with decreasing glucose concentration at constant vegetable oil concentration due to high biomass concentration. The maximum V.Oil removal efficiency was obtained at 3500 mg/L biomass concentration and without glucose addition. This result emphasize again that biomass concentration should be kept at high concentration to obtain efficiency V.Oil removal. The carbon source stress on the microorganisms in the absence of glucose devoted the microorganisms to consume V.Oil which was the only carbon source in the wastewater. However, at low biomass concentrations, microorganisms need excess amount of co-substrate to increase their population in order to tolerate V.Oil.

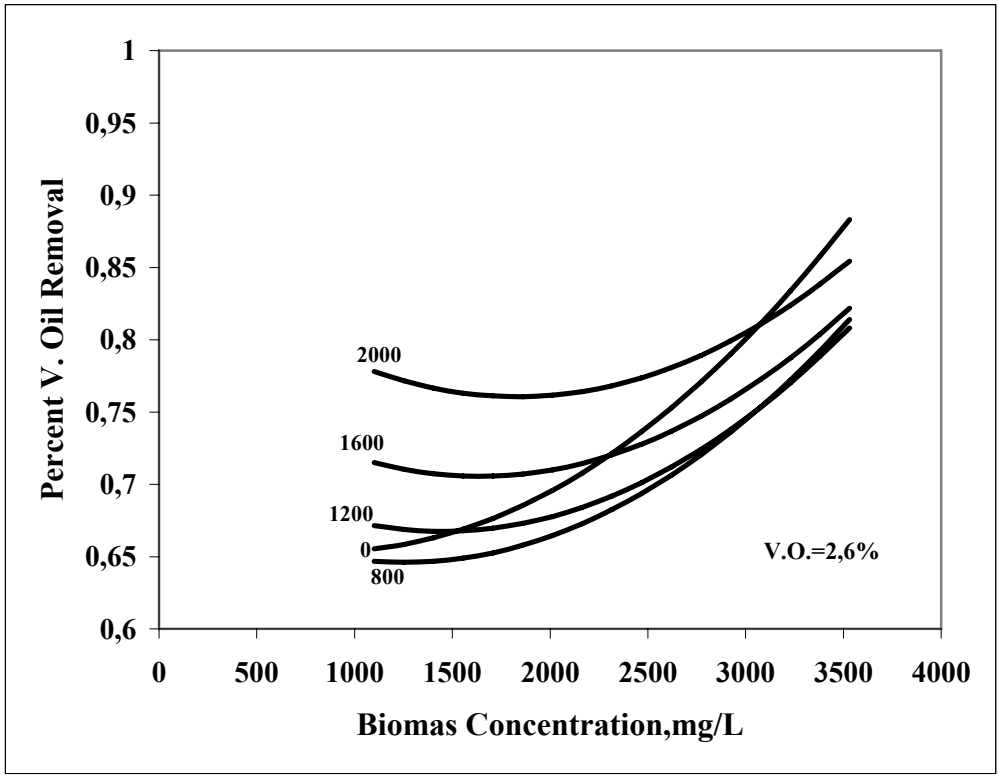

Figure 4.12 Variation of percent V.Oil removal with biomass and glucose concentration at V.O.=2.6%, V.O.(Vegetable oil concentration)

4.1.3 The Effect of Glucose, V.Oil and Biomass Concentration on COD Removal

Variation of percent COD removal with glucose concentration at different vegetable oil concentration is shown in Figure 4.13. As seen from the figure, at the maximum level of biomass concentration (3550 mg/L), the maximum COD removal was observed at the lowest concentration of the V.Oil and glucose and 82% removal efficiency was obtained. The minimum removal efficiency point was observed at 2.5% V.Oil and 1000 mg/L glucose concentration. However, keeping one of the parameter at high level while keeping the other at low level could increase the COD removal efficiency. For example, the efficiency reaches to its almost maximum value when V.Oil was at low level (1%) while glucose concentration was at maximum value (2000 mg/L). Similarly, around 78% COD removal efficiency can be obtained at high level of V.Oil (4%) and low level of glucose which is 0 mg/L. But increasing glucose concentration to maximum level at the same V.Oil concentration seems that improves the COD removal. However, it is not because of the high activity of the

organisms it is because of excess availability of easily biodegradable carbon source. Since, it is easily removed, COD removal efficiency increases.

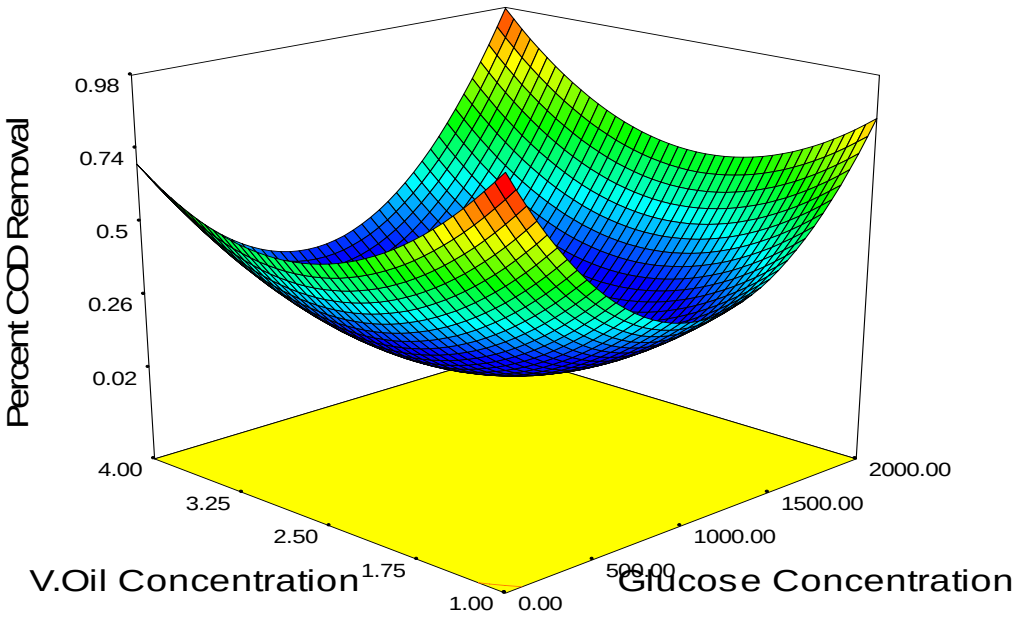

Figure 4.13 Variation of percent COD removal with glucose and V.Oil concentration at maximum biomass concentration (3550 mg/L)

Variation of percent COD removal with glucose concentration at different biomass concentration is shown in Figure 4.14. As seen from the figure, at the maximum level of vegetable oil concentration (4 %), the maximum COD removal was observed at the high concentration of the biomass and at the lowest concentration of glucose and this removal efficiency is about 95 %. At the lowest biomass and glucose concentration value, percent COD removal substantially decreased. So COD removal is significantly affected by biomass concentration and co substrate concentration. Keeping biomass at high concentrations and providing enough co-substrate at high V.Oil concentrations is essential to obtain efficient treatment. The system operates more economical at high biomass concentration and low co-substrate concentration combinations. Excess amount of co-substrate can also

be tolerated even at low biomass concentrations. But it is not essential for COD removal.

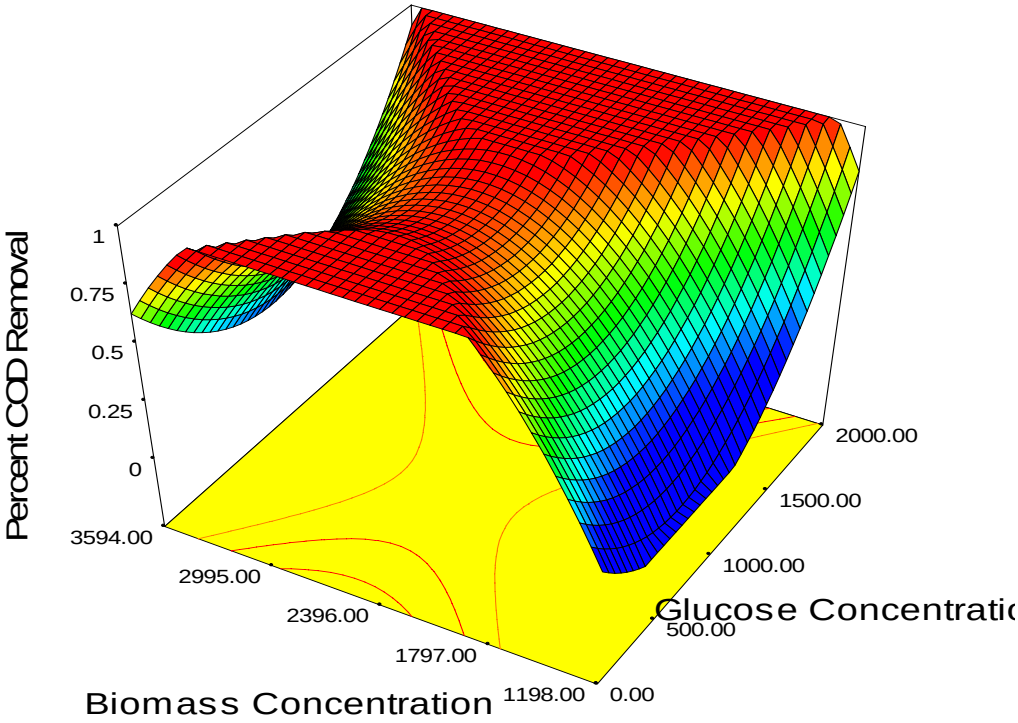

Figure 4.14 Variation of percent COD removal with biomass and glucose concentration at maximum vegetable oil concentration (4%)

Variation of percent COD removal with vegetable oil concentration at different biomass concentration is shown in Figure 4.15. As seen from the figure, at the center point of glucose concentration (1000 mg/L), the maximum COD removal was observed at the high concentration of the biomass at the lowest concentration of vegetable oil and this removal efficiency is about 83 %. At the lowest biomass and vegetable oil concentration value, percent COD removal significantly decreases.

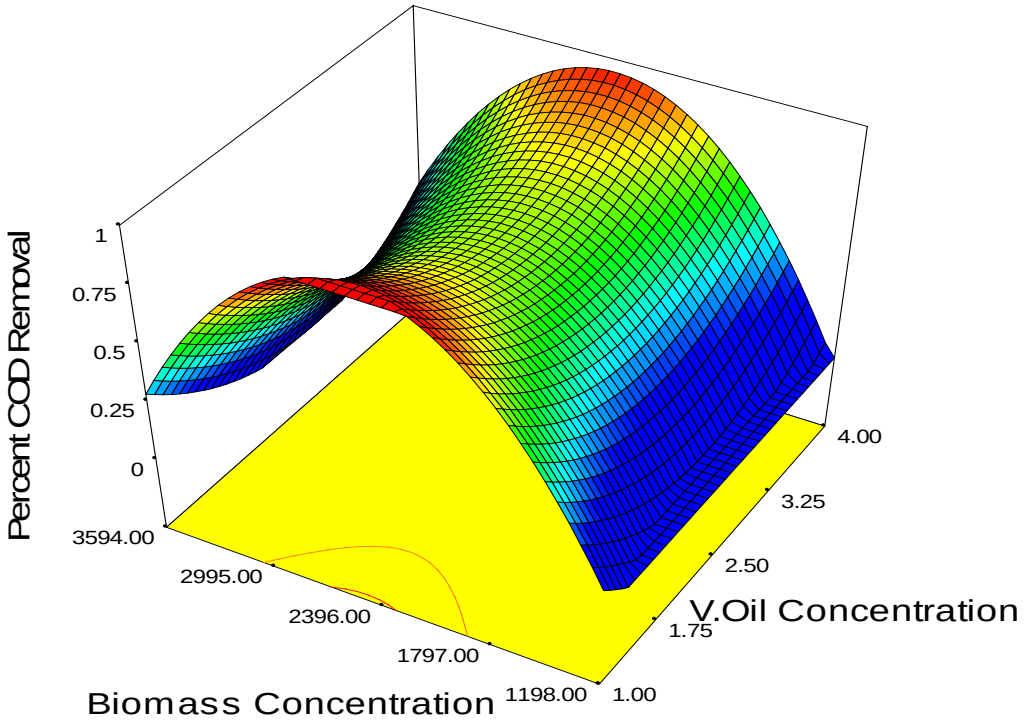

Figure 4.15 Variation of percent COD removal with biomass and vegetable oil concentration at 1000 mg/L glucose concentration

4.1.4 The Effect of Glucose, V.Oil and Biomass Concentration on Vegetable Oil Removal

Variation of percent vegetable oil removal with glucose concentration at different vegetable oil concentration is shown in Figure 4.16. As seen from the figure, the V.Oil removal decreased with increasing V.Oil concentrations at minimum glucose concentration. A similar trend was observed at high glucose concentration. The more efficient V.Oil removal can be achieved as co-substrate concentration was decreased at constant V.Oil concentrations. This result can be explained as the microorganisms attack to V.Oil when there are not enough easily biodegradable or easily available carbon sources. However, the system needs certain amount of carbon source to sustain the growth of organisms at high V.Oil concentrations. In summary, the maximum vegetable oil removal was observed as 91% at the lowest concentration of the V.Oil (1%) and glucose (0 mg/L). However, 78% V.Oil removal at 4%

vegetable oil concentration and without co-substrate addition can be evaluated as high removal efficiency.

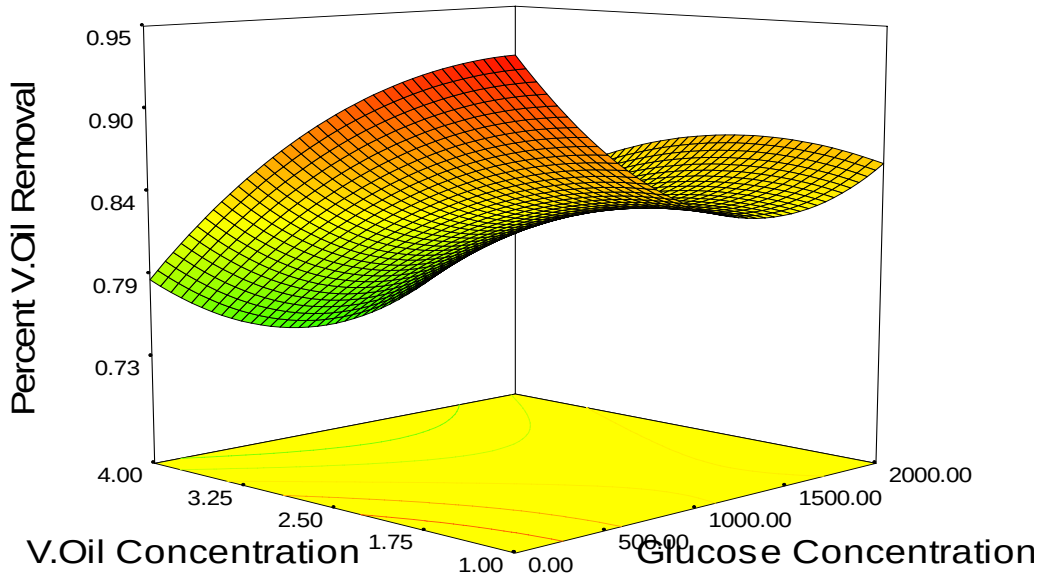

Figure 4.16 Variation of percent Vegetable Oil removal with glucose and V.Oil concentration at maximum biomass concentration (3550 mg/L)

Variation of percent vegetable oil removal with glucose concentration at different biomass concentration is shown in Figure 4.17. As seen from the figure, at the maximum level of vegetable oil concentration (4 %), the maximum vegetable oil removal was observed at the maximum concentration of the biomass and at the lowest concentration of glucose and this removal efficiency is about 77 %. These results support the previous conclusions, either biomass concentration or co-substrate concentration should be controlled for efficient V.Oil removal.

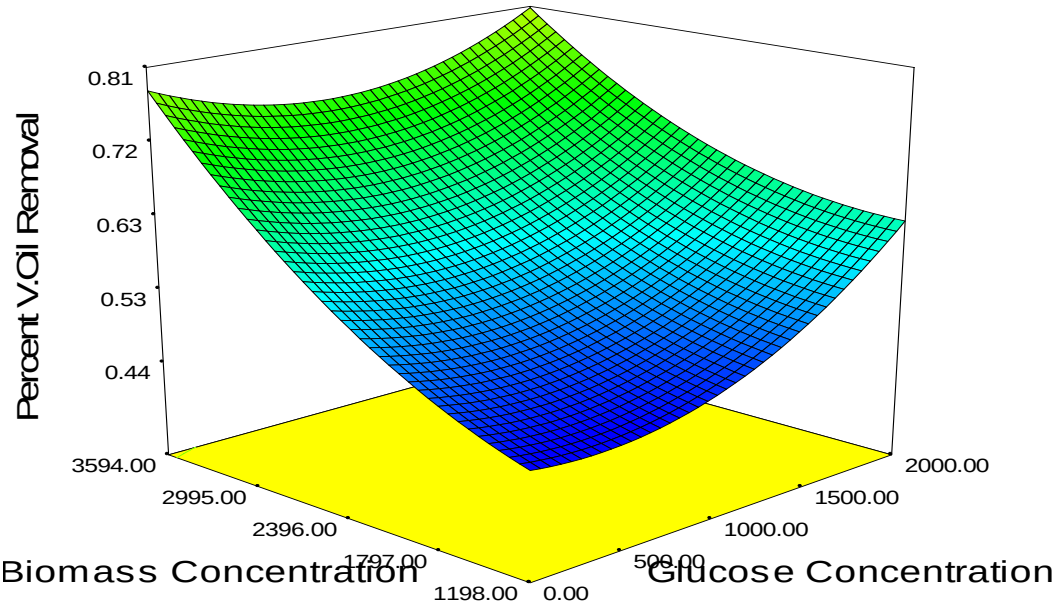

Figure 4.17 Variation of percent Vegetable Oil removal with biomass and glucose concentration at maximum vegetable oil concentration (4%)

Variation of percent vegetable oil removal with vegetable oil concentration at different biomass and glucose concentrations is shown in Figure 4.18. As seen from the Figure, at the center point of glucose concentration (1000 mg/L), the maximum vegetable oil removal was observed at the high concentration of the biomass and at the lowest concentration of vegetable oil and this removal efficiency is about 82 %.

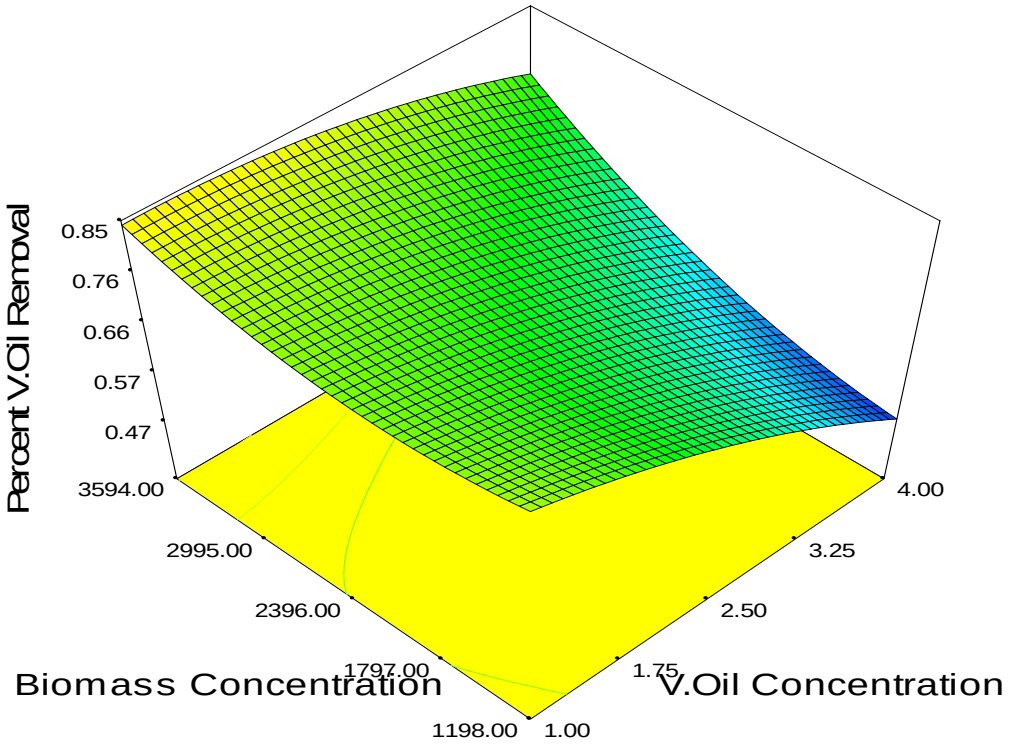

Figure 4.18 Variation of percent Vegetable Oil removal with biomass and vegetable oil concentration at 1000 mg/L glucose concentration

The optimum conditions determined by a StatEast computer program are presented in Table 4.6.

Table 4.6 Optimum condition in COD and V.Oil removal

Response	Glucose Conc. (mg/L)	Biomass Conc. (mg/L)	V. Oil Conc. (mg/L)	Efficiency (%)
COD Removal	1201.43	3.99	2574.31	1.00
	1189.41	3.95	2333.30	0.98
	332.47	3.82	2721.06	0.97
	123.21	3.98	3116.91	0.98
	86.20	3.89	2439.85	1.00
	221.53	2.36	2506.46	0.96
	99.20	4.00	1928.30	0.97
Vegetable Oil Removal	1999.99	1.79	3565.14	0.87
	0.00	2.40	3594.00	0.90
	2000.00	1.80	1198.00	0.83
	1494.67	1.31	3594.00	0.84
	14.78	1.40	3594.00	0.94

In conclusion, when all of the graphics take into consideration, we can say that biomass concentration is a significant factor in treatment of V.Oil containing wastewater. The more efficient COD removal can be observed at high biomass concentration. Also percent COD removal efficiency decreased when V.Oil concentration was lower. That is when biomass concentration is high and vegetable oil concentration is low, better COD removal was observed. Besides high vegetable oil concentration in the synthetic wastewater made inhibition to COD removal. Since the V.Oil was the only COD source in the synthetic media, COD removal can be attributed to V.Oil removal. External carbon source addition enhances the biological V.Oil degradation.

In addition, the biomass concentration significantly affects the vegetable oil removal. The effect of biomass concentration on the vegetable oil removal is more significant at high V.Oil concentration. Increasing oil concentration increased the surface tension which caused limitation in consumption of vegetable oil in the wastewater. That is biomass concentration should be kept at high concentration to obtain efficiency V.Oil removal. If biomass concentration can not to be kept high in the system, the co-substrate addition could help in improving the system performance in terms of V.Oil removal. Finally, it was shown that the biomass concentration is more important parameter compared to glucose concentration in treatment of V.Oil.

When glucose concentration was central point (1000 mg/L), biomass concentration and V.Oil concentration was maximum (3500 mg/L and 4%, respectively), maximum COD removal was observed at low oil concentration and high glucose concentration. Also at the lowest biomass and glucose concentration value, percent COD removal substantially decreased. So COD removal is significantly affected by the biomass concentration and co-substrate concentration. That is keeping biomass at high concentrations and providing enough co-substrate at high V.Oil concentration is essential to obtain efficient treatment. Similarly both biomass and co-substrate concentrations must be high for efficient vegetable oil removal.

As a result, the microorganisms attack to v.oil when there are not enough easily biodegradable or easily available carbon sources. However, the system needs certain amount of carbon source to sustain the growth of organisms at high V.Oil concentrations. That is either biomass or co-substrate concentration were high for efficient COD and V.Oil removal.

4.2 Treatment of Vegetable Oil Containing Synthetic Wastewater by Fed-Batch Operation

Batch experiments indicated that efficient V.Oil removal can be obtained by biological methods. The important parameters were determined as co-substrate, V.Oil and biomass concentration. However, the high concentrations of V.Oil (>4%) could create a problem in the treatment. By considering these facts, fed-batch operation was selected as a biological treatment approach to V.Oil removal from the synthetic wastewater. Because, fed batch operation is used for strong wastewaters and for the removal of toxic substances. The dilution of the wastewater during feeding period of the operation increases the tolerance of the microorganisms to high organic loads or to toxic substance. As a result, better removal of the substance can be obtained. Since the organisms can not remove high concentrations of V.Oil, the fed batch operation could be the best solution. Therefore, the effects of important operating parameters such as vegetable oil concentration with and without co-substrate addition, sludge age and biosurfactant addition on V.Oil removal by fed-batch operation were investigated in the second part of the thesis.

4.2.1 The Effect of Vegetable Oil Concentration on Vegetable Oil and COD Removal in the Presence of Co-substrate

The system was operated at six different vegetable oil concentrations between 0.1% (1 g/L) (w/v) and 6% (60 g/L) (w/v). Temperature and pH were 20-25 ^{0}C and pH=7-7.5, throughout the experiments. Vigorous aeration was supplied to the aeration tank to keep the dissolved oxygen (DO) concentration above 2 mg/L.

Temperature, pH and DO were monitored and manually controlled during the experiments. The initial liquid volume before the feeding was 1 liter for all experiments. The feed flow rate was kept constant at Q=0.5 L/h and fed-batch experiments continued for eight hours. The experiments were conducted with and without carbon source to investigate the need for carbon source at different concentrations of V.Oil. Molasses was used as co-substrate when carbon source added externally and its concentration was adjusted to around 2000 mg/L COD. Sludge age (θc) was controlled at 15 days. COD and V.Oil removal efficiencies were determined for each oil concentrations. The theoretical COD and V.Oil concentrations in the reactor were calculated by considering the dilution during feeding. The theoretical concentrations were considered in percent removal efficiency calculations.

Figure 4.19 depicts variation of COD concentration and percent COD removal with 7 hours of operation time at 0.14 % (1.4 g/L) (w/v) V.Oil concentration and 1600 mg/L initial COD concentration. Theoretical COD concentration increased from 1600 mg/L to 2500 mg/L with time due to accumulation of COD. However, the observed COD concentration decreased from 1600 mg/L to around 500 mg/L. As a result, the percent COD removal was 86 % at the end of the 7 hours of operation.

Variation of vegetable oil concentration and percent V.Oil removal with 7 hours of operation time is presented in Figure 4.20.Theoretical V.Oil concentration increased with time due to accumulation of vegetable oil in the reactor. The final calculated concentration of V.Oil was 1.1 g/L. However, observed V.Oil concentration decreased significantly to 0.05 g/L after 4 hours of operation. The final concentration at the end of operation was around 0.1 g/L. As a result 87% V.Oil in the system was removed biologically.

In figure 4.21, variation of biomass concentration with time is given. The initial biomass concentration in the system was around 12 g/L. Although it seems that biomass concentration was decreasing with feeding, there was an increase in the total amount of biomass. At the end of the operation the total biomass was 18 g/L. This

result means that there was around 50% increase in the biomass. So the culture was active and was able to remove both co-substrate and the V.Oil from the synthetic wastewater.

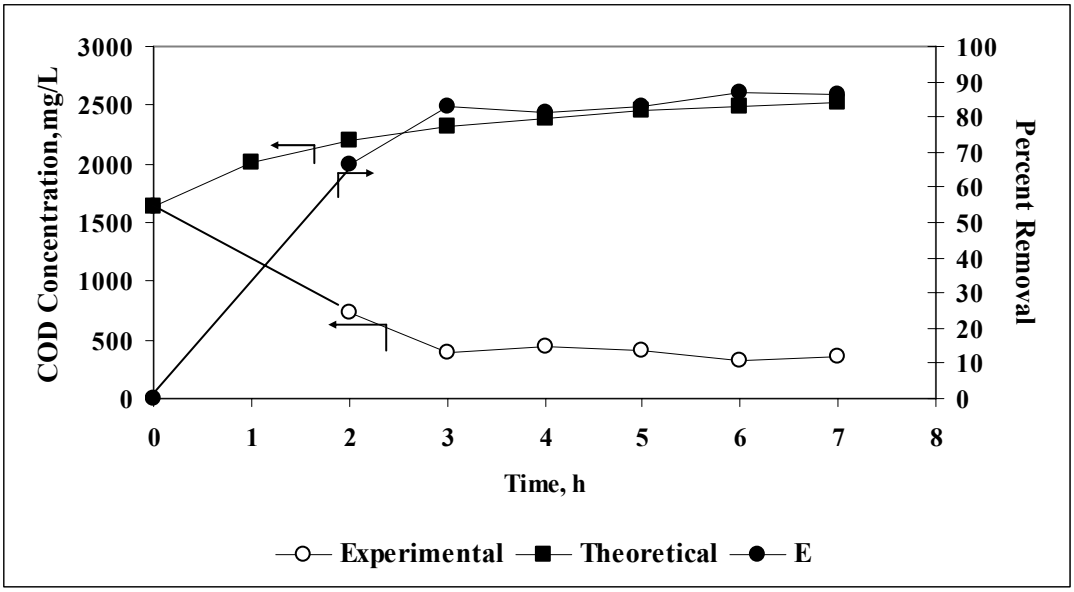

Figure 4.19 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 0.14 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

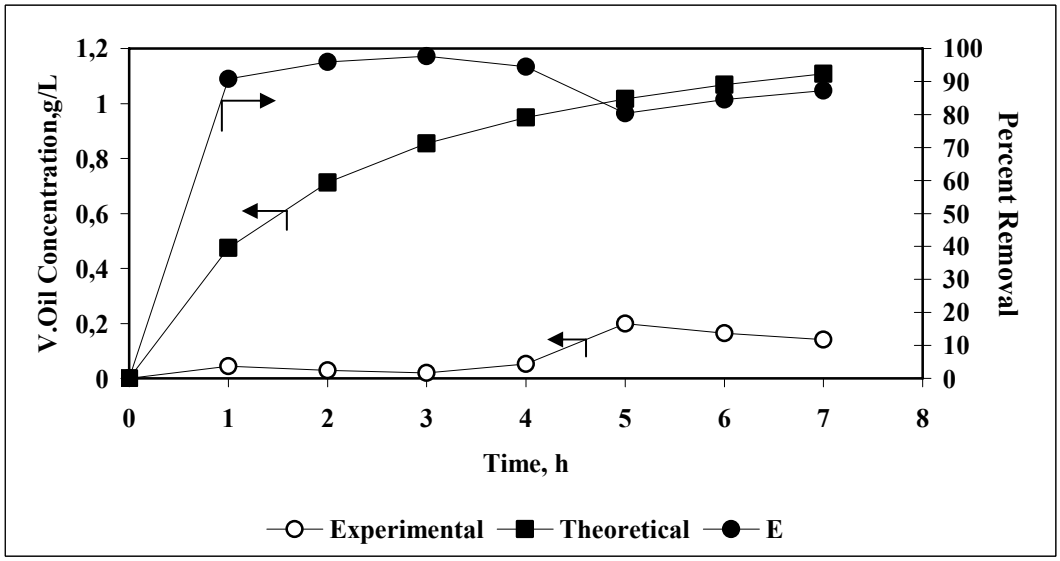

Figure 4.20 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 0.14 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

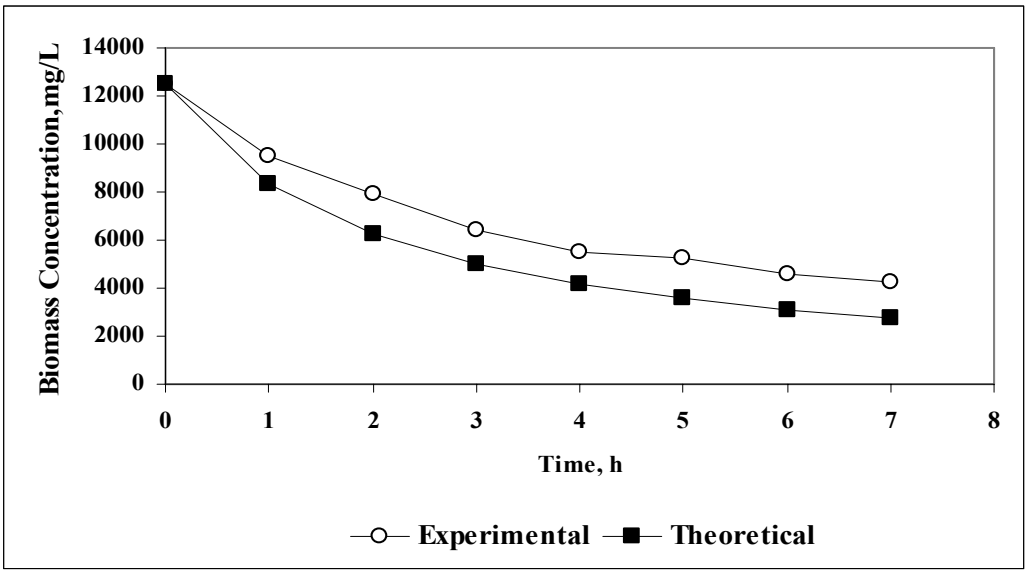

Figure 4.21 Variation of biomass concentration with time (V.Oil Concentration=0.14 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

The V.Oil concentration was increased to 1.3% (13 g/L) (w/v) in the second experiment. The contribution of co-substrate to COD in the feed was around 2000 mg/L and the sludge age was 15 days. The initial liquid volume was 1 L and the COD content of the initial volume was nearly 87 mg/L. Figure 4.22 indicates variation of COD concentration and percent COD removal with time. Theoretical COD concentration increased to 4000 mg/L at the end of operation period. Similarly, observed COD concentration increased to 2500 mg/L at 5th hour of operation and then decreased to around 1500 mg/L at the end of the operation. The percent removal was around 15% for the operation time between 1 and 5 hours. But, it increased to 58% at the end of operating period.

Variation of vegetable oil concentration and percent V.Oil removal with 8 hours of operation time is shown in Figure 4.23. Theoretical V.Oil concentration increased with time because of accumulation of oil in the system. The V.Oil concentration in the starting volume was negligible. As seen in the figure theoretical V.Oil concentration increased to 10.7 g/L in time. However, the v.oil concentration in the reactor was significantly lower compared to the theoretical one. It was observed that V.Oil concentration was around 0.2 g/L in the beginning of the operation but slightly increased to around 1 g/L. When the difference between theoretical and the observed

concentrations was considered, the percent removal was 89%. However, if the feed V.Oil concentration is taken to the consideration, the percent removal under the operating conditions can be announced as 90%.

Figure 4.24 depicts variation of biomass concentration with time. There was a parallel trend between calculated and observed biomass concentrations. An accumulation of biomass was observed in the system. The initial biomass concentration was around 25 g/L in the starting liquid volume. The final concentrations were 5.3 g/L and 8.8 g/L for calculated and experimental ones. There is a substantial difference between two values. This result indicates that the culture in the system was active. Some of the carbon source was used for biomass synthesis.

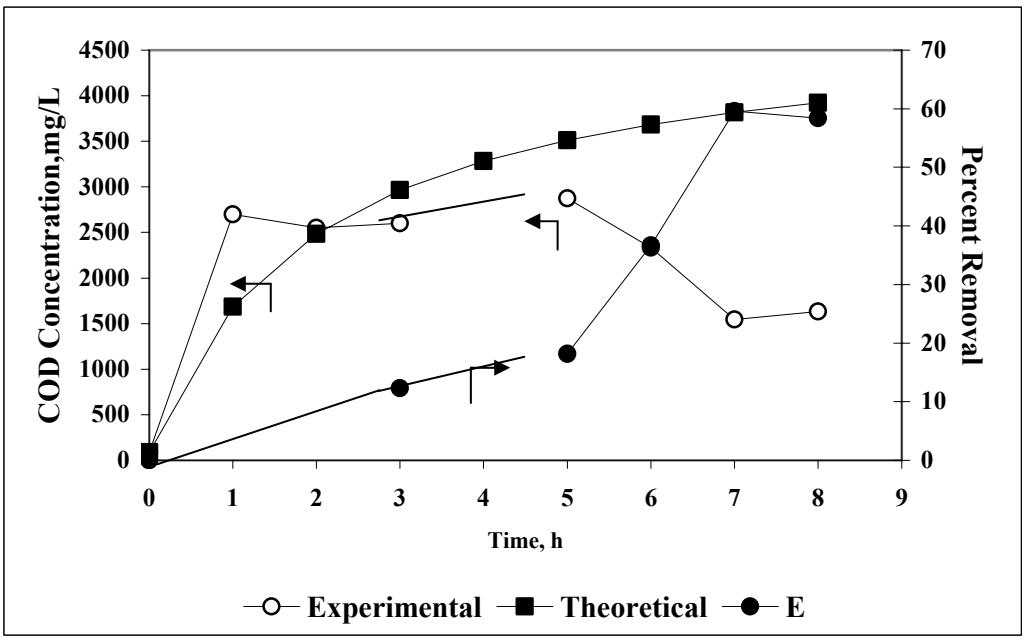

Figure 4.22 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 1.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

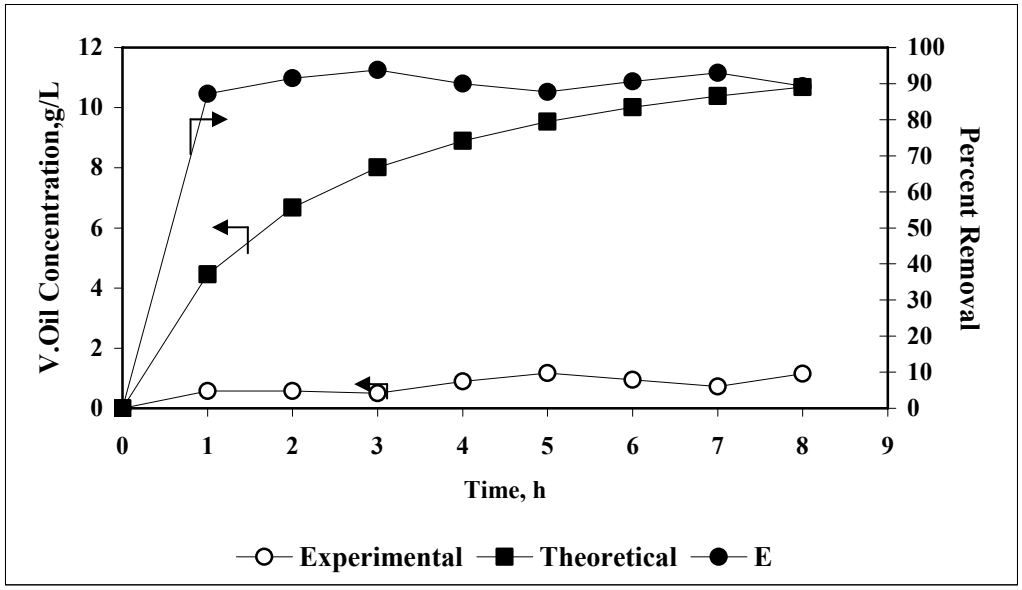

Figure 4.23 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 1.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

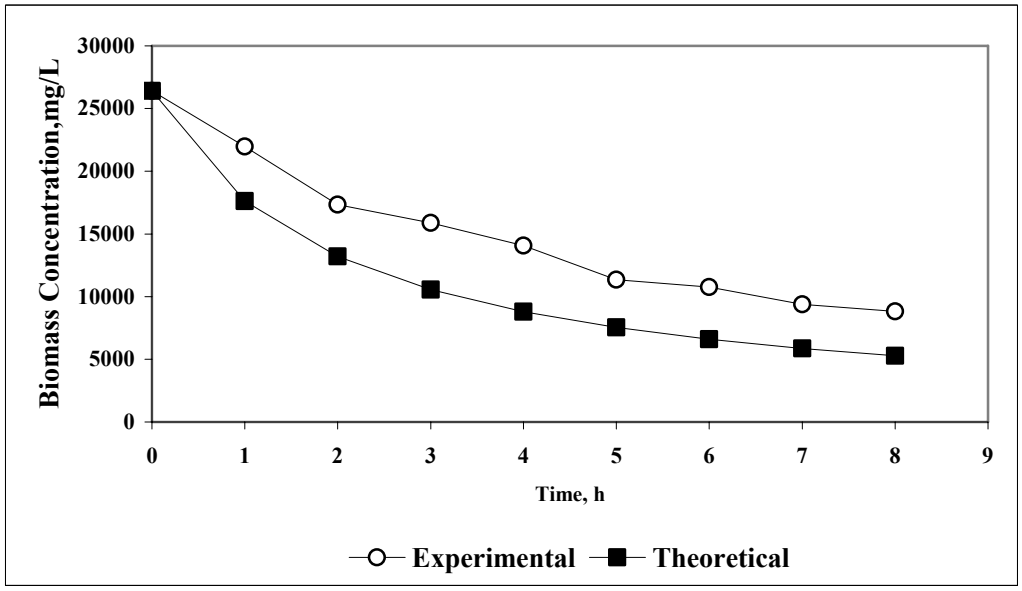

Figure 4.24 Variation of amount of biomass with time. (V.Oil Concentration= 1.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

The initial oil concentration was increased to 2 % (20 g/L) (w/v), the COD equivalent of co substrate concentration was COD=2000 mg/L. The total cycle of the operation was 8 hours. Variation of vegetable oil concentration and percent V.Oil removal with 8 hours of operation time is shown in Figure 4.25. The theoretical V.Oil concentration increased to 16 g/L. The experimental V.Oil concentration was around 1 g/L up to 4 hours of operation. As a result, the percent removal was more than 95%. However, the V.Oil concentration showed a slight increase after 4th hour.

The final concentration was around 6.5 g/L resulting in around 60% removal efficiency

Figure 4.26 indicates variation of biomass concentration with time. Theoretical biomass concentration decreased from 6000 mg/L to 2000 mg/L. However, the observed biomass concentration increased form 6000 mg/L to around 10000 mg/L. The reason of this sharp increase in the biomass concentration can be explained as starting with low concentration of biomass. As the V.Oil concentration increases, the available carbon source increases too. So, the condition was high food but less biomass concentration. In other words, there was excess amount of food for the biomass and therefore the culture increased its mass.

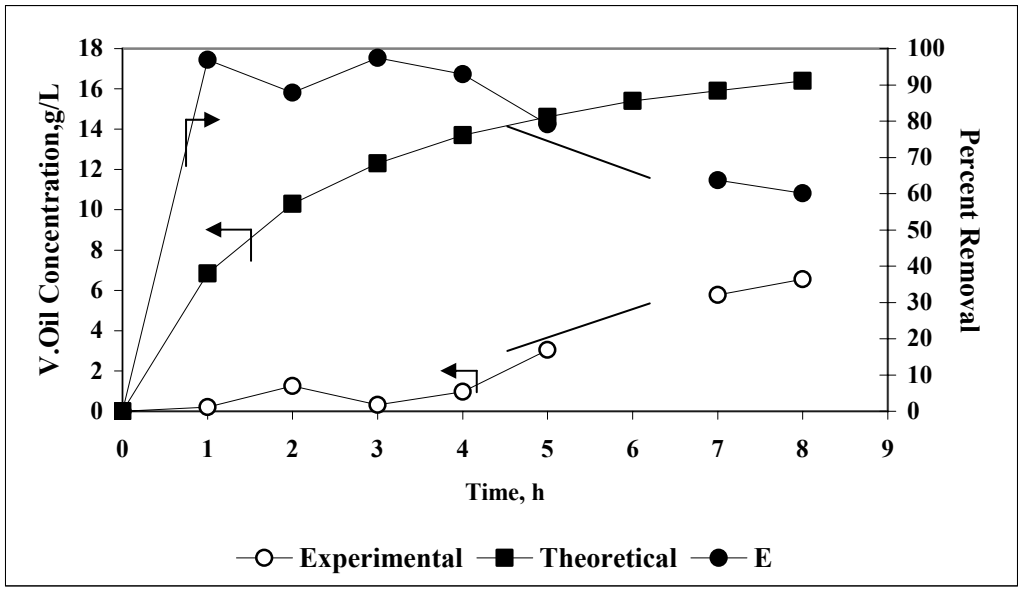

Figure 4.25 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 2 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

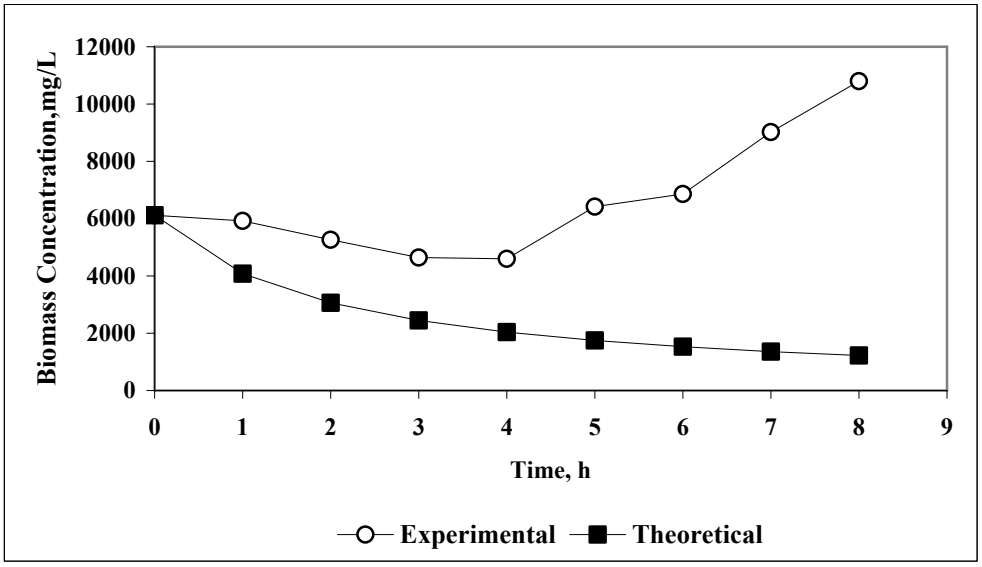

Figure 4.26 Variation of biomass concentration with time (V.Oil Concentration= 2 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

Variation of COD concentration and percent COD removal with time at 3.3% (33.3 g/L) (w/v), COD=2000 mg/L and around 1990 mg/L co-substrate concentration is depicted in Figure 4.27. Theoretical COD concentration in the aeration tank increased with time due to accumulation of COD. The final calculated concentration considering dilution was 7000 mg/L. COD concentration in the experimental tank was nearly 1179 mg/L at the end of the operation. Percent COD removal increased with time and obtained as 85 % at the end of the operation.

Variation of vegetable oil concentration and percent V.Oil removal with 8 hours of operation time is shown in Figure 4.28. Theoretical V.Oil concentration increased with time due to accumulation of vegetable oil in the system. As seen in the figure, vegetable oil concentration decreased up 1.3 g/L at the end of 4 hours of operation. A slight increase in the concentration was observed for the further operation period. The resulting concentration was 4.8 g/L as the cycle was completed at the end of 8 hours. So the percent removal reached to 90% by the 4^{th} hour of the operation but then decreased to around 82% when the cycle was completed.

Figure 4.29 depicts the variation of biomass concentration with time. The initial biomass concentration was 18 g/L when the cycle was started. Theoretical biomass concentration decreased to 3400 mg/L by the effect of dilution. However, the

experimental biomass concentration was around 7000 mg/L and it is considerably higher compared to the theoretical one. So the removal of V.Oil and COD can be attributed to the biomass. In other words, V.Oil was biodegraded and used as carbon source by the microorganisms for growth.

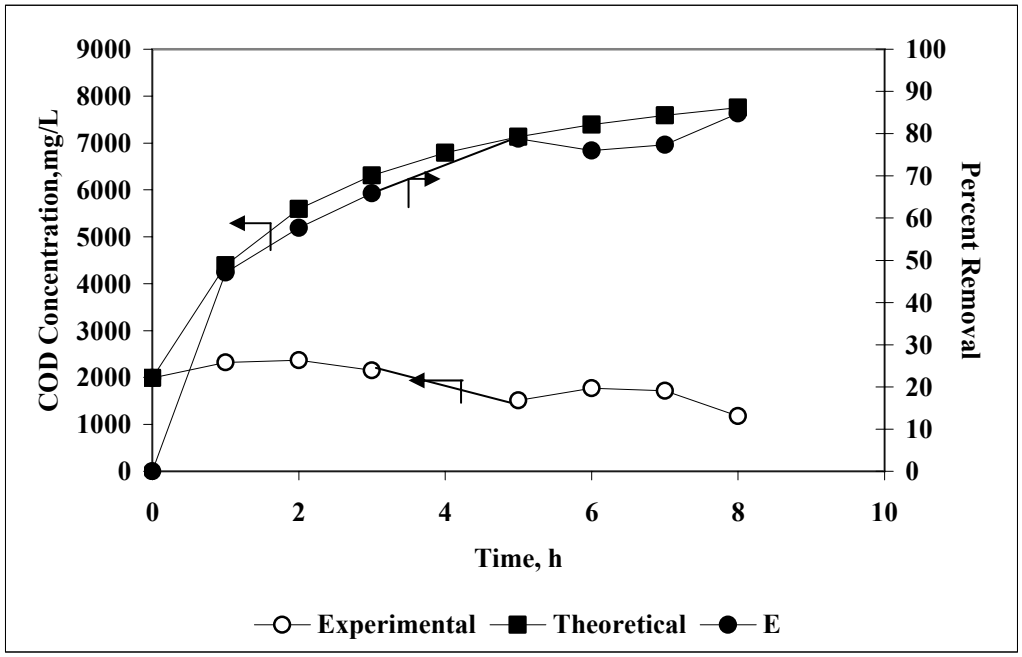

Figure 4.27 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 3.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

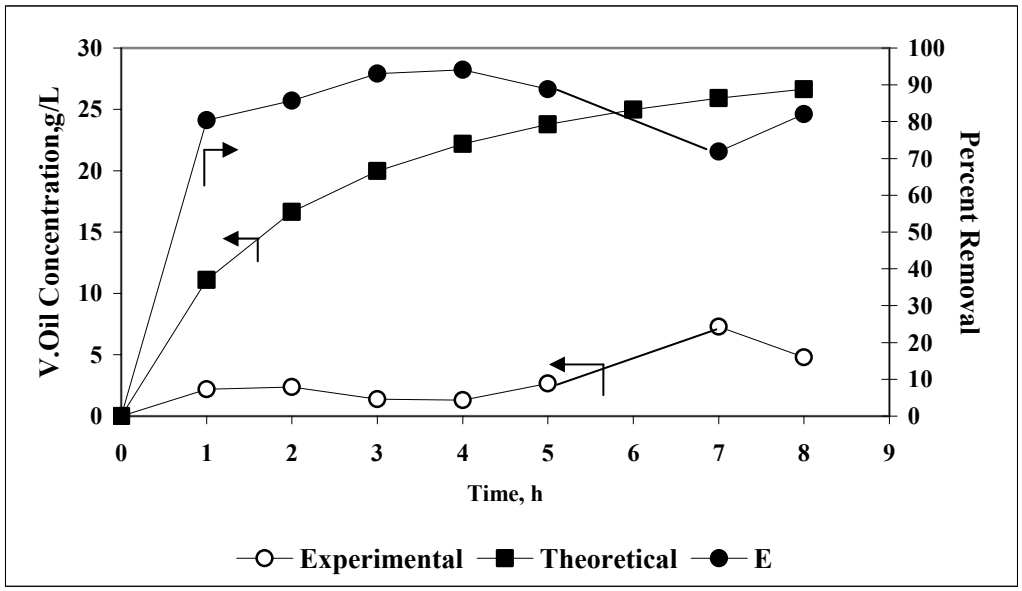

Figure 4.28 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 3.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

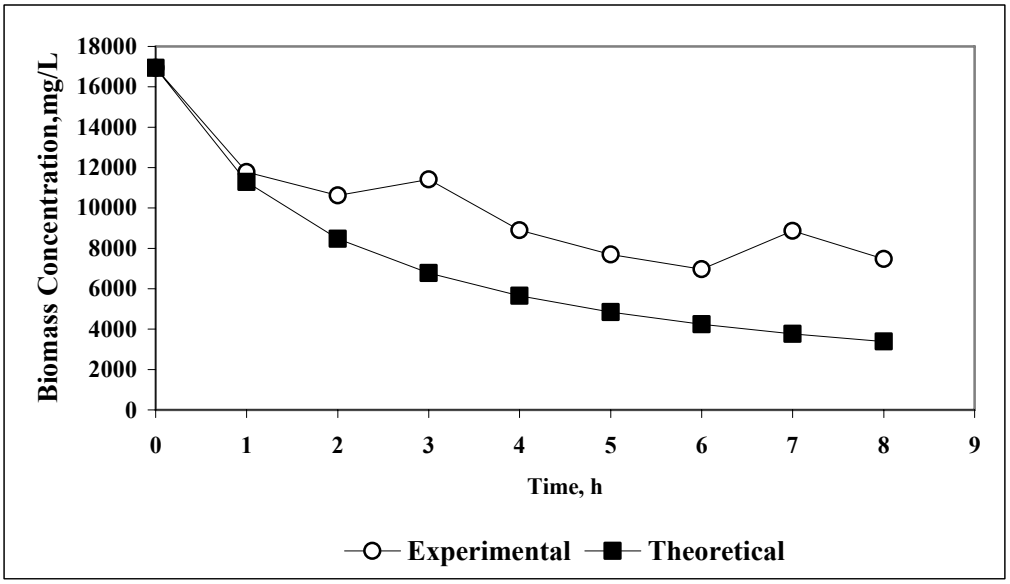

Figure 4.29 Variation of amount of biomass with time (V.Oil Concentration= 3.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

Figure 4.30 indicates variation of COD concentration and percent COD removal with time for co-substrate concentration equal to COD=2000 mg/L and oil concentration of 4.1 % (41 g/L) (w/v). Theoretical COD concentration increased with time due to accumulation of COD. The initial COD concentration in the reaction tank was only 294 mg/L and it was negligible. The theoretical calculation of COD in the system after the cycle was completed yielded 8700 mg/L while observed value was 6550 mg/L. The difference between theoretical and observed values can be explained as the consumption of either co-substrate or the V.Oil by the microorganisms. However, the percent COD removal based on this difference was only 25%.So the activity of the organisms or the biodegradation capability of the culture reduced by some reasons. One of the reasons could be the increased surface tension in the presence of high V.Oil. Surface tension could have adverse effects on the growth of the organisms, on availability of oxygen or on utilization of oxygen etc. So both carbon source and V.Oil can be consumed effectively by the culture.

Variation of vegetable oil concentration and percent vegetable oil removal with time at oil concentration of 4.1 % (41g/L) (w/v) is depicted in Figure 4.31. The V.Oil concentration at the start up was less than 1 g/L. But it slightly increased to 7 g/L at the end of 4th of operation. However a significant increased occurred when the cycle

was completed and the concentration reached to 13 g/L. The theoretical V.Oil concentration in the system was 33 g/L. If these two concentrations are compared, it can be concluded that there was a substantial decrease in the V.Oil concentration. But the percent removal was only 55% and it is lower compared to other lower concentrations studied. The adverse affects of high V.Oil concentrations on biodegradation capability of the organisms are apparent in the V.Oil removal too.

In Figure 4.32 variation of biomass concentration with time is presented. The biomass concentration at the start up was 10 g/L. A significant increase in the biomass concentration was observed as seen in the figure. The concentration would have decreased to 4 g/L by the dilution but it increased to 16 g/L by the end of 6th hour. The final biomass concentration was 12 g/L. The decrease in the biomass concentration is in parallel with the decrease in V.Oil removal efficiency. So this result supports the conclusion that high concentrations of V.Oil affect the biomass activity adversely.

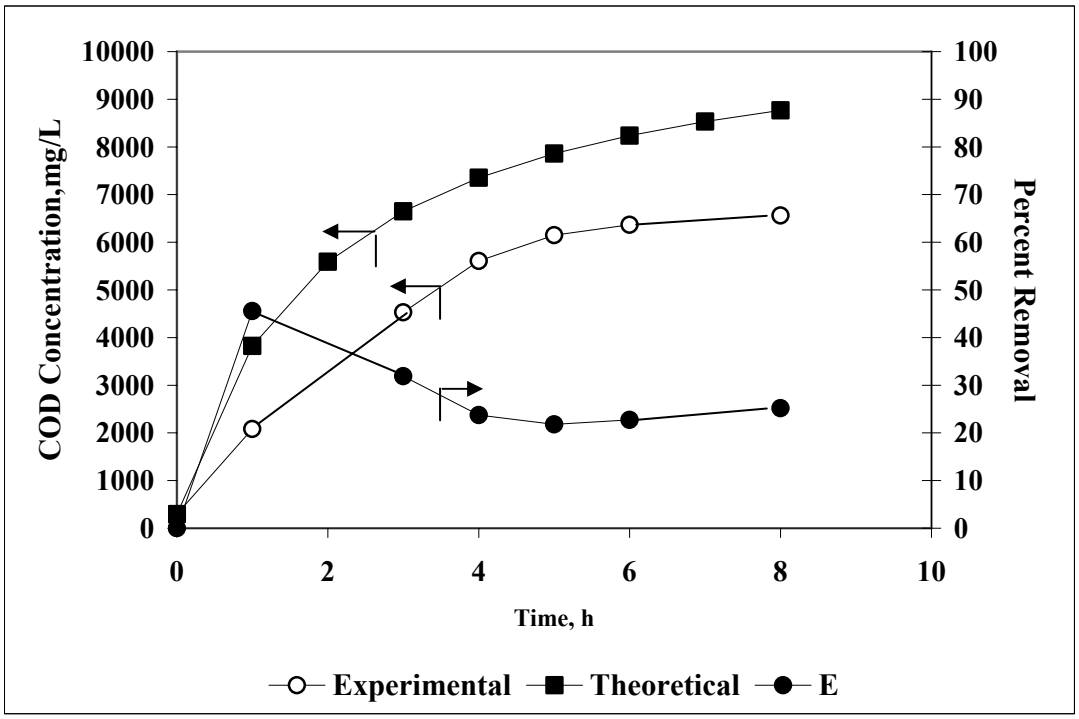

Figure 4.30 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 4.1 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

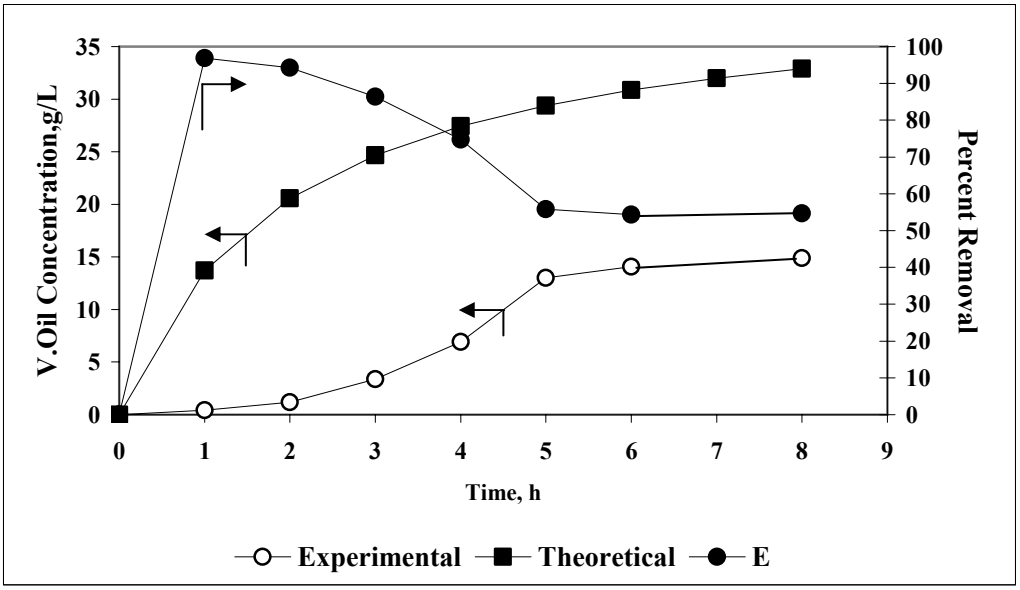

Figure 4.31 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 4.1 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

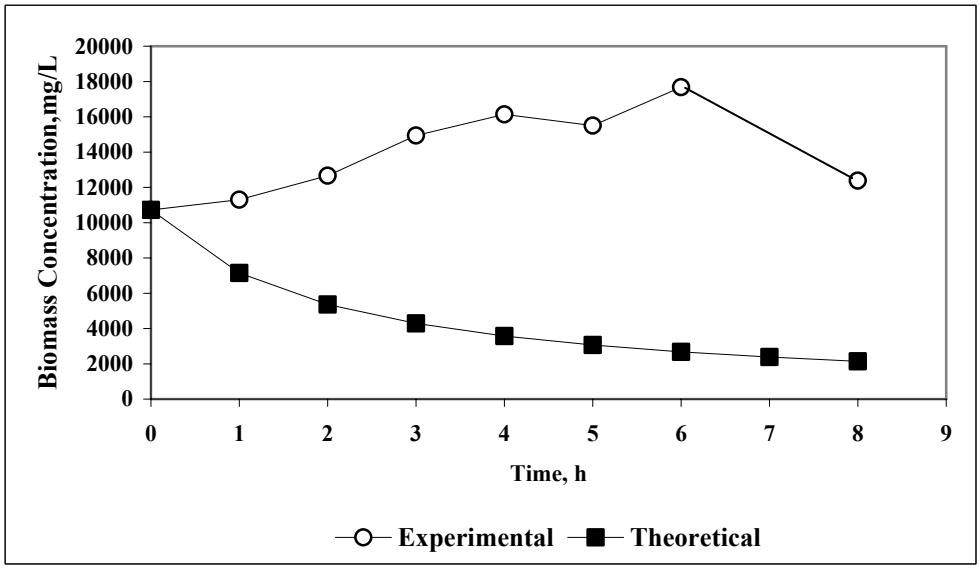

Figure 4.32 Variation of amount of biomass with time (V.Oil Concentration= 4.1 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

Figure 4.33 depicts variation of COD concentration and percent COD removal with time for oil concentration of 6.3 % (63 g/L) (w/v). As seen from the figure, experimental COD concentration increased and thus percent COD removal decreased. Theoretical COD concentration in the aeration tank increased with time due to accumulation of COD. The initial COD concentration in the reaction tank was only 1640 mg/L. Theoretical COD concentration increased to 13000 mg/L at the end of operation period. Similarly, observed COD concentration increased to 11500

mg/L at 8th hour of operation. The percent removal was around 30% for the operation time between 2 and 4 hours. But, it quickly decreased to 9% at the end of operating period. This reason could be the increased surface tension in the presence of high V.Oil. Because surface tension could have adverse effects on the growth of the organisms.

Variation of vegetable oil concentration and vegetable oil removal with time for oil concentration of 6.3 % (63 g/L) (w/v) is depicted in Figure 4.34. Theoretical oil concentration increased with time due to accumulation of oil concentration. Similarly, the observed vegetable oil concentration increased too. Microorganisms were not able to consume oil in the aeration tank. As a result, percent vegetable oil removal was only 10 % at the end of the 8 hours fed-batch operation.

Figure 4.35 depicts variation of amount of biomass with time. The expected biomass concentration due to dilution was 3389 mg/L but the observed concentration was around 6600 mg/L. If presence of rich carbon source as high V.Oil and molasses, the amount of produced biomass is low. Higher biomass concentration could have been expected. The possible explanation for the low biomass than expected could be the limitation in the growth and D.O availability because of high surface tension resulted by V.Oil.

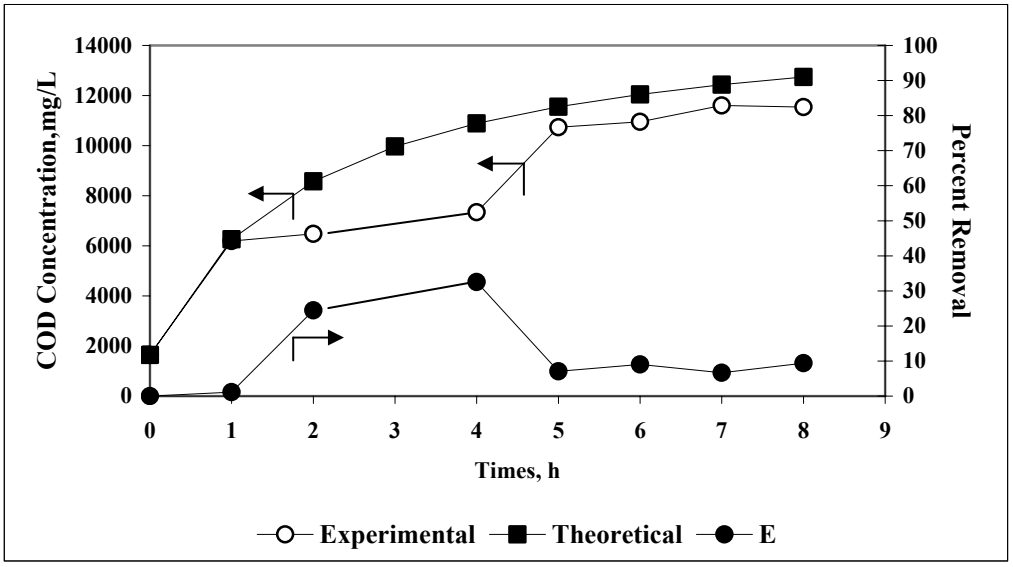

Figure 4.33 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 6.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

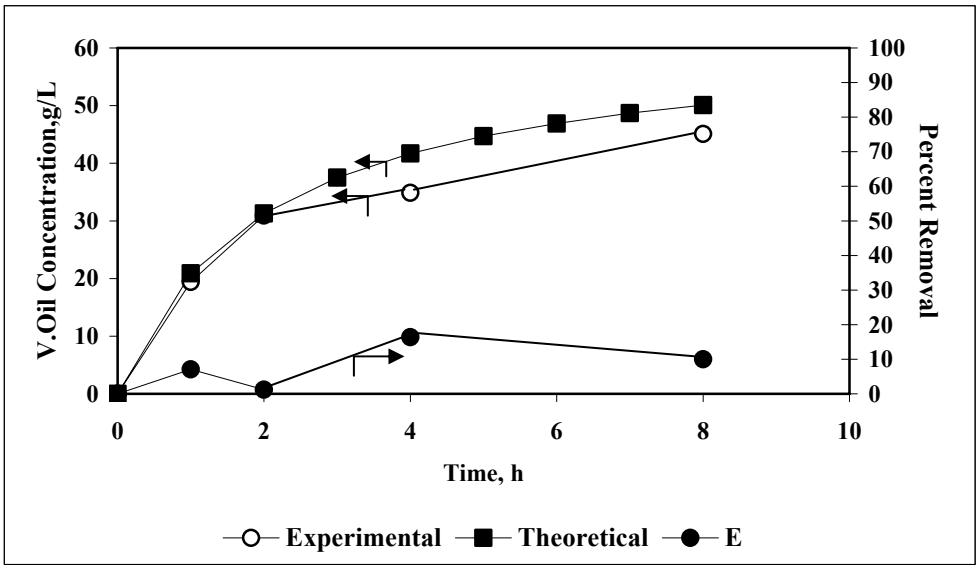

Figure 4.34 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 6.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

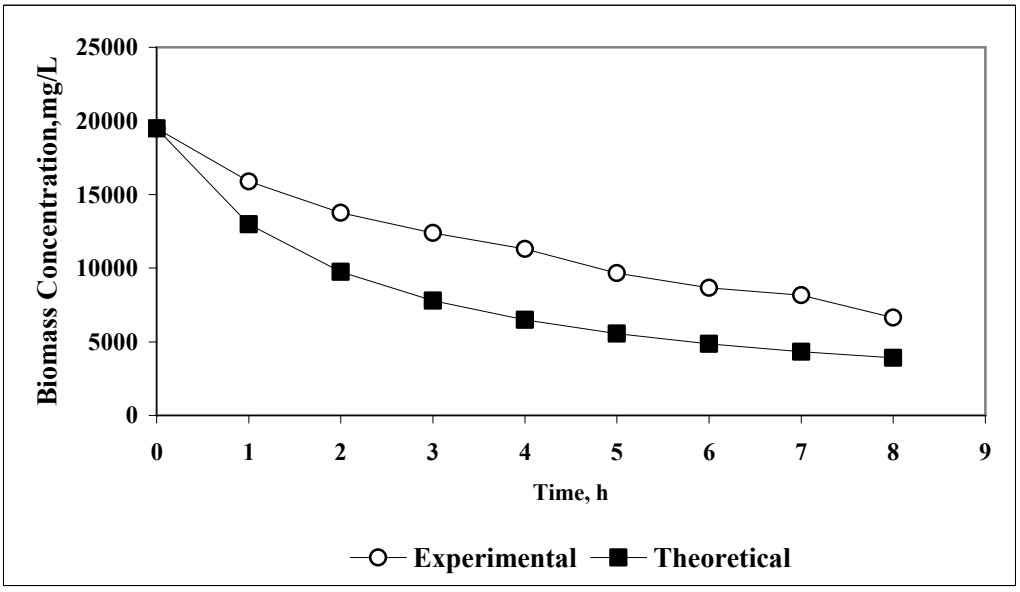

Figure 4.35 Variation of biomass concentration with time. V.Oil Concentration= 6.3 %, COD=2000 mg/L, V_0=1 L, Q=0.5 L/h)

Variation of COD concentration and percent COD removal for different oil concentration is shown in Figure 4.36. When V.Oil concentration increased, observed COD concentration increased and removal efficiency decreased. When oil concentration was higher than 3.3 % (33 g/L), percent COD removal decreased from

84 % to 12% because of increasing oil concentration. The highest COD removal efficiency was obtained, when oil concentration was 3.3 % (33 g/L).

Figure 4.37 depicts variation of vegetable oil concentration and percent vegetable oil removal according to different oil concentration. As seen from the figure, when oil concentration increased, experimental V.Oil concentration increased and due to the fact that, percent removal efficiency decreased. When oil concentration was less than 1 % (10 g/L), percent removal was over 90%. Increasing the V.Oil concentration resulted in 83% V.Oil removal. However higher concentration significantly affected the V.Oil removal efficiency and it decreased to less than 20%.

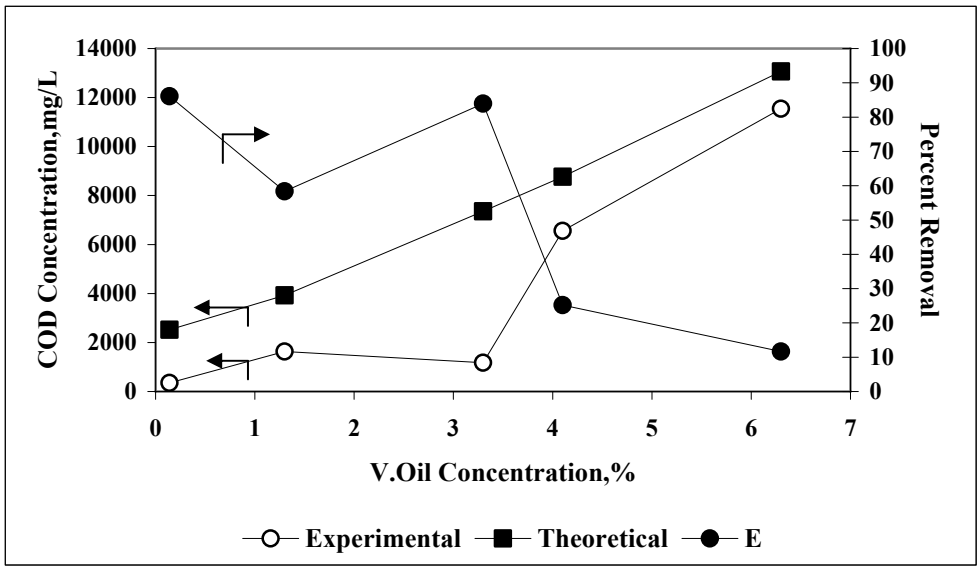

Figure 4.36 Variation of COD concentration and percent COD removal with oil concentration (with co-substrate)

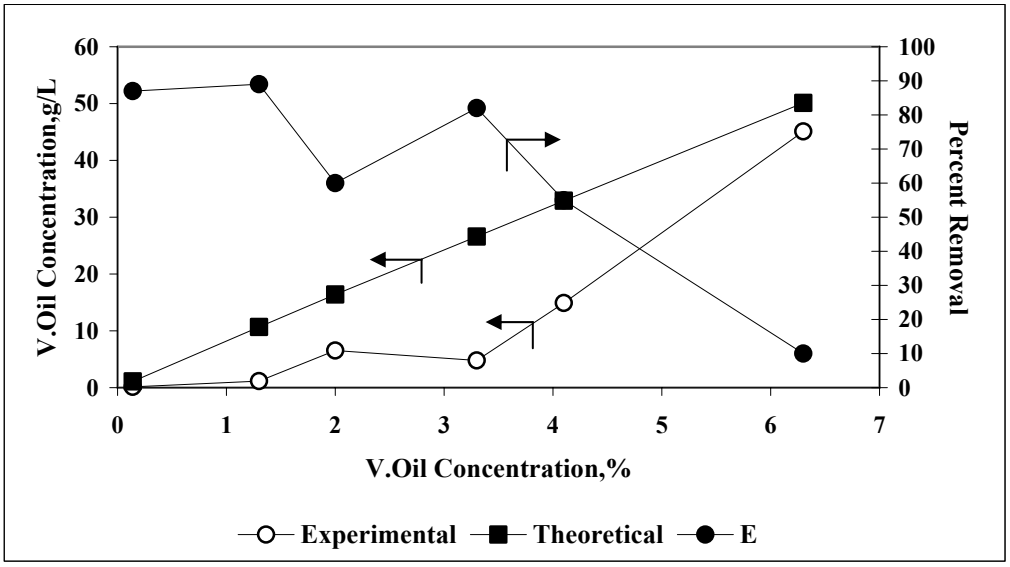

Figure 4.37 Variation of V.Oil concentration and percent V.Oil removal with oil concentration (with co-substrate)

4.2.2 The Effect of Vegetable Oil Concentration on Vegetable Oil and COD Removal without Carbon Source

The vegetable oil removal at different V.Oil concentrations without carbon addition was investigated in order to evaluate if efficient V.Oil removal can be achieved without co-substrate addition. The operation conditions were same as the previous experiments. The oil concentration was varied between 1% (10 g/L) (w/v) and 6% (60 g/L) (w/v).

Figure 4.38 indicates the variation of COD concentration and percent COD removal at 1% (10 g/L) V.Oil concentration. The COD concentration in the initial liquid volume was nearly 715 mg/L. The COD content of the feeding synthetic wastewater was nearly 2000 mg/L which is the COD content of 1% V.Oil. The theoretical COD concentration at the end of the operation cycle was around 3300 mg/L but it was only 360 mg/L when the experiment was conducted. As a result 89% COD removal was obtained.

Figure 4.39 depicts variation of vegetable oil concentration and percent vegetable oil removal at 1% (10 g/L) V.Oil concentration. There was no vegetable oil in the reactor at the start up phase. As seen from the figure, the calculated V.Oil

concentration in the system is 8 g/L when the cycle was completed in 8 hours. However, the experimental results indicate that the final V.Oil concentration was around 1 g/L in the system. So, around 7 g/L V.Oil was consumed by the microorganisms resulting in 88% percent removal.

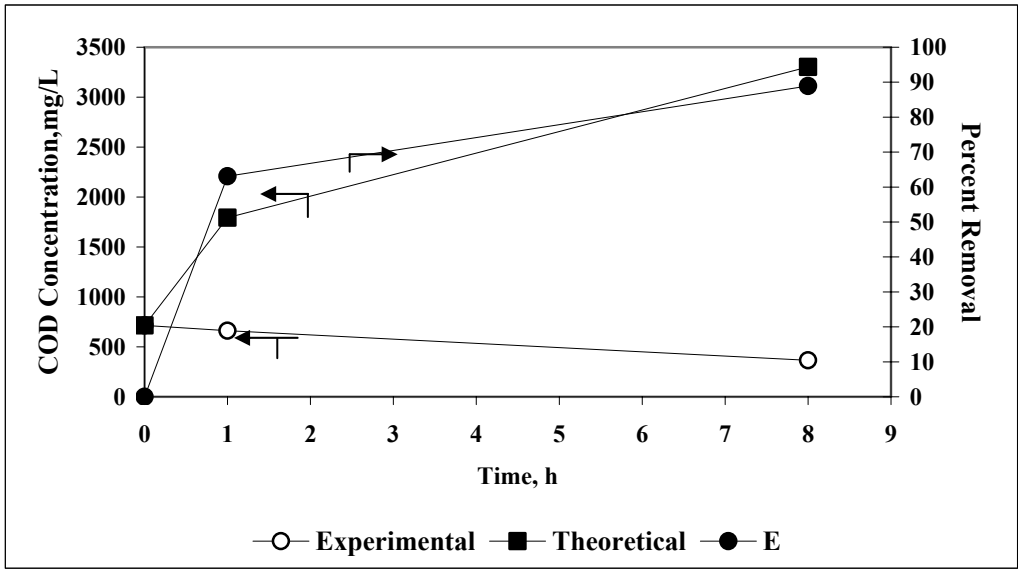

Figure 4.38 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 1 %, V_0=1 L, Q=0.5 L/h)

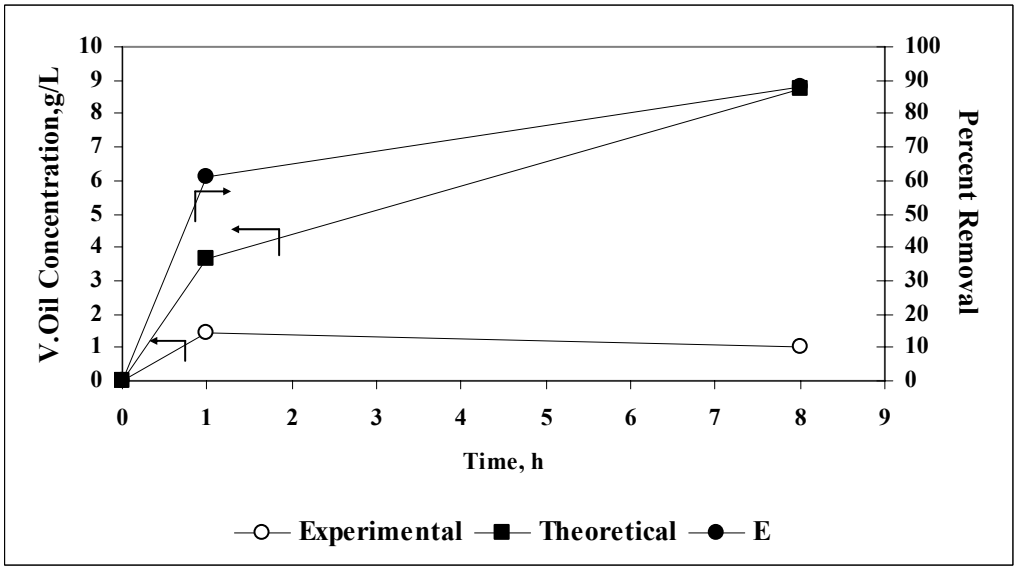

Figure 4.39 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 1 %, V_0=1 L, Q=0.5 L/h)

Figure 4.40 depicts variation of COD concentration and percent COD removal at 2 % (20 g/L) V.Oil concentration. Initial COD concentration was 5645 mg/L. The theoretical COD concentration at the end of the operation cycle was around 5850 mg/L. When the experiment was conducted, it was only 2730 mg/L. Because of this, about 53 % COD removal was obtained.

Variation of vegetable oil concentration and percent V.Oil removal at 2 % (20 g/L) oil concentration with 8 hours of operation time is shown in Figure 4.41. The V.Oil concentration in the starting volume was negligible. As seen from the figure, theoretical V.Oil concentration increased to 16.1 g/L in time. However, the V.Oil concentration in the reactor was significantly lower compared to the theoretical one. That is, the calculated V.Oil concentration in the system is about 3 g/L when the cycle was completed in 8 hours. Thus, around 13 g/L vegetable oil was consumed by the microorganisms resulting in 81 % percent removal.

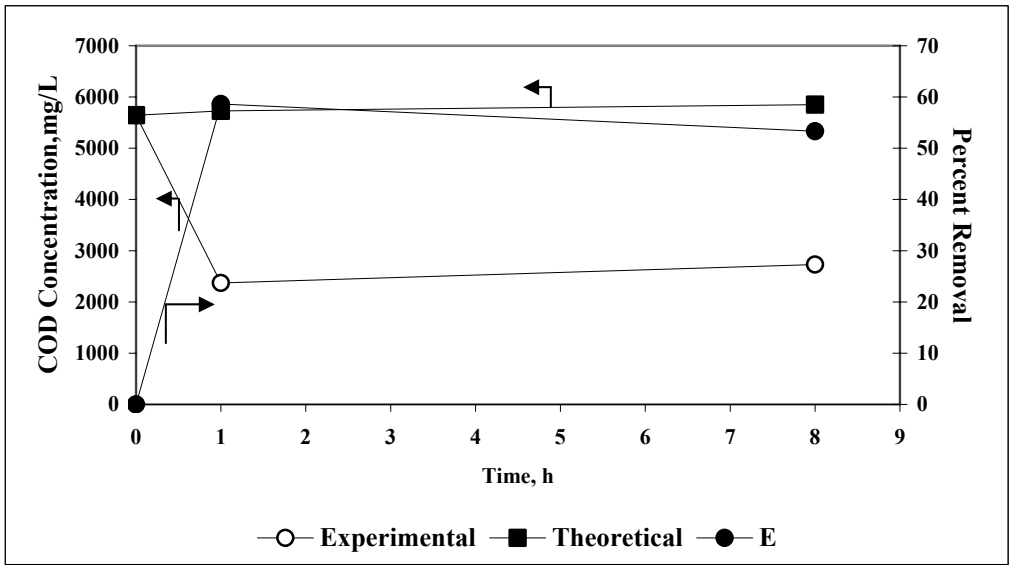

Figure 4.40 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 2 %, V_0=1 L, Q=0.5 L/h)

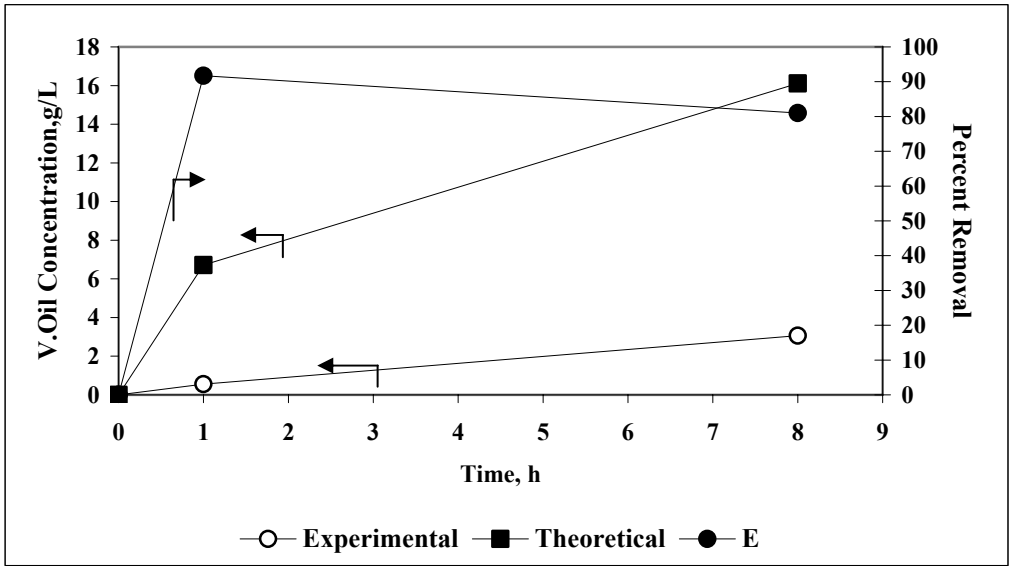

Figure 4.41 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 2 %, V_0=1 L, Q=0.5 L/h)

Figure 4.42 indicates variation of COD concentration and percent COD removal with time and oil concentration of 3.3 % (33 g/L). The initial COD content in the aeration tank was nearly 1990 mg/L. The theoretical COD concentration at the end of the operation was around 6100 mg/L. When the experiment was completed, observed value was 3663 mg/L. As a result percent COD removal was 40 % at the end of operation.

Variation of vegetable oil concentration and percent vegetable oil removal at 3.3 % (33 g/L) oil concentration is depicted in Figure 4.43. The initial vegetable oil content in the aeration tank was negligible. As seen in the figure theoretical V.Oil concentration increased to 26.6 g/L in time. But the observed vegetable oil concentration in the reactor was significantly lower compared to the theoretical V.Oil concentration. The calculated V.Oil concentration in the system is about 4.8 g/L when the cycle was completed in 8 hours. That is about 21 g/L oil was consumed by the microorganisms and as a result percent vegetable oil removal was 82 % at the end of the fed-batch operation.

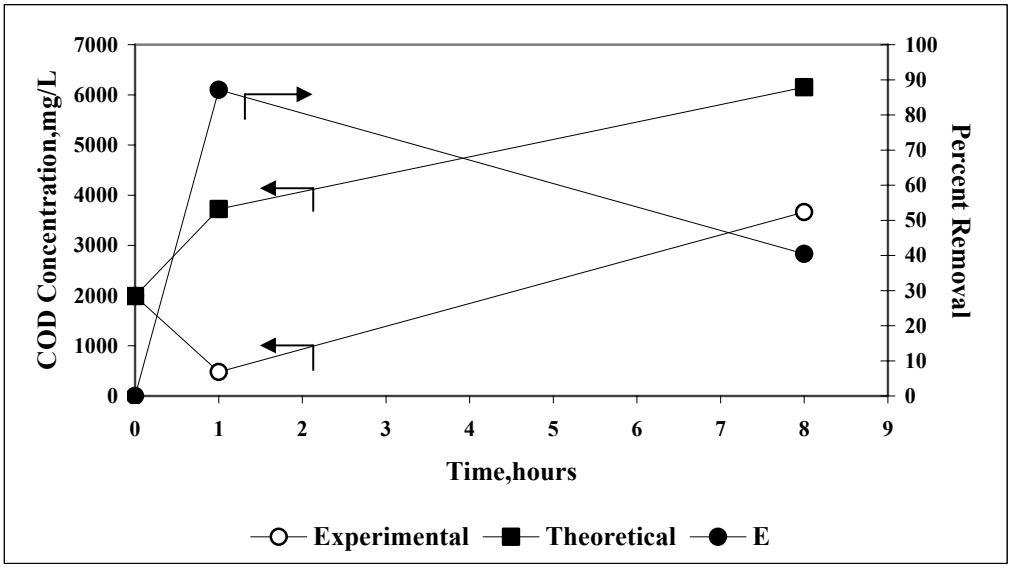

Figure 4.42 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 3.3 %, V_0=1 L, Q=0.5 L/h)

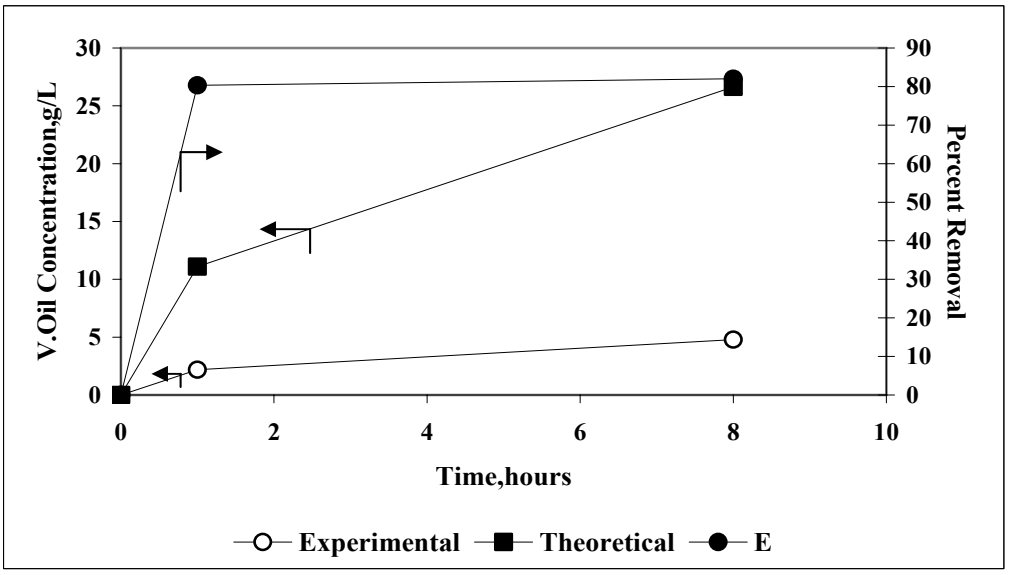

Figure 4.43 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 3.3 %, V_0=1 L, Q=0.5 L/h).

Figure 4.44 indicates variation of COD concentration and percent COD removal with time at 4% (40 g/L) vegetable oil concentration. The initial COD content in the aeration tank was nearly 3773 mg/L. Theoretical COD concentration increased from 3773 mg/L to 7770 mg/L with time due to accumulation of COD. On the other hand, the experiment COD concentration decreased from 3773 mg/L to about 2500 mg/L. As a result percent COD removal was 67 % at the end of the 8 hours of operation.

Variation of vegetable oil concentration and percent vegetable oil removal at 4 % (40 g/L) V.Oil concentration with 8 hours of operation time is shown in Figure 4.45. Theoretical V.Oil concentration increased with time because of accumulation of oil in the system. The initial vegetable oil content in the aeration tank was negligible. As seen from the figure, the theoretical V.Oil concentration in the system is 32 g/L when the cycle was completed. However, experimental results determined that the final vegetable oil concentration was about 9 g/L at the end of the system. Thus, about 23 g/L V.Oil was removed by the microorganisms and as a result 72 % V.Oil removal was obtained.

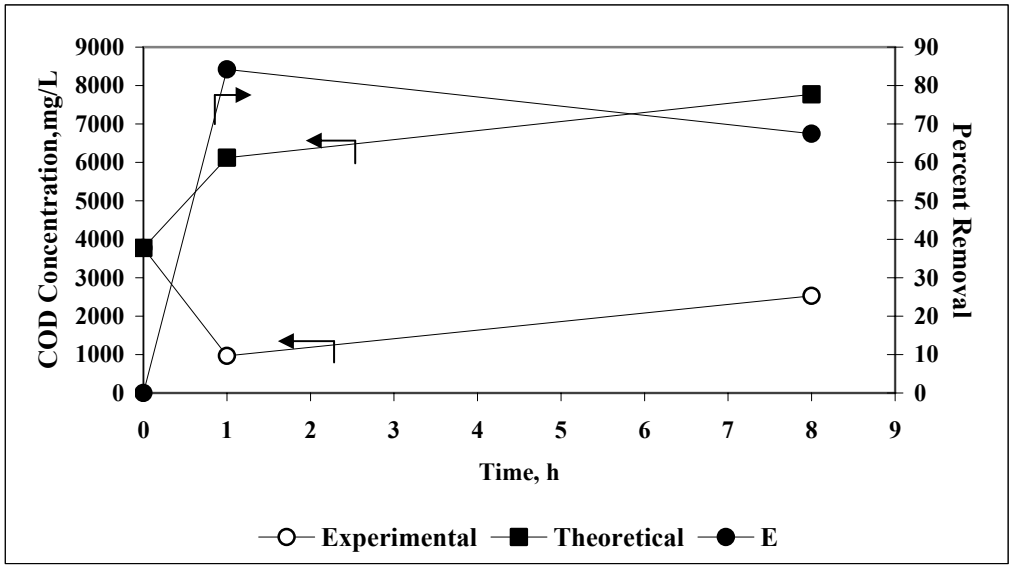

Figure 4.44 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 4 %, V_0=1 L, Q=0.5 L/h)

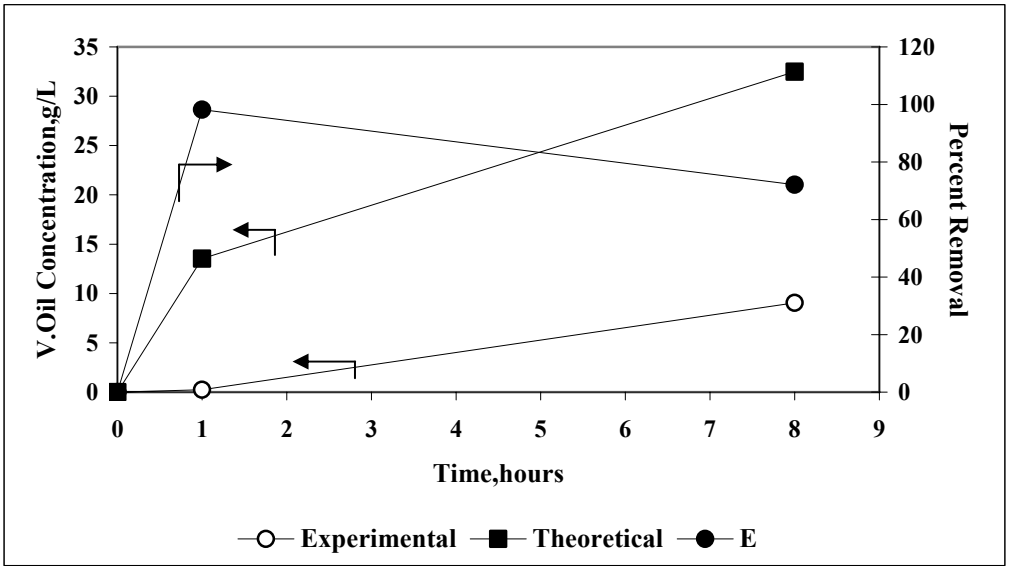

Figure 4.45 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 4 %, V_0=1 L, Q=0.5 L/h)

Vegetable oil concentration was increased to 5.7 % (57 g/L) in the final experiment. COD in the experimental tank was nearly 463 mg/L and it was negligible. Figure 4.46 depicts variation of COD concentration and percent COD removal with time. Theoretical COD concentration increased to about 10000 mg/L with time due to accumulation of COD. Similarly, measured COD concentration increased to 4500 mg/L at 8th hours of operation. As a result, percent COD removal was 53 % at the end of the operation.

Variation of vegetable oil concentration and percent vegetable oil removal with 8 hours of operation time is shown in Figure 4.47. The initial vegetable oil content in the aeration tank was negligible. As seen from the figure theoretical oil concentration increased to 46 g/L in time. But, the experimental results indicate that V.Oil concentration of the end of 8th hours was around 14 g/L. When the difference between theoretical and the observed concentrations was considered, 69 % V.Oil removal was obtained.

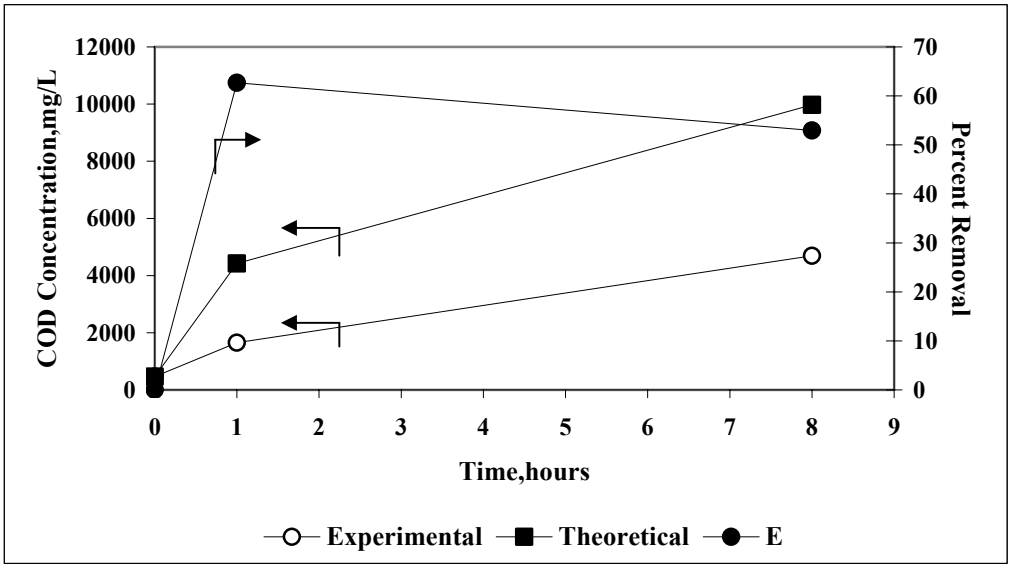

Figure 4.46 Variation of COD concentration and percent COD removal with time (V.Oil Concentration= 5.7 %, V_0=1 L, Q=0.5 L/h)

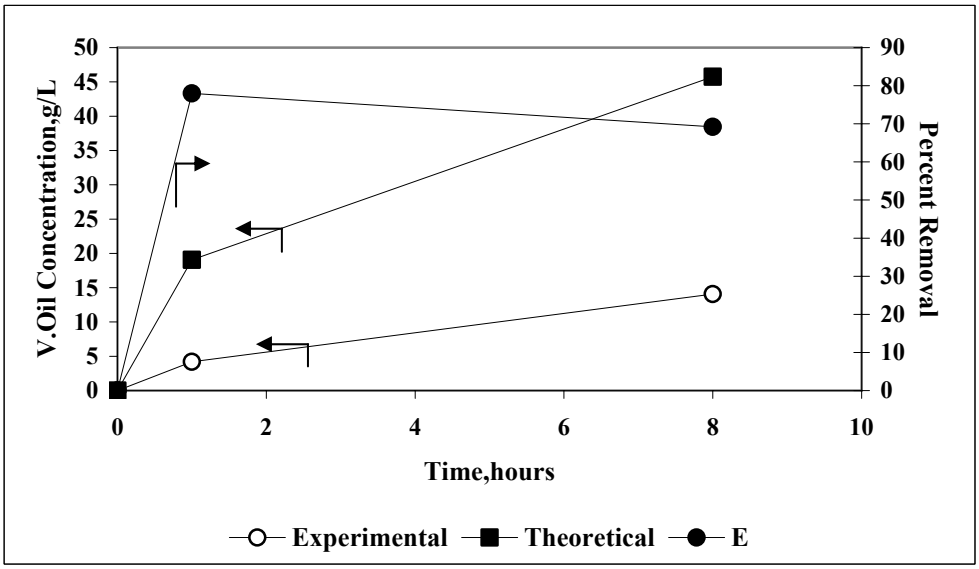

Figure 4.47 Variation of V.Oil concentration and percent V.Oil removal with time (V.Oil Concentration= 5.7 %, V_0=1 L, Q=0.5 L/h)

Variation of COD concentration and percent COD removal for different oil concentration is shown in Figure 4.48. According to figure when oil concentration increased, observed COD concentration increased and removal efficiency decreased. As seen from the figure when oil concentration was higher than about 3 % (30 g/L), percent COD removal decreased from 89 % to 40 % because of increasing V.Oil concentration. The highest COD removal efficiency was obtained when oil concentration was 1 % (10 g/L).

Figure 4.49 depicts variation of vegetable oil concentration and percent removal according to different oil concentration. As seen from the figure when oil concentration increased, observed vegetable oil concentration increased and due to the fact that, percent removal efficiency decreased. When oil concentration was less than about 3 % (30 g/L), vegetable oil removal was over 82 %. With increasing oil concentration, V.Oil removal significantly was affected and it decreased from 82 % to 69 %.

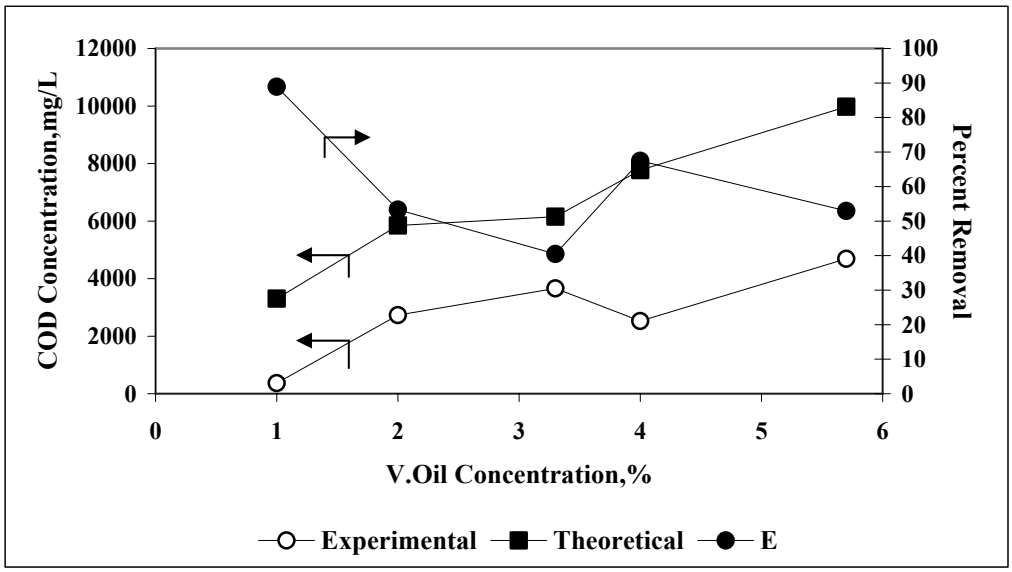

Figure 4.48 Variation of COD concentration and percent COD removal with oil concentration (without co-substrate)

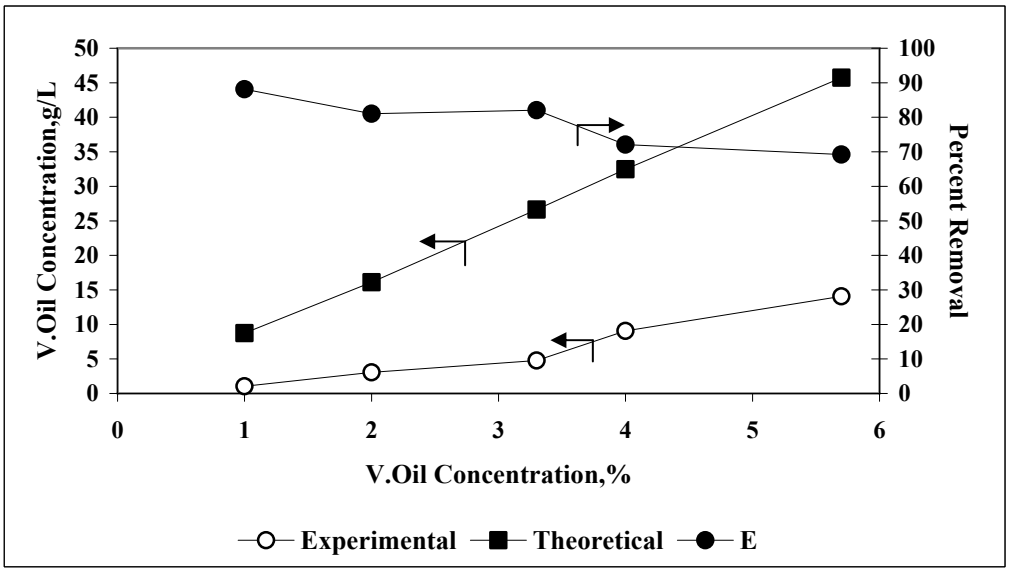

Figure 4.49 Variation of V.Oil concentration and percent V.Oil removal with oil concentration (without co-substrate)

Comparison of COD percent and V.Oil removal with and without co-substrate is shown in Figure 4.50, Figure 4.51, Figure 4.52 and Figure 4.53. When co-substrate was not used, the higher removal was obtained at both percent COD and vegetable oil removal. On the other hand, when V.Oil concentration was about 6 % (60 g/L), COD and V.Oil removal efficiency was 12 % and 10 % with co-substrate, respectively (in Figure 4.50 and Figure 4.51) and COD and V.Oil removal efficiency was 53 % and 69 % without co-substrate, respectively (in Figure 4.52 and Figure 4.53). As a result, when oil concentration was between 3 % (30 g/L) and 4 % (40 g/L), maximum removal efficiency was obtained in both with co-substrate and without co-substrate.

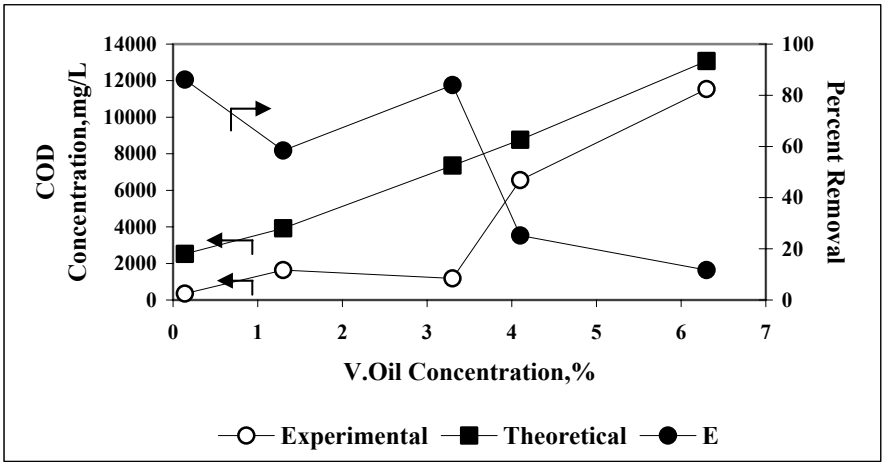

Figure 4.50 Variation of percent COD removal with co-substrate

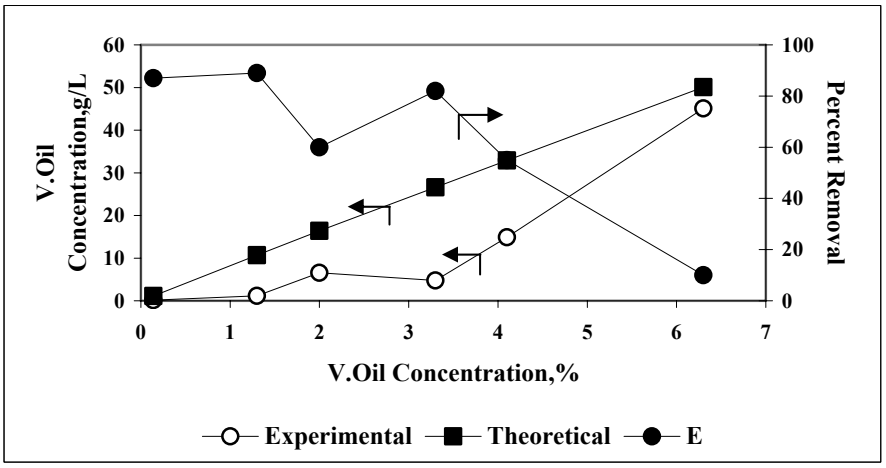

Figure 4.51 Variation of percent V. Oil removal with co-substrate

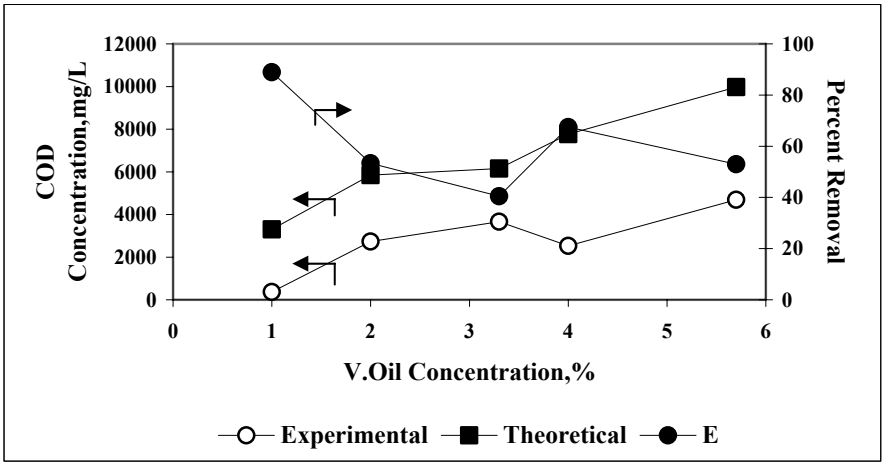

Figure 4.52Variation of percent COD removal without co-substrate

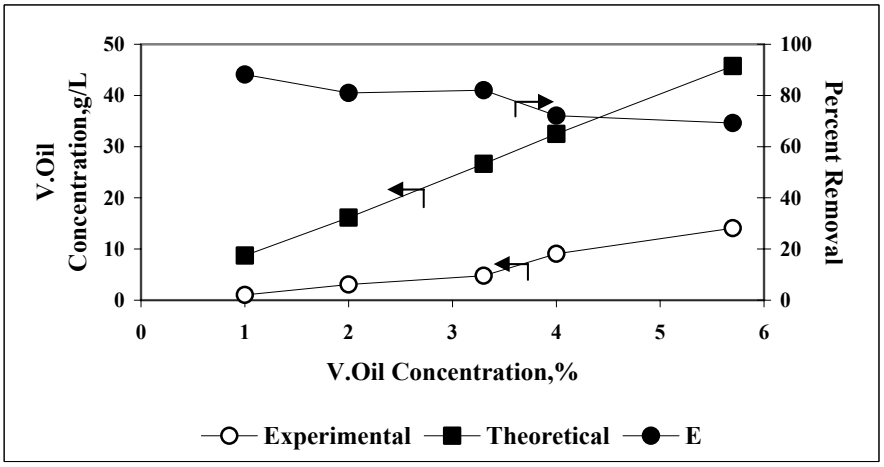

Figure 4.53 Variation of percent V. Oil removal without co-substrate

4.2.3 The Effect of Sludge Age (θc) on Vegetable oil and COD Removal

In order to determine the maximum COD and vegetable oil removal, the system was operated at three different sludge retention times between θc=18 days and θc=30 days. Vegetable Oil and COD concentrations and removal efficiencies for each retention times were determined. In this experiment, the feed flow rate was kept constant at Q=0.5 L/h and COD contents of the feed were adjusted to 2000 mg/L. Molasses was used as carbon source. Fed-batch experiments continued for eight hours. Operation time was 8 hours. Starting volume of the aeration tank was 1 liter during all of the experiments. Temperature and pH were 20-25 ^{0}C and pH=7-7.5, throughout the experiments. Oil concentration was 4 % (w/v) for the all the

experiments. The 1st and 8th hours of the V.Oil, biomass and COD concentrations were taken into consideration in the experiments.

The experiments were repeated three times for θc=18, 25 and 30 days. The mean and the standard deviations of COD, Oil and biomass concentrations for the repeated experiments at different sludge ages were given in Table 4.7.

Tablo 4.7 Values of average and standard deviation for each one of COD, Oil, and biomass concentration

	Vegetable Oil Concentration								
	θc=18 day			θc=25 day			θc=30 day		
	Theor.	E	Observ.	Theor.	E	Observ.	Theor.	E	Observ.
Average	32	51	15,56	34,33	33	23,3	35,03	34	23,03
Standard Deviation	0	12	3,72	2,08	16	6,77	2,64	12	4,28
	COD Concentration								
	θc=18 day			θc=25 day			θc=30 day		
	Theor.	E	Observ.	Theor.	E	Observ.	Theor.	E	Observ.
Average	8557	44	4836	8758	30	6104	8683	28	6269
Standard Deviation	23,58	13	1099	50,81	22	1938	36,67	4	257
	Biomass Concentration								
	θc=18 day			θc=25 day			θc=30 day		
	Theor.		Observ.	Theor.		Observ.	Theor.		Observ.
Average	3401		8667	11948		21893	5321		8707
Standard Deviation	762,37		1137	735		9217	1913		3029

Figure 4.54 indicates variation of COD concentration and percent COD removal at sludge ages 18 d, 25 d and 30 d. As seen from the figure, when sludge age increased, percent COD removal decreased. The percent removal was around 45% at 18 days sludge age, but it was around 30% for 25 days sludge age and decreased further to 25% for 30 days sludge age. The general approach in biological wastewater treatment is better COD removal as sludge age increased. But this was not the case in our experiments. The highest COD removal was observed at 18 days sludge age.

Variation of vegetable oil concentration and percent V.Oil removal at sludge age=18, 25 and 30 days in the system is depicted in Figure 4.55. When sludge age was increased, percent vegetable oil removal decreased. Percent V.Oil removal was around 55% at 18 days sludge age, but it decreased to 35% when sludge age was 25 and 30 day. Increasing sludge age did not improve the V.Oil removal at 4% V.Oil concentration but reduced the removal of the substrate.

In Figure 4.56 variation of amount of biomass at sludge age=18, 25 and 30 days in the system is presented. As seen from the figure, there is significant difference between theoretical and observed biomass concentrations at the end of the operation. Therefore, this result indicates that there was biomass synthesis in the system.

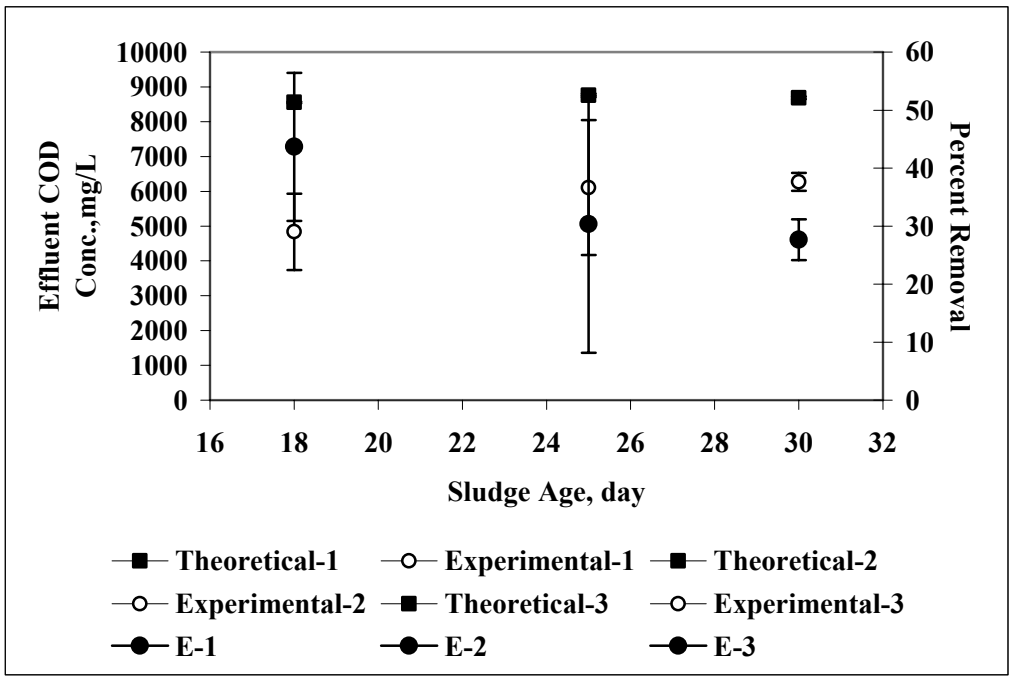

Figure 4.54 Variation of COD concentration and percent COD removal at sludge age= 18, 25 and 30 days

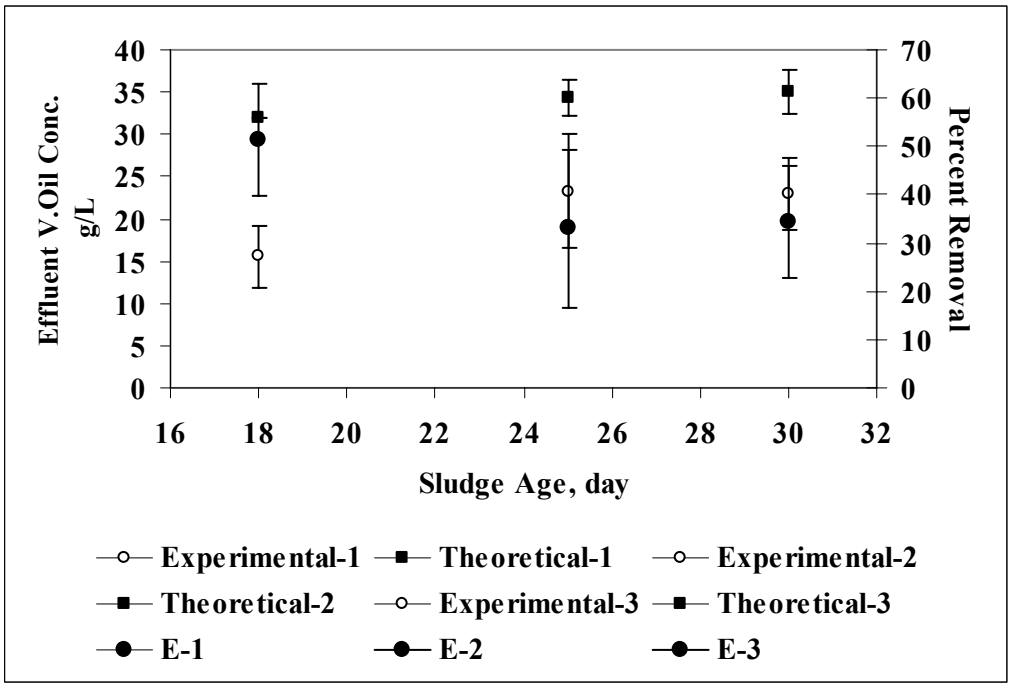

Figure 4.55 Variation of V.Oil concentration and percent V.Oil removal at sludge age= 18, 25 and 30 days

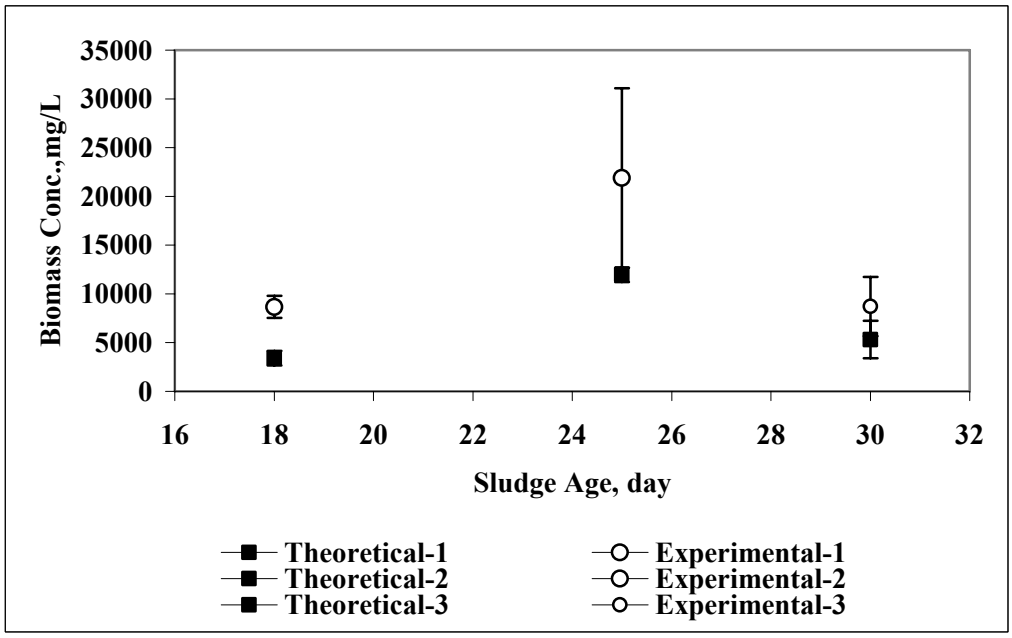

Figure 4.56 Variation of amount of biomass at sludge age= 18, 25 and 30 days

4.2.4 The Effect of Biosurfactant Addition on Vegetable oil and COD Removal

In this study, the effect of biosurfactant on biodegradability of oil was investigated by fed-batch operation. The variables were the biosurfactant and oil concentration, and the effect of those operation conditions on vegetable oil and COD

removal and biomass concentration were studied. In this experiment, the feed flow rate was kept constant at Q=0.5 L/h and the sludge age (θc) were adjusted to 15 days. Molasses was used as carbon source and JBR 210 rhamnolipid was used as biosurfactant. Fed-batch experiments continued for eight hours. Starting volume of the aeration tank was 1 liter during all of the experiments. Temperature and pH were 20-25 ^{0}C and pH=7-7.5, throughout the experiments. Vigorous aeration was supplied to the aeration tank to keep the dissolved oxygen (DO) above 2 mg/L. The system was operated at three different biosurfactant concentration (20, 40, and 100 mg/L). COD and V.Oil removal efficiencies for each biosurfactant concentration were determined.

4.2.4.1 Operation with Carbon Source

The oil concentration was adjusted to 4 % (40 g/L) and 6 % (60 g/L) and the external carbon source was provided as COD=2000 mg/L. The biosurfactant concentration was varied between 20-100 100 mg/L for each V.Oil concentrations. Table 4.7 depicts the percent COD, V.Oil removal and increase in the biomass concentration when biosurfactant added.

As seen from the Table 4.8, it seems that oil concentration increases with biosurfactant addition. This result can be explained as the contribution of oil content of biosurfactant to the concentration of V.Oil. Therefore, there was negative effect of biosurfactant on the system rather than improvement in the removal of the V.Oil. The similar effect was observed in COD removal. The COD content of the biosurfactant significantly contributed to the total COD load. As a result, the microorganisms were not able to removal COD because of high COD load.

Tablo 4.8 Effect of biosurfactant on COD, Oil, and biomass removal with addition carbon source

Oil Conc. (%)	BS Conc. (mg/L)	Theor. Oil (mg/L)	E (%)	Observ. Oil (mg/L)	Theor. COD (mg/L)	E (%)	Observ. COD (mg/L)	Theor. Biomass (mg/L)	Observ. Biomass (mg/L)
4	100	32	-15	36,84	10002	4	9645	4508	7540
6	20	48	21	38,058	12097	22	9459	2164	9060
6	40	48	-16	55,8	13350	6	12550	2628	9280
6	100	48	6	45,166	12185	12	10755	2452	9840

4.2.4.2 Operation without Carbon Source

The second stage of the experiments with biosurfactant is the operating the system without adding any readily biodegradable carbon source. The oil concentration was 6 %, and biosurfactant concentration=20 and 40 mg/L. The results of the experiments were depicted in Table 4.9. Almost the same results were obtained as the previous experiments. The results indicated that biosurfactant addition does not improve the oil and COD removal.

Table 4.9 Effect of biosurfactant on COD, Oil, and biomass removal without addition carbon source

Oil Conc. (%)	BS Conc. (mg/L)	Theor. Oil (mg/L)	E (%)	Observ. Oil (mg/L)	Theor. COD (mg/L)	E (%)	Observ. COD (mg/L)	Theor. Biomass (mg/L)	Observ. Biomass (mg/L)
6	20	53,78	15	45,65	12527	17	10360	4240	6720
6	40	48	-17	56	13376	6	12600	7036	8740

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study was performed to investigate the vegetable oil containing wastewater treatment by biological methods. Two sets of experiments; batch flask and fed-batch operation of activated sludge process were performed. Oil and COD removal was studied by using activated sludge culture acclimated to the V.Oil biodegradation. The effects of significant operating parameters on removal of V.Oil and COD were determined and the effect of biosurfactant addition on V.Oil removal was evaluated.

Following results was obtained.

1. Biomass concentration is a significant factor in treatment of vegetable oil containing wastewater. COD and vegetable oil removal is significantly affected by the biomass concentration and co-substrate concentration. External carbon source addition enhances the biological vegetable oil degradation. Keeping biomass at high concentration and providing enough co-substrate at high vegetable oil concentration is essential to obtain efficient treatment. When oil concentration is high, COD and vegetable oil removal efficiency decrease but when oil concentration is low, COD and vegetable oil removal increase. Maximum percent COD and v.oil removal was obtained about between 77% and 95%.

2. In the fed-batch operation with co-substrate, as the V.Oil concentration increases, the available carbon source increases too. So the condition was high food but less biomass concentration. In other words, there was excess amount of food for the biomass and therefore the culture increased its mass. When oil concentration was increased, the activity of the organisms or the biodegradation capability of the culture reduced by some reasons. One of the

reasons could be the increased surface tension in the presence of high V.Oil. Surface tension could have adverse effects on the growth of the organisms, on availability of oxygen or on utilization of oxygen etc. So both carbon source and V.Oil can be consumed effectively by the culture. Also the adverse affects of high vegetable oil concentrations on biodegradation capability of the organisms are apparent in the V.Oil removal too. High concentration of vegetable oil affects the biomass activity, adversely.

3. In the fed-batch operation without co-substrate, increasing oil concentration caused to increase COD concentration and decrease removal efficiency. When oil concentration was increased from 1% to 5.7%, COD removal efficiency decreased from 89% to 53%. The highest COD removal efficiency was obtained with 4% oil concentration. When oil concentration was higher than 3%, vegetable oil removal decreased because of increasing oil concentration. On the other hand, when carbon source was not used, maximum removal efficiency was obtained at both percent COD and vegetable oil removal. As a result when oil concentration was 3.3% and 4%, maximum COD and V.Oil efficiency were obtained in both with co-substrate and without co-substrate experiments.

4. Increasing sludge age from θ_C=15 days to θ_C=30 days significantly affect the COD removal performances. The most significant effect was observed at 18 days sludge age. When sludge age increased, percent COD removal decreased. That is when sludge age was 25 and 30 days, COD removal efficiency was 30 % and 25 %, respectively. Similarly, increasing sludge age from θ_C=15 days to θ_C=30 days negatively affect the oil removal performances. The most significant effect was observed at 18 days sludge age. When sludge age increased, percent vegetable oil removal decreased. That is when sludge age was 25 and 30 day, percent vegetable oil removal was 33 % and 35 %, respectively. Increasing sludge age did not improve the V.Oil removal at 4% V.Oil concentration but reduced the removal of the substrate. Also increasing sludge age from θ_C=15 days to θ_C=30 days

significantly affect the biomass concentration. Biomass concentration increased by increasing sludge age. So this result indicates that there was biomass synthesis in the system.

5. The effect of biosurfactant on biodegradability of oil concentration was investigated by fed-batch operation with changing biosurfactant concentration and oil concentration, and the effect of that operation condition on vegetable oil and COD removal was studied. The fed-batch experiments with addition of biosurfactant concentration were carried out both with carbon source and without carbon source. With carbon source, there was negative effect of biosurfactant on the system rather than improvement in the removal of the V.Oil. The similar effect was observed in COD removal. That is the COD content of the biosurfactant significantly contributed to the total COD load. Without carbon source, almost the same results were obtained as the previous experiments. Finally, there was no significant effect of biosurfactant on vegetable oil and COD removal. Biosurfactant addition does not enhance the vegetable oil and COD removal.

5.2 Recommendations

Following recommendations can be made for future studies on investigation of vegetable oil containing wastewater treatment by biological methods.

1. The lipid removal under anaerobic conditions can be evaluated.

2. The lipid removal in continuous operations should be studied.

3. The performance of suspended and immobilized biological systems in lipid removal should be investigated.

4. The effect of biosurfactant on lipid removal should be investigated in detail and optimum biosurfactant concentration should be determined.

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APPENDIX

RAW DATA OF EXPERIMENTAL STUDIES

6.1 Raw Data for Box-Wilson Experimental Design

Table 6.1 Variation of glucose, vegetable oil, and biomass concentration on COD and V.Oil removal.

Sample no	Glucose Conc. (mg/L)	V.Oil Conc. (%)	Biomass Conc. (mg/L)	Effluent COD Concentration (mg/L)	Experimental COD Conc. (mg/L)	E (%)	Effluent V.Oil Concentration (g/L)	Experimental V.Oil Conc. (g/L)	E (%)
1	1578	2.5	2396	8177	643	92	22.5	5.85	74
2	421	2.5	2396	1684	238	86	22.5	7	69
3	1000	1.6	2396	580	74	87	14.4	4.5	69
4	1000	3.4	2396	517	137	74	30.6	9.7	68
5	1000	2.5	3091	6628	3324	50	22.5	5.175	77
6	1000	2.5	1701	6637	4303	35	22.5	7.875	65
7	2000	4	3594	1684	86	95	36	7.2	80
8	0	4	3594	643	244	62	36	8.3	77
9	2000	1	3594	1274	308	76	9	1.3	86
10	2000	4	1198	1463	279	81	36	14.08	61
11	0	1	3594	580	55	91	9	0.32	96
12	2000	1	1198	1306	260	80	9	1.01	89
13	0	4	1198	896	690	23	36	19.8	45
14	0	1	1198	612	291	52	9	1.56	83
15	1000	2.5	2396	7166	2378	67	22.5	6.3	72
16	1000	2.5	2396	7622	2126	72	22.5	7.425	67
17	1000	2.5	2396	7451	2252	70	22.5	6.975	69

6.2 Raw Data for Fed-Batch Experiments

6.2.1. The effect of vegetable oil concentration on COD and V.Oil removal in the presence of co-substrate

Table 6.2 V.Oil concentration= 0.14 % (w/v), θ_H = 7 h, θc= 15 days, and COD= 2000 mg/L.

Time (h)	**COD-Theoretical (mg/L)**	**COD-Experiment (mg/L)**	**E (%)**	**V.Oil-Theoretical (g/L)**	**V.Oil-Experiment (g/L)**	**E (%)**	**Biomass-Theoretical (mg/L)**	***Biomass-Experiment (mg/L)***
0	1635	1635	0	0	0	0	12480	*12480*
1	2014	2229	0	0,475	0,044	91	8320	*9520*
2	2203	735	67	0,712	0,029	96	6240	*7880*
3	2317	393	83	0,854	0,02	98	4992	*6420*
4	2392	447	81	0,949	0,053	94	4160	*5460*
5	2446	411	83	1,017	0,2	80	3566	*5240*
6	2487	322	87	1,068	0,165	85	3120	*4560*
7	*2519*	*1688*	*33*	*1,108*	*0,141*	*87*	*2773*	*4220*

Table 6.3 V.Oil concentration= 1.3 % (w/v), θ_H = 8 h, θc= 15 days, and COD= 2000 mg/L

Time (h)	**COD-Theoretical (mg/L)**	**COD-Experiment (mg/L)**	**E (%)**	**V.Oil-Theoretical (g/L)**	**V.Oil-Experiment (g/L)**	**E (%)**	**Biomass-Theoretical (mg/L)**	***Biomass-Experiment (mg/L)***
0	3558	3558	0	0	0	0	26400	*26400*
1	4601	6760	0	4,45	0,57	87	17600	*21980*
2	5123	2550	50	6,68	0,57	91	13200	*17320*
3	5436	535	90	8,01	0,5	94	10560	*15880*
4	5645	1651	71	8,90	0,89	90	8800	*14060*
5	5794	2874	50	9,54	1,17	88	7543	*11340*
6	5906	2334	60	10,01	0,942	91	6600	*10760*
7	5992	1543	74	10,38	0,726	93	5867	*9380*
8	*6062*	*4889*	*19*	*10,68*	*1,148*	*89*	*5280*	*8820*

Table 6.4 V. Oil concentration= 2 % (w/v), θ_H = 8 h, θc= 15 days, and COD= 2000 mg/L

Time (h)	**V.Oil-Theoretical (g/L)**	**V.Oil-Experiment (g/L)**	**E (%)**	**Biomass-Theoretical (mg/L)**	***Biomass-Experiment (mg/L)***
0	0	0	0	6120	*6120*
1	6,83	0,212	97	4080	*5920*
2	10,3	1,25	88	3060	*5260*
3	12,3	0,31	97	2448	*4640*
4	13,7	0,97	93	2040	*4600*
5	14,6	3,052	79	1749	*6420*
6	15,4	12,18	21	1530	*6860*
7	15,9	5,778	64	1360	*9020*
8	*16,4*	*6,548*	*60*	*1224*	*10800*

Table 6.5 V.Oil concentration= 3.3 % (w/v), θ_H = 8 h, θc= 15 days, and COD= 2000 mg/L

Time (h)	**COD-Theoretical (mg/L)**	**COD-Experiment (mg/L)**	**E (%)**	**V.Oil-Theoretical (g/L)**	**V.Oil-Experiment (g/L)**	**E (%)**	**Biomass-Theoretical (mg/L)**	***Biomass-Experiment (mg/L)***
0	1990	1990	0	0	0	0	16940	*16940*
1	2134	479	78	11,1	2,182	80	11293	*11780*
2	2206	2368	0	16,7	2,382	86	8470	*10620*
3	2249	263	88	20,0	1,38	93	6776	*11420*
4	2278	1234	46	22,2	1,302	94	5647	*8900*
5	2299	587	74	23,8	2,656	89	4840	*7700*
6	2314	1774	23	25,0	16,2	35	4235	*6960*
7	2326	1720	26	25,9	7,284	72	3764	*8860*
8	*2336*	*3663*	*0*	*26,6*	*4,782*	*82*	*3388*	*7480*

Table 6.6 V.Oil concentration= 4.1 % (w/v), θ_H = 8 h, θc= 15 days, and COD= 2000 mg/L

Time (h)	**COD-Theoretical (mg/L)**	**COD-Experiment (mg/L)**	**E (%)**	**V.Oil-Theoretical (g/L)**	**V.Oil-Experiment (g/L)**	**E (%)**	**Biomass-Theoretical (mg/L)**	***Biomass-Experiment (mg/L)***
0	2010	2010	0	0	0	0	10720	*10720*
1	4577	2082	55	13,7	0,43	97	7147	*11300*
2	5860	1147	80	20,6	1,184	94	5360	*12660*
3	6630	4529	32	24,7	3,37	86	4288	*14940*
4	7143	5609	21	27,4	6,93	75	3573	*16140*
5	7510	6148	18	29,4	12,99	56	3063	*15500*
6	7785	967	88	30,9	14,08	54	2680	*17680*
7	7999	3234	60	32,0	6,88	78	2382	*23280*
8	*8170*	*1686*	*79*	*32,9*	*14,9*	*55*	*2144*	*12380*

Table 6.7 V. Oil concentration= 6.3 % (w/v), θ_H = 8 h, θc= 15 days, and COD= 2000 mg/L

Time (h)	**COD-Theoretical (mg/L)**	**COD-Experiment (mg/L)**	**E (%)**	**V.Oil-Theoretical (g/L)**	**V.Oil-Experiment (g/L)**	**E (%)**	**Biomass-Theoretical (mg/L)**	***Biomass-Experiment (mg/L)***
0	1450	1450	0	0	0	0	19480	*19480*
1	6056	5390	11	20,9	19,42	7	12987	*15880*
2	8359	4365	48	31,3	30,93	1	9740	*13760*
3	9740	12731	0	37,5	13,49	64	7792	*12400*
4	10661	15105	0	41,7	34,86	16	6493	*11300*
5	11319	1720	85	44,7	49,7	0	5566	*9660*
6	11813	9438	20	46,9	50,72	0	4870	*8660*
7	12197	3069	75	48,7	53,71	0	4329	*8160*
8	*12504*	*1936*	*85*	*50,1*	*53,44*	*0*	*3896*	*6640*

6.3 The effect of vegetable oil concentration on COD and V.Oil removal without carbon sources

Table 6.8 V.Oil concentration= 1 % (w/v), θ_H = 8 h, and θc= 15 days

Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
0	715	715	0	0	0	0	15620	15620
1	763	175	77	3,64	1,412	87	---------	---------
8	9593	2854	70	8,728	1,034	91	6520	3124

Table 6.9 V.Oil concentration= 2 % (w/v), θ_H = 8 h, and θc= 15 days

Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
0	5645	5645	0	0	0	0	9120	9120
1	5429	2370	56	6,71	0,556	92	---------	---------
8	8840	2730	69	16,12	3,06	81	4240	1824

Table 6.10 V.Oil concentration= 3.3 % (w/v), θ_H = 8 h, and θc= 15 days

Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
0	1990	1990	0	0	0	0	16940	16940
1	2134	479	78	11,10	2,182	80	---------	---------
8	2336	3663	0	26,64	4,782	82	7480	3388

Table 6.11 V.Oil concentration= 4 % (w/v), θ_H = 8 h, and θc= 15 days

Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
0	3773	3773	0	0	0	0	8820	8820
1	6124	571	91	13,53	0,241	98	---------	---------
8	9415	1147	88	32,48	9,058	72	4460	1764

Table 6.12 V.Oil concentration= 5.7 % (w/v), θ_H = 8 h, and θc= 15 days

Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
0	463	463	0	0	0	0	19980	19980
1	859	1651	0	19,06	4,198	78	---------	---------
8	1413	1255	11	45,744	14,078	69	4980	3996

6.4 The effect of sludge age (θc) on COD and V.Oil removal

Table 6.13 θc= 18 days, θ_H = 8 h, V.Oil concentration= 4 % (w/v), and COD= 2000 mg/L.

	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
Replicate 1	0	1830	1830	0	0	0	0	12660	12660
	8	8537	3018	65	32	19,782	38	9900	2532
Replicate 2	0	355	355	0	0	0	0	18580	*18580*
	8	2975	1219	59	32	12,73	60	8440	*3716*
Replicate 3	0	1794	1794	0	0	0	0	19780	*19780*
	8	*4155*	*3342*	*20*	*32*	*14,182*	*56*	*7660*	*3956*

Table 6.14 θc= 25 days, θ_H = 8 h, V.Oil concentration= 4 % (w/v), and COD= 2000 mg/L.

	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
Replicate 1	0	1526	1526	0	0	0,948	0	62840	62840
	8	2763	1148	58	32	15,876	51	30900	12568
Replicate 2	0	1085	1085	0	0	14,504	0	60700	*60700*
	8	2574	1179	54	35	24,91	29	12480	*12140*
Replicate 3	0	1085	1085	0	0	18,722	0	55680	*55680*
	8	*2195*	*959*	*56*	*36*	*29,12*	*19*	*22300*	*11136*

Table 6.15 θc= 30 days, θ_H = 8 h, V.Oil concentration= 4 % (w/v), and COD= 2000 mg/L.

	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
Replicate 1	0	643	643	0	0	0	0	19020	19020
	8	2208	328	85	32	21,166	34	12120	3804
Replicate 2	0	990	990	0	0	21,32	0	23440	*23440*
	8	2000	1022	49	36,264	27,93	23	6340	*4688*
Replicate 3	0	927	927	0	0	24,138	0	37200	*37200*
	8	*1836*	*990*	*46*	*36,8276*	*19,99*	*46*	*7660*	*7470*

6.5 The effect of biosurfactant (BC) addition on Vegetable oil and COD removal with carbon source

Table 6.16 BS= 100 mg/L, V.Oil concentration= 4 % (w/v), θc= 15 days, θ_H = 8 h, and COD= 2000 mg/L.

BS Conc. (mg/L)	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
100	0	3450	3450	0	0	0	0	22540	22540
	8	10934	7588	31	32	36,838	-15	7540	4508

Table 6.17 BS= 20 mg/L, V.Oil concentration= 6 % (w/v), θc= 15 days, θ_H = 8 h, and COD= 2000 mg/L.

BS Conc. (mg/L)	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
20	0	643	643	0	0	0	0	10820	10820
	8	10833	9459	13	48	38,058	21	6980	2164

. Table 6.18 BS= 40 mg/L, V.Oil concentration= 6 % (w/v), θc= 15 days, θ_H = 8 h, and COD= 2000 mg/L.

BS Conc. (mg/L)	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
40	0	2910	2910	0	0	0	0	13140	13140
	8	8782	8307	5	48	55,8	-16	9280	2628

. Table 6.19 BS= 100 mg/L, V.Oil concentration= 6 % (w/v), θc= 15 days, θ_H = 8 h, and COD= 2000 mg/L.

BS Conc. (mg/L)	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
100	0	283	283	0	0	0	0	12260	12260
	8	4342	13704	-216	48	45,166	6	9840	2452

6.6 The effect of biosurfactant (BC) addition on Vegetable oil and COD removal without carbon source

Table 6.20 BS= 20 mg/L, V.Oil concentration= 6 % (w/v), θc= 15 days, θ_H = 8 h, and COD= 2000 mg/L.

BS Conc. (mg/L)	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
20	0	4745	4745	0	0	0	0	21200	21200
	8	10387	8703	16	53,7848	45,652	15	6720	4240

Table 6.21 BS= 40 mg/L, V.Oil concentration= 6 % (w/v), θc= 15 days, θ_H = 8 h, and COD= 2000 mg/L.

BS Conc. (mg/L)	Time (h)	COD-Theoretical (mg/L)	COD-Experiment (mg/L)	E (%)	V.Oil-Theoretical (g/L)	V.Oil-Experiment (g/L)	E (%)	Biomass-Theoretical (mg/L)	Biomass-Experiment (mg/L)
40	0	11042	11042	0	0	0	0	35180	35180
	8	6638	10934	-65	48	56	-17	8740	7036