OPTIMISATION OF EDIBLE OIL EFFLUENT DEGRADATION BY MICROORGANISMS

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I hereby declare that the dissertation represents my own work. It has not been submitted before for any diploma/degree or examination at any other Technikon/University.

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SWASTIKA SURUJLAL

I hereby approve the final submission of the following dissertation.

_____ _____

Dr. FAIZAL BUX
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this 14 day of MAY, 2003, at Durban Institute of Technology

DEDICATION

This dissertation is dedicated to my parents ...
... this is for you mum and dad!

ABSTRACT

Water is a precious and very valuable resource. Water resource shortages are problems that are plaguing the world. There is therefore a high demand for tightening water quality standards for both potable water and waters in the environment. It is becoming a necessity to treat and reuse wastewaters, especially wastewaters from industries, as these waters are of concern due to their toxic effects on the environment. In South Africa one of the industries of major concern is that of the edible oil industry and there are over a dozen of these industries in South Africa alone. Each of these industries utilises approximately 1.75 million m^3 of water and discharges approximately 0.61 million m^3 to the sewer each year. This wastewater that is being released has very high organic, inorganic and fats, oils and greases loads. Depending on the type of refinery process conducted on the crude oil, the effluent quality and quantity varies on a day to day basis. The two types of refinery procedures are the physical refining, where water is used or chemical refining where caustic soda is used. The organic load of the untreated effluent can range from 1 100 to 8 990 mg COD/L, the oils and greases can range from 80 to 1 360 mg/L and the pH can range from 1.8 to 10.5.

The industries do employ treatment methods like gravity separators or "fat traps", for removal of the fats and oils that are not emulsified, or neutralisation to bring the acidic or alkaline effluent to a neutral pH. Other processes are screening and acid splitting. Coagulation and dissolved air flotation(DAF) is also one of the treatment methods employed for fats and oils that are emulsified. Even though one or more of these methods are applied, the removal efficiency is not adequate enough to reduce the high organic loads to comply with environmental standards. It was then decided to integrate the physico-chemical treatments with biological treatment processes to specifically reduce organic and FOG loads to comply with environmental standards. Therefore the aim of this research project was to investigate, activated sludge treatment of edible oil effluent and selection of appropriate pretreatment technology to prepare effluent for fungal and yeast biological treatment, in order to reduce the organic and FOG loads in the effluent.

For the activated sludge process a 10 L pyrex reactor was used with a total working volume of 6 L. The following parameters were investigated to optimise treatment: 1) temperature, 2) supplementation of nutrients (C:N:P of 100:5:1) and 3) lack of nutrients. The experiments 1 and 2 were conducted at 21°C without and with nutrients respectively, while experiments 3 and 4 were conducted at 31°C without and with nutrients respectively. These four experiments were carried out with diluted effluent at ~2000 mg/L. The 5th experiment was conducted using optimised conditions determined from the investigation of the first four experiments. This experiment was carried out using undiluted effluent at ~ 3000 mg/L. All the processes were carried out using a semi fed-batch and batch reactor.

Two types of pretreatment were conducted. The first pretreatment was coagulation with ferric chloride at 10ppm followed by DAF and the second was ozonation for 3 minutes. Each pretreatment was then subjected to fungal and yeast biological treatment. There were three fungal isolates viz.,

Alternaria sp, Mucor sp 1 and *Mucor sp* 2. The two yeast isolates were *Rhodosporidium sp* and *Candida succiphila*. The fungal and yeasts processes were conducted using 2 L conical flasks as batch reactors with a total working volume of 1L. The pretreated fungal batch tests were carried out at a pH of 5 at 31°C, while the pretreated yeast batch tests were carried out at a pH of 8 at 31°C. The COD for all fungal and yeast batch tests were at ~2000 mg/L.

The results show that the activated sludge produced substantial COD removal, with diluted effluent, at 21°C with and without nutrients being 87 and 85 % respectively. For the undiluted effluent which used parameters of 21°C with nutrients, accomplished an average of 95 % COD removal and 82 % FOG removal.

The coagulation with DAF yielded a higher efficiency than the ozone. Comparison of the findings showed that fungal isolates exposed to effluent without pretreatment showed an overall better performance with regards to COD removal than those fungal isolates that were exposed to pretreated effluent.

Comparison of the degradative potential for edible oil effluent showed the yeast displayed a lower potential, for reducing COD and FOG loads.

PREFACE

Some of the material presented in this dissertation has/will been/be published and presented elsewhere:

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ABBREVIATIONS

BAS	:	Batch Activated Sludge
BOD	:	Biological Oxygen Demand
C	:	Carbon
CO_2	:	Carbon Dioxide
COD	:	Chemical Oxygen Demand
DAF	:	Dissolved
DMDAAC	:	dimethyldiallylammonium chloride
DO	:	Dissolved Oxygen
FA	:	Fatty Acids
FBR	:	Fed-Batch Reactor
$FeCl_3$	:	Ferric Chloride
FOG	:	Fats, Oils and Greases
g	:	grams
GC	:	Gas Chromatography
GCMS	:	Gas Chromatography Mass Spectrophotometry
H	:	Hydrogn
H_2O	:	Water
H_3P04	:	Phosphoric Acid
HCL	:	Hydrochloric Acid
K	:	Potassium
KI	:	Potassium Iodide
kpa	:	kilo pascals
L	:	Liter
MCRT	:	Mean Cell Retention time
MEA	:	Malt Extract Agar
Mg	:	Magnesium

mL	:	mililitre
MLSS	:	Mixed Liquor Suspended Solids
MLVSS	:	Mixed Liquor Volatile Suspended Solids
N	:	Nitrogen
N_2	:	Nitrogen
NA	:	Nutrient Agar
$Na_2S_2O3.SH_20$	:	Sodium thiosulphate
NOM	:	Natural Organic Matter
NaOH	:	Sodium Hydroxide
O_2	:	Oxygen
O_3	:	Ozone
OUR	:	Oxygen Utilisation Rate
P	:	Phosphorus
PA	:	Polyaluminium
PACl	:	Polyaluminium chloride
PC	:	Polymeric Coagulants
ppm	:	parts per million
rpm	:	rounds per minute
S	:	Sulphur
SDA	:	Saboraud Dextrose Agar
So/Xo	:	Initial substrate concentration
SOG	:	Soaps, Oils and Greases
SPL	:	Specific Pollution Load
SRT	:	Solid Retention time
SV	:	Sludge Volume
SVI	:	Sludge Volume Index
TDS	:	Total Dissolved Solids
TKN	:	Total Kjeldahl Nitrogen

TN	:	Total Nitrogen
TP	:	Total Phosphorus
TSS	:	Total Suspended Solids
US	:	United States
VSS	:	Volatile Suspended Solids
Wt	:	Weight
Xo	:	nitial biomass concentration

CHAPTER ONE

INTRODUCTION

1.1 WATER - A PRECIOUS RESOURCE

Water is a natural resource that is becoming increasingly valued and there is a growing perception that the pattern of rainfall, and hence the supply of good quality water, is changing (Terrel & Holmes, 1994). With this there is a growing awareness of the impact of pollution, an enhanced ability to measure minute concentrations of contaminants and a demand for ever-tightening water quality standards for both potable water and waters in the environment and hence there is increased pressure for action and improvement. (Terrel & Holmes, 1994; Clouston, 1994).

Wastewaters from industries is of much concern due to their toxic effects on the environment, and one of the industries concerned is that of the edible oil industry. Sengül (1990), stated that in Turkey, the edible oil industry is one of the most important industrial sectors and as part of the agro-industry, the oil production makes a significant contribution to environmental pollution. There are over a dozen of these industries in South Africa alone and each of these industries discharges approximately 0.61 million m^3 of water each year. The wastewater discharged from the edible oil industry contains various organic and inorganic contaminants, that need to be removed before being discharged in to the sewer (Steffen *et al.*, 1989).

Selection of wastewater treatment processes must be based on several factors, but the three most important ones are probably: (a) the wastewater characteristics, (b) the effluent standards to be met, and (c) the cost of treatment (Ødegaard, 2001).

1.2 EFFLUENT QUALITY FROM AN EDIBLE OIL PLANT

The effluent from an edible oil plant varies in both quality and quantity. Table 1.1 shows the specific pollution loads (SPL) of the final effluent and is based on the total tonnage of refined oil produced monthly in the edible oil industry (Steffen *et al.*, 1989). The lipids in wastewater are characteristics as fats, oils and greases (FOG) or as separable oil and grease. Although domestic sewage does contain lipids, it is the industrial effluents that are of greater concern when considering problems associated with this type of material (Forster, 1992).

Table 1.1 Specific Pollution Loads in the Edible Oil Industry (Steffen *et al.*, 1989)

Average monthly effluent discharge (m^3)	Average monthly oil production (t)	Specific Pollution Load		
		COD (kg/t)	SOG (kg/t)	TDS (kg/t)
Range	Range	Range	Range	Range
2180 - 8200	1000 - 3600	4,3 - 13.8	0,2 - 3,3	10,1 - 24,8
Mean	Mean	Mean	Mean	Mean
5130	2520	9,8	1,6	15,4

Sengül, (1990), found that sunflower oil effluents are oily, turbid, and yellowish brown in color and that the most important pollutants in the effluent were oil and grease and chemical oxygen demand (COD) which are present in excessive amounts.

Control of oil and grease is a stringent requirement of municipal authorities responsible for permitting connection of industrial wastewaters in to the sewer system. Oil and grease tend to clog the sewer pipes and pumps and create difficulties in the municipal wastewater plants. Therefore the oil and grease limits are be considered with other parameters such as pH, total suspended solids in sewer discharge standards. Industries are therefore requested to remove the oil and grease material

from the effluents before they discharge the effluent to the sewer. (Sengül, 1990).

1.3 TREATMENT METHODS EMPLOYED BY AN EDIBLE OIL INDUSTRY

With regard to removal of oils and fats that is not emulsified in the water, poses no serious problem as it can float and agglomerate. Gravity separators or fat traps is one method of removing the flotable fats in the wastewater, where the fats can then be skimmed off the surface mechanically. However for emulsified oils and fats is more difficult to remove. Here chemical methods are employed. Coagulants used for breaking emulsions include alum, ferrous sulfate, ferric sulfate or chloride, sodium and commercial organic treating chemicals. Once chemical addition freed the oil a physical process is employed such as dissolved air flotation (DAF) (Steffen *et al.*, 1989; Sengül, 1990).

Dissolved air flotation was widely put to use for water and wastewater treatment during the 1960's and 1970's in the Scandinavian countries. The DAF systemwas an overwhelming efficient unit operation for removal of light suspended solids created by coagulation and flocculation of humic substances from water by trivalent metal salts (Kiuru, 2001).

More recently DAF is used in the physical separation of the oil and grease from the water with the use of injected air. The air is added to the waste stream under pressure by a pump and then discharged to atmospheric pressure in a tank. The oil and grease and small particles of solids cling to the surface of the minute air bubbles and float to the surface where they are skimmed off (Garcia & Louch, 1994; Paris, 2001).

A factor often overlooked when evaluating flotation performance is coagulant type. Coagulant selection should depend on water temperatures and raw water quality characteristics such as particle concentrations and types, and the concentration and nature of natural organic matter (NOM) (Bunker *et al.*, 1995).

Besides the above mentioned processes, screening, acid splitting and final neutralisation are the other treatment method that can be conducted in the edible oil industry. Further details of these process is discussed in the chapter to follow.

For this research project two types of pretreatment methods were conducted, i.e., coagulation with DAF and ozonation.

Ozone is known to be a very powerful oxidant appropriate to treat water and wastewater. It is widely used also as a disinfectant. The main factors governing its efficiency are: specific selectivity for target compounds, pH, and temperature (Lopez *et al.*, 1998). Research by Song, *et al.*, (1998), showed that with demulsification of wastewaters containing emulsified oils, found that the demulsification efficiency was 40 % higher with treatment of ozone.

A rather innovative approach is provided by the so called "integrated-processes" where the effectiveness of combining biological and physico-chemical treatments is specifically designed to reduce organic and FOG loads to comply with environmental standards.

1.4 BIOLOGICAL TREATMENT PROCESSES

Bioremediation, requires the use of microorganisms or microbial processes to detoxify and degrade environmental contaminants, attempts to accelerate the natural degradations rates by overcoming factors that limit microbial degradation. Conditions for biodegradation are optimized by modifying environmental factors, such as pH and temperature control, aeration and nutrient addition (Margesin & Schinner, 1998).

Biological treatment of wastewater can be accomplished in a number of ways, the determining factors depend upon efficiency and economics of wastewater contact with the microorganisms concerned. The three types of biological treatment conducted in this research project were the use

of activated sludge, 3 isolates of fungi and 2 isolates of yeast.

1.4.1 Activated Sludge

Garcia & Louch (1994), stated that the use of biological treatment, in the direct discharge of edible oil refining wastewater as a "best practicable treatment" and that the treatment method is utilised in the activated sludge processes and aeration basins and not in grease traps.

Activated sludge systems are based on suspended growth processes. The process relies on the dense growth of microorganisms in a reactor where air is continuously supplied to allow for carbonaceous oxidation. An aerobic slurry of microorganisms which can be removed from the process through sedimentation and returned, in quantifiable amounts, to the wastewater stream can be referred to the term "activated sludge". Microbial metabolism of the organic matter present results in the production of oxidised end-products such as carbon dioxide, nitrates and phosphates, as well as the biosynthesis of new microbial biomass (Grady & Lim, 1980, cited in Atkinson, 1999).

Microbial floc formation is essential to the success of the activated sludge processes as it allows for the rapid and efficient separation of sludge from the treated wastewater. Even though the exact mechanism of floc formation is not well understood, it seems to be almost entirely bacterially mediated (Muyima *et al.*, 1997, cited in Atkinson, 1999). Bitton's (1999), research shows that the filamentous microorganisms form the matrix or backbone of the structure to which zoogleal (floc-forming) microorganisms attach to. This attachment is thought to be brought about by exopolysaccharides, present in the form of a capsule or discrete slime layer (Horan, 1990).

The size and physical structure of the flocs are important parameters since they influence the settling properties of the sludge. A major operational problem with the activated sludge process is bulking in which sludge becomes difficult to settle. As a result some sludge may be discharged in the effluent which reduces the quality of the effluent. At the same time, appreciable quantities of sludge

may be lost from the system. Although some causes of bulking are known, such as organic overloading, under-aeration and sudden increased loadings of carbonaceous wastes, the phenomenon of bulking is still not fully understood (Curds, 1992).

The organisms present in the activated sludge include protozoa, nematodes, rotifers and worms. Protozoa can reach populations of up to 50 000 cells per milliliter (Curds, 1992).

1.4.2 Fungi

The green growth on a spoiling orange, the mildew on a moist cloth, the mushrooms on a lawn, and the bubbling or frothing of a glass of unrefrigerated juice, all represents the growth and activities of fungi. Fungi consists of morphologically different types of microbes ranging from unicellular and filamentous forms to the fleshy macroscopic types we know as the mushrooms. Most of the fungi, that we call molds, are filamentous in nature. Fungi possess cell walls and obtain nutrients by diffusion and transport across their cytoplasmic membranes. They are however not photosynthetic and like the protozoa, they utilise organic materials for energy and nutrients (Denmark & Batzing, 1987).

The degradation of phenolic compounds by *Fusarium flocciferum*, the degradation of cyanide by *Gibberella sp* and the successful treatment of industrial wastewater with *Aspergillus niger* and *Chaetomium cuprem* have been reported. Recent studies have also shown the treatment of industrial effluents containing fatty acids with fungi can lead to the production of viable biomass (Wainwright, 1992).

There are various studies that indicate that certain conditions must be satisfied for the biodegradation of environmental pollutants to occur. These include the following:

- The organism producing the necessary enzymes or appropriate catabolic potential to bring about the biodegradation must exist;
- The chemical or pollutant must be made accessible to the organism having the requisite

enzymes;

- If extracellular enzymes are bringing about the degradation, then the bonds acted upon by that enzyme must be exposed for the catalyst to function efficiently.
- If it is intracellular enzymes, then the molecule must be able to penetrate the surface of the cell to the internal sites where the enzyme acts; and
- Due to the initial biomass concentration acting upon various synthetic compounds is small, the environmental conditions should be made favorable to induce the proliferation of the potentially active microorganisms (Alexander, 1999).

1.4.3 Yeast

The classification of yeasts uses cells, ascospores, colony characteristics for distinguishing genera, and physiological fermentation characteristics of individual sugars, to identify species. They are heterotrophic, lack chlorophyll and are characterised by a wide dispersion of natural habitats (SGD, 2001, cited in Reddy, 2001). Yeasts are also fungi that grow as single cells and they differ from most other fungi which grow as thread - like hyphae. This distinction is however, not a fundamental one as some fungi can alternate between a yeast phase, and hyphal phase, depending on environmental conditions (Reddy, 2001). The morphology exhibited by a particular yeast is directly associated with the mechanisms it employs for asexual reproduction. Yeasts are able to grow at pH values between 3 and 8 and at temperatures ranging from 21 to 30°C (Walker, 1998).

Yeasts secrete specific enzymes in to there micro - environment in order to degrade compounds for nutrients and energy, e.g., they secrete the enzyme lipase to degrade lipids (Hunter, 1999). Lipase belong to a group of enzymes classified as ester hydrolyze. The substrates that the lipase degrade are hydrophobic, therefore the highest activity of the enzyme is based at an oil - water interface. The function of lipase is to provide a source of energy under carbon limiting conditions by supplying carbon compounds, amino acids and fatty acids. Also the composition of nitrogen and carbon sources, inorganic salts, presence of lipids, temperature and availability of oxygen all determine the

amount of lipase produced (Lazar & Schröder, 1992).

Due to the poor results emanating from just the physico-chemical treatment of the edible oil effluent, more attention is focused on the use of microorganisms and the physico-chemical treatment coupled with biological treatment to reduce the high organic and FOG loads in these wastewaters.

1.5 AIMS AND OBJECTIVES

The aim of this research project was to investigate, activated sludge treatment of edible oil effluent and select appropriate pretreatment technology to prepare effluent for fungal and yeast biological treatment, in order to reduce the organic and FOG loads in the effluent.

The objectives were:

- To optimise parameters such as nutrient supplementation to maximize COD and FOG removal using fed batch/batch tests for activated sludge processes and batch tests for fungi and yeast;
- To optimise parameters such as temperature to maximize COD and FOG removal using fed batch/batch tests for activated sludge processes and batch tests for fungi and yeast;
- To compare the efficacy of the two pretreatment methods, i.e., coagulation with DAF and ozonation, in preparing the effluent for biological treatment using fungi and yeast.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE WATER PROBLEM

The development and expansion of water supply systems are continuing problems for the highly industrialized countries as well as the transition countries on the threshold of the 21st century. Even though the magnitude and the complexities of the problems vary from country to country, the fundamental issues of demand, planning concepts, water sources, pollution control and wastewater reuse are universal (Maksimovic, *et al.*, 1996).

Since history was first written, man has been reluctant to use his wastewater, usually disposing of it in the nearest river, lake or ocean. In the mid 1800's the relationship between disease and contaminated drinking water was established and the reluctance to using wastewater today continues even though the technology exists to treat it to a quality exceeding existing drinking water standards (Maksimovic, *et al.*, 1996).

The amount of world water use has tripled over the last half century. Seventy percent of water that is withdrawn from rivers or from underground sources is used for irrigation, 20% used by industry and 10% for residential purposes (Brown, 2001; Veltri, 1996).

The limits to sustainable development in the supply and use of freshwater resources have been reached during the last decades in many parts of the world. It is believed that all countries with improving life conditions will use more water consumption facilities and in addition, an increase in water demand for irrigation and industries has to be expected. However these tendencies are in conflict with the inadequacy of water resources in terms of both quality and quantity (Veltri, 1996).

Water resource shortages are problems that are plaguing the world. Three fifths of the earth is covered by water. This may appear to be in abundance but most of this water is not available for most uses. The ocean accounts for 97.2% of the earth's water, but due to its high salinity this water is of no use (Sanders, *et al.*, 1996). Of the remaining 2.8% left, 2% is reserved in the polar ice caps and glaciers and of the 0.8% fresh water, only 0.05% can actually be utilized for human consumption (Atkinson, 1999).

Holtzhausen, (2002), stated that the fresh water ecosystems said to be most in danger are those regions with high human populations and large fresh water withdrawals or large amounts of wastewater discharge.

2.2 EFFECTS OF WASTEWATER DISCHARGE

In natural streams there is a balance between plant and animal life, with considerable interdependence among the various life forms. Waters of good quality are characterized by multiplicity of species with no dominance. The introduction of excessive quantities of pollutants can upset this natural balance in a variety of ways (Sanders, *et al.*, 1996).

Irrigated farming often returns water to rivers with a high saline and agrochemical content, which is costly to households, other farmers and fisher folk. Releases of untreated industrial effluent can also poison fish, spoil rivers and lakes for recreation, and impose treatment costs on society (Winpenny, 1994).

The general term applied to liquid wastes collected from residential, commercial and industrial areas and conveyed by means of a sewerage system to a central location for treatment is called "municipal wastewater" (Appleyard & Lander, 1994). These liquid wastes have to be disposed of in some manner, whether it's by reuse, discharge to surface waters, by injection or percolation to groundwater, or by evaporation to the atmosphere. In all cases the wastewater must first be treated to remove bulk of the contaminants. Table 2.1 shows the types of wastewater treatment carried out

at the municipal works (Ramalho, 1983). For more detailed information on the treatment process the works of (Henze *et al*, 1997, McGhee, 1991, Ramalho, 1983 and Qasim, 1994) should be consulted. The effects of various pollutants on the environment to which they will be discharged need to be considered, in order to determine the degree of treatment required. Also any statutory or regulatory requirements which may have been established need to be considered (McGhee, 1991). Table 2.2 shows the acceptance limits of effluents for discharge into the sewage disposal system for the Durban Metropolitan area (Wastewater Departments, 2001).

Table 2.1: Types of Wastewater Treatment

Primary Treatment
- Screening
- Sedimentation
- Flotation
- Oil Separation
- Equalization
- Neutralization
Secondary Treatment
- Activated Sludge Process
- Extended aeration (or total oxidation) processes
- Contact stabilization
- Other modifications of the conventional activated sludge process: step aeration, complete mix activated sludge process, tapered aeration, high-rate activated sludge, and pure oxygen aeration
- Aerated lagoons
- Wastewater stabilization ponds
- Trickling filters
- Rotating biological contactors
- Anaerobic treatment: anaerobic contact process and anaerobic (submerged) filters
Tertiary Treatment (or "advanced treatment")
- Microscreening
- Filtration (sand, anthracite, and diatomaceous beds)
- Precipitation and coagulation
- Adsorption (activated carbon)
- Ion Exchange
- Reverse osmosis
- Electrodialysis
- Chlorination and ozonation
- Nutrient removal processes
- Sonozone process

Adapted from Ramalho, 1983.

Table 2.2: Acceptance Limits of Trade Effluents for Discharge into the Sewage Disposal System, Durban Metro, South Africa

General quality Limits	Large Works > 25 Ml/d	Small Works < 25 Ml/d	Units
1. Temperature (°C)	< 44	< 44	Degree Celsius
2. pH	6 < pH < 10	6.5 < pH < 10	pH units
3. Oil, greases, waxes	50	50	mg/l
4. Vegetable Oils, greases, waxes	250	250	mg/l
5. Total sugar and starch (as glucose)	1 000	500	mg/l
6. Sulfates in solution	250	250	mg/l
7. Sulfides, hydrosulphides, polysulphides	1	1	mg/l
8. Chlorides (as Cl^{-1})	1 000	500	mg/l
9. Fluoride (as F^-)	5	5	mg/l
10. Phenols (as phenol)	10	5	mg/l
11. Cyanides (as CN^-).	20	10	mg/l
12. Settleable Solids	Charge	Charge	ml/l
13. Suspended Solids	2 000	1 000	mg/l
14. Total Dissolved Solids	1 000	500	mg/l
15. Electrical Conductivity	-	400	mS/m
16. Anionic Surfactants	-	500	mg/l
17. C.O.D.	Charge	Charge	mg/l

Special Limitations

1. No calcium carbide, radio active waste or isotopes
2. No yeast and yeast wastes, molasses spent or unspent
3. No cyanides or related compounds capable of liberating HCN gas or cyanogen
4. No degreasing solvents, petroleum spirit, volatile flammable solvents or any substance which yields a flammable vapor at 21°C.

Adapted from (Wastewater Departments, 2001)

Wastewaters discharged represents an extremely complex mixture of organic and inorganic material (Horan, 1990, McGhee, 1991 and Steffen, *et al.*, 1989). The sunflower oil industry is one of the major polluters of the environment as its effluent contains high quantities of fats, oils and greases,

sulfates, phosphates and other pollutants. All of these contribute to the high organic and inorganic loads (Mkhize, *et al.*, 2000, Mkhize & Bux, 2001 and Steffen, *et al.*, 1989). Most of this effluent is processed by the receiving municipal treatment works (Mkhize & Bux, 2001).

2.3 THE SUNFLOWER OIL INDUSTRY

Sunflower oil is obtained from the seed of the plant *Helianthus annus*. The seed has an oil content of 22 - 36% (Formo *et al*, 1979). Sunflower oil has become the number one oil by preference between consumers and chefs. Its health benefits, high smoke point, bland flavor and light clear consistency makes it an excellent choice for baking, frying and salad dressing. It is also an excellent source of vitamin E (∝-Tocopherol). For health reasons it is desirable to use an oil with a low level of saturated fat, and sunflower oil contains a low level of saturated fats. It has a mono unsaturated fatty acid, oleic acid, and a high percentage of the polyunsaturated acid, linoleic acid. Both of these unsaturated fatty acids are believed to be beneficial for health. Sunflower oil also contains <0.5% linolenic acid. Oils containing more than 2% linolenic acid must be partially hydrogenated, as the oil can develop an off-flavor over a period of time (Hui, 1996 b).

2.3.1 Chemistry of Vegetable Oils

Lipids are biological compounds that are insoluble in water but soluble in organic solvents (Palleros, 2000). The types of lipids that are of concern in this study are fats and oils. The other types of lipids are waxes, phospholipids, terpenes and steroids.

2.3.1.1 Fats and Oils

Fats and oils are hydrophobic (from Greek: *hydor*, water; *phobos*, fear) therefore they are insoluble in water (Palleros, 2000). They consist mainly of triglycerides or acylglycerols (Devnath, 2001 and Palleros, 2000).

2.3.1.2 Triglycerides

Sunflower oil is considered a mixed triglyceride. Figure 2.1 shows that a triglyceride is a reaction production of one molecule of glycerol with three molecules of fatty acids to yield three molecules of water and one molecule of a triglyceride. Simple triglycerides are a result from the three fatty acids being identical and a mixed triglyceride is a result of the fatty acids being dissimilar (Devnath, 2001). The triglycerides that are solid at room temperature are called fats and those that are liquid are termed oils (Devnath, 2001 and Palleros, 2000).

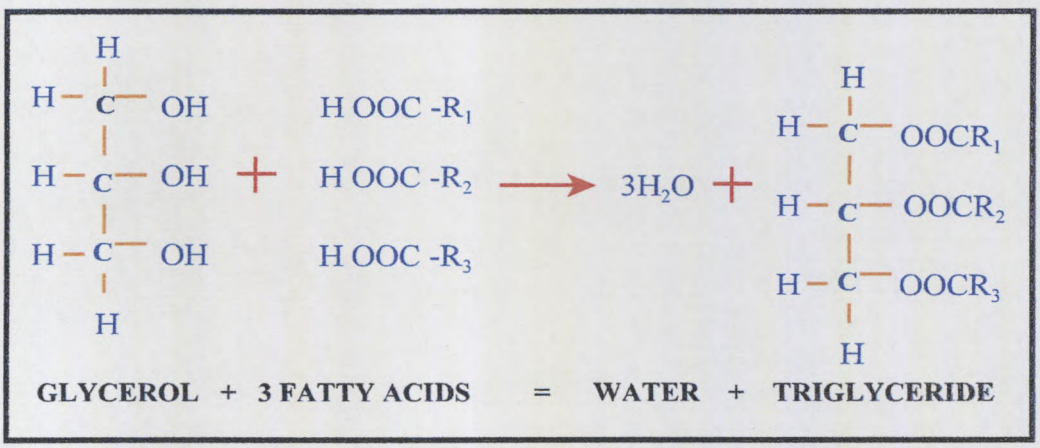

Figure 2.1: Formation of a triglyceride (Nawar, 1996, cited in Devnath, 2001).

2.3.1.3 Fatty Acids

The carboxylic acids found in natural fats and oils are called fatty acids. Fatty acids are long chain organic acids which have an even number of carbon atoms ranging from 4 to 22 (Heinzen, *et al.*, 1985 and Palleros, 2000). Fatty acids having no carbon-carbon double bonds are termed saturated, e.g., Palmitic acid and those that have one or more double bonds are called unsaturated, e.g., linoleic acid (Heinzen, *et al.*, 1985). Table 2.3 shows the F.A. composition of crude sunflower oil (Hui, 1996 b). Fatty acids are sometimes abbreviated by giving the total number of carbon atoms followed by the number of double bonds Hui, (1996a). Reference can be made to Appendix 1 which shows the common and systematic name, number of carbons, abbreviation and structure of some common natural fatty acids (Palleros, 2000).

2.3.2 Sunflower Oil Processing

In South Africa the total quantity of oil refined are approximately 250 000 tons/annum, but is expected to increase by about 3% per annum (Steffen, *et al.*, 1989). The first stage in the production of refined vegetable oil is - crude oil production conducted in an oil mill and the second stage is processing the oil which is conducted in a refinery.

Table 2.3: Fatty acid Composition of Sunflower Oil.

Fatty Acid	Range Wt %
< C14	< 0.1
C14:0	<0.5
C16:0	3.0 - 10.0
C16:1	< 1.0
C18:0	1.0 - 10.0
C18:1	14.0 - 65.0
C18:2	20.0 - 75.0
C18:3	< 0.7
C20:0	< 1.0
C20:1	< 0.5
C22:0	< 1.0
C22:1	< 0.5
> C24	< 0.02

Adapted from Formo, *et al.*, 1979; Hui, 1996 b

2.3.2.1 Crude Oil Production

The procedure is as follows:

- The first operation is the removal of all foreign matter, such as sticks, stones and metal articles, from the seeds.
- The sunflower seeds are then decorticated and the meats are separated from the hulls.

- The separated meats, which contain the oil, may be rolled in to thin flakes. The flakes are then subjected to live steam.
- The conditioned seed is then passed to screw presses (expellors) where about 75% of the oil is squeezed from the flakes.
- The expelled meat or cake still contains 25% of oil and this oil is extracted using a solvent extraction method. The solvent used is hexane.
- The extracted oil is then sent to a crude oil storage tank. (Steffen, *et al.*, 1989)

2.3.2.2 Refining of Crude Oil

There are number of impurities which must be removed before in can be considered fit for human consumption. These impurities can be classified in to four broad categories:

a) Gums:a collective term referring to phosphatides, sugars, resins and other proteinaceous material. The gum content of sunflower oil is 2%.

b) Free fatty acids: these usually range between 0.5% and 8%, but they can reach extremely high quantities (as high as 40%), depending on the oil type.

c) Pigment: usually of chlorophyllic or carotenic base.

d) Taste and odor: this is a result of volatile aldehydes and ketones (Steffen, *et al.*, 1989)

Figure 2.2 shows the refinery process for crude oil. Crude oil is refined to remove all the impurities mentioned above. The "chemical" method of refining crude oil is neutralisation with caustic soda followed by bleaching and deodorising of the neutralised oil. There is also another method called "physical " refining. The basis of physical refining is the use of deodorises for the steam distillation of free fatty acids without prior neutralising stage. The advantages of physical refining over the conventional chemical method are firstly reduction in oil loss and elimination of soapstock and its associated effluent treatment problem. Physical refining can handle oils having high free fatty acid content. This comprises of the addition of phosphoric or citric acid prior to physical refining followed by high speed centrifugation, drying of centrifuged oil and subsequently bleached and

winterised before being physically refined (Steffen, *et al.*, 1989).

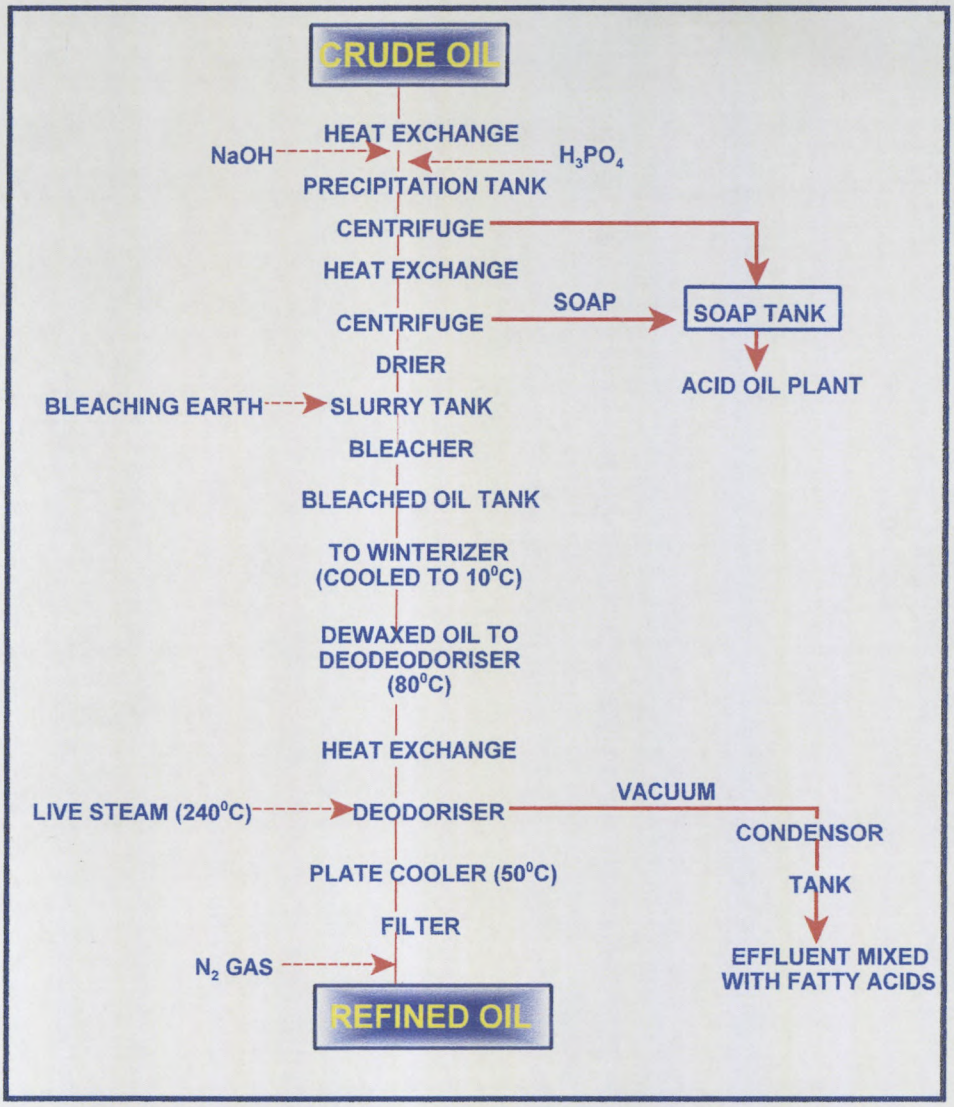

Figure 2.2: Diagram of Refinery Process of Crude Oil (Surujlal, 1999)

For more detailed information on the refinery process (such as Degumming, Neutralisation, Bleaching, Winterising and Deodorising), works of (Devnath, 2001, Steffen, *et al.*, 1989, Hui, 1996 b, Surujlal, 1999 and Gawad & Shafy, 2002, Mkhize, 2002) may be consulted.

2.4 PROBLEMS CREATED WITH VEGETABLE OIL EFFLUENT

The environmental challenge for the vegetable oil industry is associated with liquid wastes, solid waste and soil contamination. However, the main problem faced by a vegetable oil industry, both quantitatively and qualitatively, is the wastewater (Pakistans Edible Oil Sector, 2001).

An oil plant discharges approximately 35% of the incoming water to the sewer and the remaining 65% is vaporised in the cooling circuits, or it leaves the site in one of a number of secondary products and by-products (Steffen, *et al.*, 1989).

The wastewater that is generated from a vegetable oil industry is mainly from neutralisation of oil, soap section, acid oil production and washing processes. Wastewaters generated from these sources vary in pollution load and concentration. Process wastewater contributes to most of the pollution load in the effluent, with high amounts of BOD, COD, Oil and Grease, TSS, TDS, oil, phosphate, sulfate etc. (Pakistans Edible Oil Sector, 2001). The effluent thus has a high inorganic as well as high organic loading (Steffen, *et al.*, 1989).

2.5 EFFLUENT TREATMENT

In recent years, there has been an increasing awareness of the need to protect the environment. This has obliged those working in the vegetable oil processing industry to change their waste disposal methods. Laws, regulations, restrictions and limits for waste disposal enacted by governmental pollution control authorities have required taking strict measures to minimize or prevent atmospheric pollution, pollution by waste materials and water pollution (Bauer, 1991).

When considering effluent from oil mills, there are a few points which should be considered:

a) whether the finished oil is produced from seed or whether the factory only produces crude oil or only refines purchased oil.

b) whether chemical or physical refining is the predominant method

c) whether other products such as margarine etc., are manufactured on site.

d) whether representative sampling of individual process effluents is allowed from existing effluent drainage system.

e) whether the effluent is treated before being discharged (Steffen, *et al.*, 1989).

2.5.1 Wastewater Treatment Processes Employed by a Vegetable Oil Industry

Effective treatment of refinery effluent may be achieved by a combination of screening, acid splitting of oil emulsions, skimming of fats and oils and final neutralisation.

2.5.1.1 Screening

Defatted meal and husk, which may have entered the effluent system may be removed using a rotary, vibrating or a static "self-cleaning" screen. Both require periodic cleaning (Steffen, *et al.*, 1989).

2.5.1.2 Acidification

Most of fatty matter contained in soapy water, deodoriser effluent and final effluent is in an emulsified form. This fatty matter cannot be removed by the direct application of gravity separators. The emulsified fats need to be destabilised. This is achieved by using sulphuric acid and the advantage of this is that the fatty material can be recovered. Once destabilisation of emulsions has occurred, the fatty matter can then be removed in a gravity settler system (Steffen, *et al.*, 1989).

2.5.1.3 Gravity Separation or "Fat Traps"

For many years "fat traps" were used in the removal of separable fatty matters. The gravity fat trap is the simplest form of physical treatment and is installed as standard equipment on all process

effluent streams. However this gravity fat trap cannot reduce emulsified fatty matters less than 500 mg/L, which is very high to discharge to municipal sewerage systems (Eroglu *et al*, 1990).

Since fats and oils are less dense than water, the simplest way of separation is flotation. This flotation system may consist of a tank containing a series of baffles beneath which the aqueous phase can flow freely. There is a manometric arm which maintains the liquid level at a baffle height so that the fatty material is trapped, however periodically the fat has to be removed manually from the surface. How effective a gravity separator is, will depend upon the water temperature, the density and size of the oil globules and the amount of suspended matter in the effluent.

There are limitations in the use of gravity separators. These are:

a) they will not separate stable emulsions as they can only remove gravity-separable oils

b) other parameters only undergo limited reduction

c) the efficiency is reduced if any emulsifiers, spent caustic or detergents are present

d) if there are suspended solids attached to oils, they may not settle

e) the efficiency can be further reduced if hydraulic overloading occurs (Steffen, *et al.*, 1989).

2.5.1.4 Dissolved Air Flotation (DAF)

DAF was recognised as a method of separating particles in the early 1900's. A US patent was issued in 1905 for a process using pressurized aeration followed by pressure release. From these early beginnings DAF has found many applications including: mineral separation, clarificatin of paper mill wastewaters, refinery wastewaters, combined sewer and storm waters, municipal wastewaters in tertiary treatment and wastewater reclamation (Edzwald, 1995). Longhurst & Graham, 1987, Wortel, 1991 (cited in Edzwald, 1995) deduced that DAF was first used in South Africa in the 1960's. Dissolved air flotation techniques are much more widely applied to fatty effluents (Eroglu *et al.*, 1990). This is an alternative or subsequent treatment stage to gravity separation (Steffen, *et al.*, 1989). In terms of operation the DAF process is more robust than the conventional clarifier in that it will readily withstand stop/start conditions, the ramping of flows and some variation in water quality. Its has the added advantage in that it can be started and be on-line within 30 mins and can

tolerate being out of service for several hours without any deteriorations in water quality (Schofield, 2001).

DAF is a technique that used minute air bubbles to enhance the flotation of oils and suspended materials (Hui, 1996 c). Air is dissolved in the effluent in a pressure vessel before the solution is released into an open flotation tank. The sudden reduction in pressure causes the air to leave solution as fine bubbles, which then adhere to any oil, fat or other suspended solids in the effluent and bring them to the surface. The entrained air causes the resulting agglomerates to have very fast vertical-rise rates, about 0.0025 to 0.005 m/s. The layer of frothy solids, fats and oils which form is swept into an inclined exit chute by a rotating arm or other skimming device (Steffen, *et al.*, 1989). This process is enhanced by the addition of coagulants/flocculants. Common agents are ferric chloride, sodium aluminate, aluminum sulphate, lime polymers and other flocculants (Hui, 1996 c and Steffen, *et al.*, 1989). In fats and oils applications, this technique has been shown to be very effective in removing fats, oils and greases(FOG) (Hui, 1996 c).

2.5.1.5 Filtration

Using diatomaceous earth for in-line filtration is an effective way for reducing FOG and associated insoluble BOD. However this process has not found extensive use in fats and oils wastewater treatment, but it does provide an effective treatment process, particularly as an alternative to DAF. The main disadvantage of filtration is that it also produces another waste sludge to be disposed of (Hui, 1996 c).

2.5.1.6 Neutralisation

Effluents with a low pH will needs to be neutralised before being mixed with the remaining effluent. The liquor discharged must be neutralised in order to bring the pH value up to local statutory requirements. This final neutralisation can be affected with either caustic soda or calcium hydroxide. By using calcium hydroxide, a further increase in the sodium content of final discharge would be avoided, however this would give rise to a sludge handling problem which will require the use of

a filter. Although an additional stage of treatment would be required, this filtration could provide further removal of fatty matter, COD and suspended solids (Steffen, *et al.*, 1989).

In this research study coagulation followed by DAF and ozonation were used as pretreatment methods prior to biological treatment of the oil effluent.

2.5.1.7 Ozonation

The name "ozone" is derived from the Greek word *ozein*, "to smell". Ozone (O_3) was first identified by Van Marum in 1785. The first important use of ozone was in disinfection of water. Ozone has thus been used for over a century for water treatment. More than a thousand municipal water treatment plants use ozone as part of their chemical treatment (Kloos, 2001).

- *Ozone Chemistry:*

Ozone is a highly reactive gas formed by electrical discharges in the presence of oxygen. Ozone is an allotrope of oxygen (Hesby, 1998). Figure2. 3 shows its chemical structure. Each resonance is composed of one single bond and one double bond (Kloos, 2001)

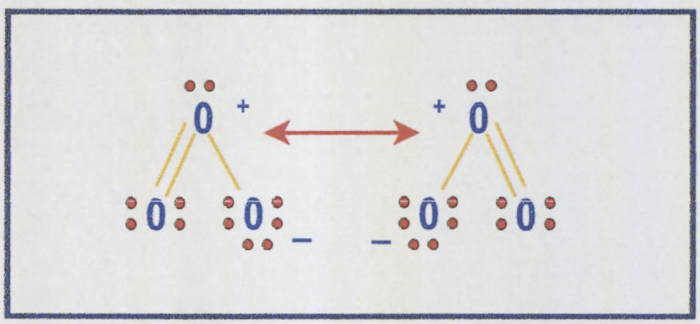

Figure 2.3: Ozone Resonance Structures (Kloos, 2001)

- *How Ozone Operates*:

Ozone readily reacts with most substances that contain unsaturated bonds. It does not react readily with saturated bonds, as there is no easy chemical pathway for the oxidation to take place (Kloos, 2001).

There are three ways in which ozone reacts when introduced to organic materials:

- ❑ Direct oxidation of ozone: this occurs at low pH and is slow and very selective.
- ❑ Indirect oxidation of ozone: this occurs when free radicals are formed. This is highly reactive and may lead to auto oxidation of organic matter. This occurs at high pH values and is fast and non-selective.
- ❑ Ozonolysis: occurs when all three oxygen atoms are added at the site of double or triple bonds which results in the production of ozonides. Decomposition of these ozonides, at the position of the unsaturated bond forms aldehydes, ketones and acids (White, 1999). This can be seen in Figure 2.4.

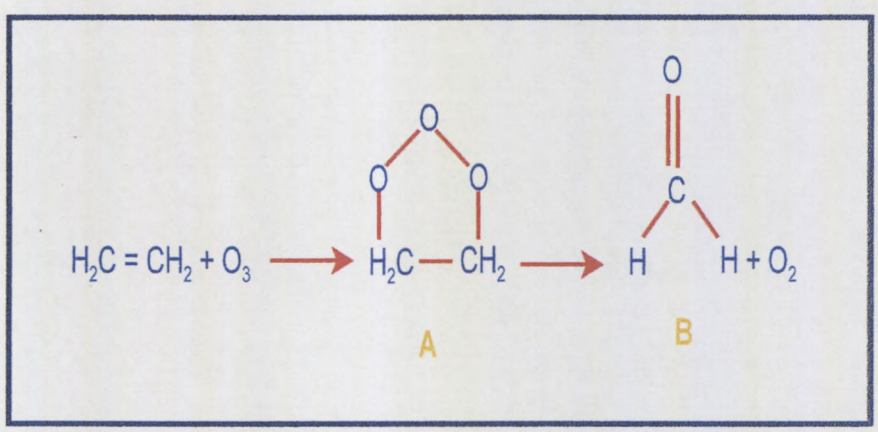

Figure 2.4: Reaction of Ethylene with Ozone (Kloos, 2001).

– *How ozone is generated*

When a high voltage alternating current is imposed across a discharge gap in the presence of air or pure oxygen, ozone is produced. Between the electrodes, there are stray electrons, which become excited and accelerated within the high-energy field created between the 2 electrodes. The current changes the polarity, which then causes the negatively charge electrons to be attracted to one electrode and then the other. This causes the electrons to increase in velocity and gain energy to split O_2 molecules in two, which in turn combine with other O_2 molecules to form O_3 (White, 1999). Figure 2.5 shows a basic ozonator configuration (Hesby, 1998).

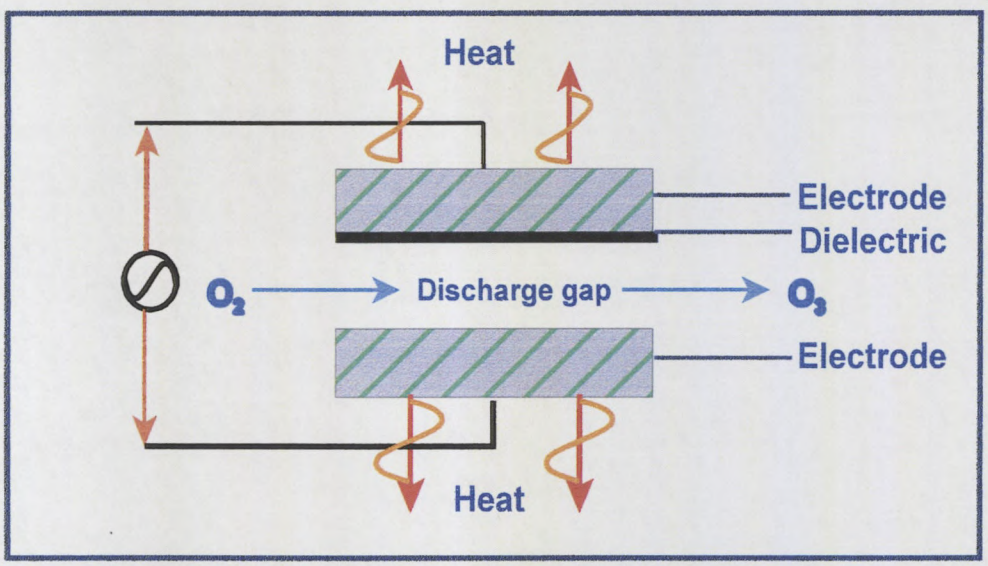

Figure 2.5: Basic Ozonator Configuration (Hesby, 1998).

- The Advantages and Disadvantages of Ozone are shown in table 2.4.

Table 2.4: The Advantages and Disadvantages of Ozone

ADVANTAGES	DISADVANTAGES
1. Generated on site	1. Relatively costly
2. Rapidly decomposes to oxygen, leaving no traces.	2. Produces biodegradable material that must be controlled
3. Reactions in potable water do not produced toxic compounds	3. Produces bromate in bromide rich waters
4. Breaks down organic compounds, allowing them to be easily removed	
5. Increases dissolved oxygen	
6. Decreases turbidity	
7. Have no undesirable effects on marine organisms.	

Adapted from White, 1999; Singer & Reckhow, 1999 and Spellman, 1999

2.5.1.8 Other Processes

- Membrane technology has been applied. However, while pollutant removals are good in some applications, the membrane life and regeneration have presented problems. Therefore, the operating costs for periodic membrane replacement are quite high.
- Attempts were made to use activated carbon to remove residual organics, but the nature of the soluble components in the acidulation stream is such that they cannot be effectively removed.
- Technology from petroleum waste application was adopted, where processors attempted to use a mixed media (sand, anthracite) filter to remove residual oils. Unfortunately it did not function well as there was an inability to backwash these units.
- Application of inclined plate and other packaged media separator systems have been tried. Failure of this was due to the highly viscous nature of these oils coating the separator media (Hui, 1996 c).

2.6 BIOLOGICAL TREATMENT

In nature all things eventually become decayed. Many molecules degrade by the action of oxygen, halogens and radicals found naturally in the environment. One reason why materials become degraded is that they are subjected to the action of enzymes. However, it is unfortunate that the wastes humans produce do not decay as fast as we would like. The wastes end up polluting the air, land and water. There are two main factors that prevent the wastes from decaying rapidly. The first one being that the waste produced is so much that the rate of natural decay is insignificant compared to the amount present. The second is that most of the wastes are dumped in areas that are not conducive to rapid degradation. Hence bioremediation was developed as a way to accelerate or encourage the degradation rate of pollutants. Bioremediation is the use of microorganisms to reduce, eliminate or detoxify environmental pollutants (Shmaefsky, 2002).

The principles of modern bioremediation are not new, dating back to the 1960's. Even though sewage treatment using rock filtration was the earliest application of bioremediation some, of the first studies were conducted in 1972 on using microbes to degrade oil in sea water. The principles are based on the idea that all organisms remove substances from the environment to carry out growth and metabolism. Bacteria, protista and fungi are very good at degrading complex molecules and incorporating the breakdown products into their metabolisms. Bioremediation can be used to clean unwanted substances from air, soil, water and raw materials from industrial processing. Living organisms or just their enzymes can be used to do this (Shmaefsky, 2002).

2.6.1 Microbial Utilization of Fats, Oils and Greases

The long term stability of fats and oils during storage without biodegradation led to the belief that fats and oils were difficult substrates for microorganisms to utilise. However, if the substrates concentration can be kept below a critical level and the culture conditions optimized, then a large number of microorganisms can then grow on and degrade fats and oils. Majority of work on

microbial utilisation of fats and oils has only been conducted since 1982, therefore the realization that lipid degradation is a more generalized phenomenon than at first appreciated has arisen mainly from the continuing and developing interest in microbial lipases. Most of the work done on the utilisation of lipid materials was mainly on growth of microorganisms on various fats, oils and greases (Ratledge, 1994). Pan *et al.* (1959), (cited in Ratledge, 1994) showed that vegetable oils could be utilised as a sole source of carbon and energy. In studies by Tan and Gill (1985b) (cited in Ratledge, 1994), observed that for successful growth of a microorganism to occur on a lipid, the culture medium had to be maintained close to the optimum pH of the lipase that was needed to hydrolyse the oil. The initial degradation of triglycerides by microorganisms is extracellular and involves the hydrolysis of ester bonds by lipolytic enzymes (lipase) which removes the fatty acids from the glycerol molecules of the triglycerides. After the entry into the cell, the fatty acids are either catabolized or directly incorporated into complex lipids (Wakelin & Forster, 1997).

As mentioned previously, edible oil effluent can be treated by a number of physico-chemical means, however, these treatments still cannot reduce the high organic loads and FOG content. Biological treatment is now being given more attention. In this research study aerobic biological treatment using activated sludge, fungi and yeast were conducted.

2.7 ACTIVATED SLUDGE

Activated sludge can be defined as " a mixture of microorganisms which can contact and digest biodegradable materials (food) from wastewater" (Department of Natural Resources, 2001 a). It is a suspended growth process that began in England (Bitton, 1999; Horan, 1990). Droste, 1997, stated that the process was developed by Arden and Lockett in the early 1900's. This process has since been adopted worldwide as a secondary biological treatment for domestic and industrial wastewaters (Bitton, 1999; Ramalho, 1983). The process consists of an aerobic treatment that oxidises organic matter to CO_2, H_2O and new cell biomass. Air is introduced by diffused or mechanical aeration (Bitton, 1999). At the same time a flocculant sludge is formed. Microscopic examination of this

sludge shows that is formed by a heterogenous population of microorganisms. This population changes continually in nature in response to variation in the composition of the wastewater and environmental conditions (Ramalho, 1983).

2.7.1 The Formation of the Floc

The bacterium that is in the sludge develops into small chains or clumps as they grow. They are still very active and motile, which makes it difficult for them to settle. The reason for this is that the slime layer which helps them to stick together has not yet developed. As the sludge is allowed to age the bacteria lose their motility and accumulates more slime. This helps the chains and clumps to stick together which then grows bigger until a floc is formed. If the organisms develop properly, under the right conditions, the flocs get larger and more compact and begin to settle (Department of Natural Resources, 2001 a).

The reason why flocs are formed is still partly inexplicable. However it is known that there are various factors that play a part in the formation of an activated sludge floc, *viz.*:

- many bacteria form slime capsules. The slime, which is built up mainly of polymeric compounds, glues the cells together as it was;
- bacteria have a negative charge and the positively charge ions around them contribute to the joining of the cells;
- networks of extremely thin filaments, known as fibrils, are formed by some bacteria around the cell. These are mostly composed of cellulose or another polysaccharide. It is this network that contributes to further joining of cells and trapping of bacteria (Eikelboom & Buijsen, 1983).

2.7.2 Microscopic Investigation of the Floc

To an untrained eye every activated sludge floc can look like a conglomerate almost without structure and irregularly shaped. The color of the floc components may vary from yellowish-grey to brownish-black. There are however, visually observable, differences which are related to:

a) firmness, shape, structure and size of the floc;
b) composition of the floc: is there a clearly observable variation of microorganisms, are there many (in)organic nonbacterial particles in the floc?
c) filamentous microorganisms: are these organisms present in the sludge; what kinds can be distinguished; does the population change in time?
d) dispersed growth of bacteria: are many free bacterial cells present between the flocs?
e) "higher" organisms: which protozoa etc., are present in the sludge and how many?
f) are many Spirochetes and/or Spirils present? (Eikelboom & Buijsen, 1983).

Microscopic sludge investigation provides information about a number of visually observable characteristics of the floc and the sludge population. The way in which a treatment plant functions is determined by the quality of the biomass in the system. Many operational problems are caused directly by the fact that the sludge floc is not in an optimum condition. Microscopic sludge investigation is a good aid for determining this (Eikelboom & Buijsen, 1983).

2.7.3 Morphology of the Floc

A sludge floc can vary from a globular and compact unit to open and irregularly shaped structure. The flocs are characterised on the basis of shape, firmness and structure.

2.7.3.1 Shape

There are no truly spherical flocs in activated sludge, however those flocs that have a more or less spherical shape are characterised as "rounded" and those flocs where large parts stick out and the

shape differs, are called "irregular". An irregular shaped floc is due to the presence of filamentous bacteria (Eikelboom & Buijsen, 1983).

2.7.3.2 Firmness

A floc can either be weak or firm. In a weak floc the cohesion between the bacterial cells is low, therefore a firm center of the floc is absent. The floc structure can be easily destroyed. There is lot of dispersed cell material with a weak floc. The firmness of the floc is determined by the sludge loading of a treatment plant. If there is a low food to microorganism ratio there is then insufficient nutrients available to keep the floc-forming organisms (Eikelboom & Buijsen, 1983).

2.7.3.3 Structure

The structure of the floc is characterised by whether they are compact or open. If the flocs are compact, then there are very few open spaces within the floc, however in an open floc parts of the floc are clearly separated from each other by open spaces. The presence of filamentous organisms can sometimes but not always cause the open floc structure (Eikelboom & Buijsen, 1983).

The morphology of the floc in a particular activated sludge must then be described by means of a combination of the above mentioned features, eg., a firm, irregular and compact sludge floc.

2.7.4 Dimensions of the Floc

Using a calibrated eyepiece micrometer, the approximate diameter of the flocs can be determined. This is done by taking two points which are farthest apart and measuring it, however the filamentous organisms that protrude from the floc must not be taken in to account. Table 2.5 shows the three groups that may be distinguished:

Table 2.5: Types of flocs and the respective diameters.

TYPE OF FLOC	DIAMETER
1. Large Flocs	> 500 μm
2. Middle-sized Flocs	150 μm < diameter < 500 μm
3. Small Flocs	< 150 μm

Adapted from Eikelboom & Buijsen, 1983

When the floc is too small a turbid effluent occurs. Small flocs occur due to very low sludge loading, very violent turbulence in the aeration tank, poisoning of the floc population, the presence of high concentrations of complexing compounds etc. (Eikelboom & Buijsen, 1983).

2.7.5 Composition of the Floc

A sludge floc consists of a whole range of microorganisms. It is this great diversity which makes an activated sludge system so flexible and enables a large number of different compounds to be simultaneously metabolized. Figure 2.6 shows the microbial composition of an activated sludge floc (Horan, 1990) .

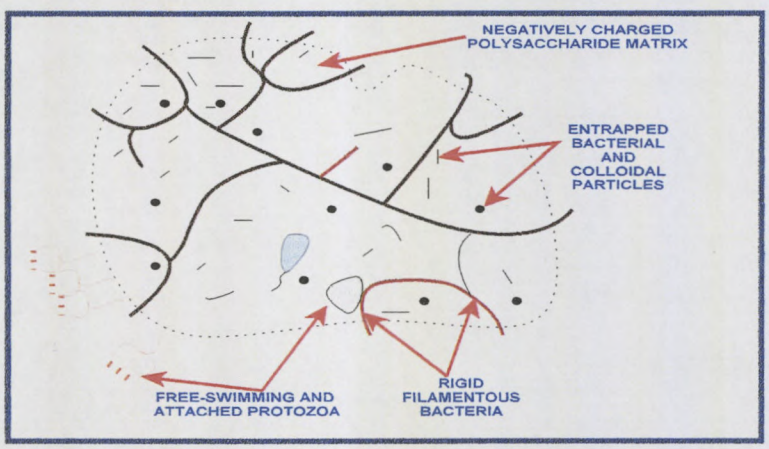

Figure 2.6: The microbial composition of an activated sludge floc (Horan, 1990).

2.7.5.1 Bacteria

Bacteria make up the largest component of the microbial community in all biological wastewater treatment process. The numbers are in excess of one million bacteria per mL (Horan, 1990). It is mainly the gram negative bacteria that make up this major component. The bacteria is responsible for oxidation of organic matter and nutrient transformations and produce polysaccharides and other polymeric materials which aid in the flocculation of microbial biomass. Filamentous microorganisms are also found. Table 2.6 shows the bacterial genera found in standard activated sludge, using culture based techniques (Bitton, 1999).

Table 2.6: Distribution of Aerobic Heterophic Bacteria in Standard Activated Sludge

GENUS OR GROUP	% OF TOTAL ISOLATES
Comamonas-Psuedomonas	50.0
Alcaligenes	5.8
Pseudomonas (flourescent group)	1.9
Paracoccus	11.5
Unidentified (gram negative rods)	1.9
Aeromonas	1.9
Flavobacterium - Cytophaga	13.5
Bacillus	1.9
Micrococcus	1.9
Coryneform	5.8
Arthrobacter	1.9
Aurebacterium - Microbacterium	1.9

Adapted from Hiraishi et al, 1987 (cited in Bitton,1999).

2.7.5.2 Fungi

Even though fungal filaments are occasionally observed in activated sludge, the growth of fungi is not usually favored. They may grow abundantly but only under specific conditions of low pH,

toxicity and nitrogen-deficient wastes. The predominant genera found are *Geotrichum, Penicillium, Cephalosporium, Cladosporium* and *Alternaria* (Bitton, 1999).

2.7.5.3 Protozoa

Protozoa being eukaryotic organisms demonstrates a wide diversity in form and mode of life. They are generally unicellular, motile and classified on the basis of their morphology, more particularly their mode of locomotion (Horan, 1990). Protozoa are also significant predators of bacteria in activated sludge (Bitton, 1999). The presence of particular types of protozoans is related to effluent quality and plant performance. They play secondary but important role in purification of aerobic wastewater. The protozoans in activated sludge fall in to three classes, viz; *Sarcodina*: amoeba; *Mastigophora*: the flagellates and *Ciliophora:* the ciliates (free–swimming, crawling and stalked) (Department of Natural Resources, 2001 a).

a) *Sarcodina* - the amoeba: they possess pseudopodial structures (false feet), which are used for movement and also for feeding by means of protoplasmic flow (Bitton, 1999 and Horan, 1990). They feed on small organic particles. When they are present in large numbers in the aeration basin, this indicates that there has been some sort of shock loading (i.e., there is a lot of food available). They can also tolerate low amounts of dissolved oxygen (Department of Natural Resources, 2001 a).

b) *Mastigophora* - the flagellated protozoa: these possess one or more flagella which are used for both locomotion and feeding (Horan, 1990). Flagellates and bacteria both feed on organic nutrients. If they are present in large amounts in the later stages of activated sludge development, this indicates that the wastewater contains a large amount of soluble organic nutrients.

c) *Ciliophora* - the ciliates : the *Ciliophora* is the largest of the three phyla in terms of the number and species it represents (Horan, 1990). Ciliates feed on bacteria not on dissolved organics while the bacteria and flagellates compete for dissolved nutrients, ciliates compete with other ciliates and rotifiers for bacteria. The presence of ciliates indicate a good sludge, because they dominate after the floc has been formed and after most of the organic nutrients have been removed (Department of Natural Resources, 2001 a). There are three types of ciliates:

- Free-swimming: these have cilia arranged uniformly over their whole body (Horan, 1990). They appear as flagellates disappear. As the bacterial population increases a lot of bacteria is available for feeding, it is then that the free-swimming ciliates begin to dominate and feed on the bacteria (Department of Natural Resources 2001 a). Examples of free-swimming ciliates are *Blepharisma sp., Chilodonella cuculatus, Colpidium colpodem, Euplotes sp., and Paramecium candatum* (Eikelboom & Buijsen, 1983).
- Crawling Ciliates: as the flocs begin to enlarge and stabilize, crawling ciliates graze on the floc. They then out compete the free swimming ciliates for food as they can find food from within the floc (Department of Natural Resources, 2001 a). Examples of crawling ciliates are *Aspisdisca, Trachelophylum pusillum* (Eikelboom & Buijsen, 1983).
- Stalked Ciliates: these appear in mature sludge. Here the crawling and stalked ciliates compete for dominance (Department of Natural Resources, 2001 a). The stalked ciliates have funnel or bell shaped bodies which are mounted on a stalk. The wide end of the bell acts as an oral aperture and they have cilia arranged around this which aid in feeding (Horan, 1990). Examples of stalked ciliates are *Vorticella sp, Carchesium sp, Opercularia sp* and *Epistylis sp.* (Eikelboom & Buijsen, 1983).

2.7.5.4 *Rotifiers*

These are large, somewhat stretched multicellular organisms (Eikelboom & Buijsen, 1983). They are rarely found in large numbers. Their principal role is removal of bacteria and aiding in floc formation by secretion of a mucous. Rotifiers require a longer time to become established and their presence indicates increasing stabilization of organic wastes (Department of Natural Resources, 2001 a).

2.7.6 Effect of the Filamentous Bacteria on Floc

Three different types of activated sludge flocs can be distinguished based on the amount of filamentous organisms that they contain. Figure 2.7 shows the different types of flocs. Figure 2.7a shows the ideal floc. In this floc the filamentous organisms and floc forming organisms grow in "balance". Here the filamentous organisms grow inside the floc providing it with structure and strength. There are a few filaments that protrude out from the floc surface, but these are in sufficient quantities and would not cause settling problems in the activated sludge, creating a clear supernatant. Figure 2.7b is a pinpoint floc. In this type of floc there are either no filaments present or a very few. The floc is very small and if the glycolax is not properly developed, the flocs are weak and easily sheared and broken. The large flocs settle and compact quickly while these smaller aggregates do not settle well creating a turbid supernatant.

Figure 2.7c is a filamentous bulking sludge. Here the filamentous organisms are predominant and they grow both inside and outside the flocs, penetrating the bulk solution. With this type of sludge, poor settling rates are known to occur. However when they do settle a clear supernatant is produced (Jenkins, *et al.*, 1993).

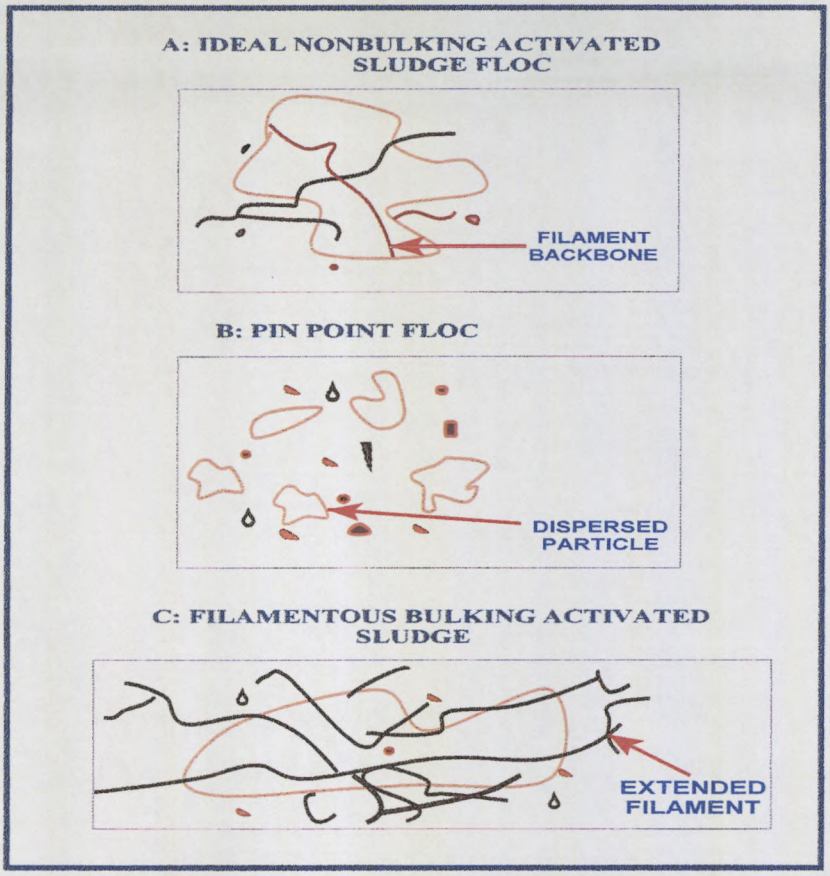

Figure 2.7: Effect of filamentous organisms on activated sludge floc structure (Jenkins, *et al.*, 1993)

The overall filamentous organism level in activated sludge is extremely important in determining its settling and compacting characteristics (Jenkins, *et al.*, 1993). In operation of a treatment plant the principal aim is always the production of a clear effluent, this cannot always be achieved. Table 2.7 provides information on the problems regarding solids separation in activated sludge. Two of the major problems affecting biological waste treatment today is filamentous bulking and foam production caused by filamentous microorganisms (Dueholm, *et al.*, 2001).

TABLE 2.7: Causes and Effects of Activated Sludge Separation Problems

NAME OF PROBLEM	CAUSE OF PROBLEM	EFFECT OF PROBLEM
DISPERSED GROWTH	Microorganisms do not form flocs but are dispersed, forming only small clumps or single cells	Turbid effluent.
SLIME (jelly); VISCOUS BULKING;(also referred to as nonfilamentous resulting in bulking)	Microorganisms are present in large amounts of extracellular slime	Reduced settling and compaction rates. Virtually no solids separation.
PIN-FLOC (OR PINPOINT FLOC)	Small, compact, weak, roughly spherical flocs are formed, the larger of which settle rapidly. Smaller aggregates settle slowly.	Low sludge volume index (SVI), and a cloudy, turbid effluent.
BULKING	Filamentous organisms extend from flocs into the bulk solution and interfere with compaction and settling.	High SVI- very clear supernatant
RISING SLUDGE	Denitrification causes release of N_2 gas which attaches to flocs and causes them to float to surface of clarifier	A scum of activated sludge forms on the surface of secondary clarifier.
FOAMING/SCUM FORMATION	Caused by nondegradable surfactants or the presence of filamentous bacteria, such as, *Nocardia sp* or *Microthrix parvicella.*	Foam causes the activated sludge solids to float to surface of treatment unit. Foam accumulate and putrefy. Solids can also overflow in to secondary effluent.

Adapted from (Bitton,1999 and Jenkins, *et al.*, 1993)

2.7.6.1 Bulking Sludge

Bulking sludge may defined as sludge which only settles slowly and compacts poorly because of an excessive growth of filamentous and/or Zooglea organisms (Eikelboom & Buijsen, 1983). Bulking problems were known already before World War II (Wanner, 1997). A widely used sludge settleability parameter is the sludge volume index (SVI) (Casey, 1995). The following formula shows the way in which this parameter is determined:

$$SVI = \frac{V \times 1\,000}{MLSS}$$

where V = volume of settled sludge after 30minutes (mL/L), and MLSS = mixed liquor suspended solids (mg/L). The SVI is expressed in mL per gram and is thus the volume occupied by one gram of sludge. A high SVI (>150 mL/g) indicates bulking conditions, whereas an SVI below 70 mL/g indicates the predominance of pin (small)flocs (Bitton, 1999).

2.7.7 Parameters Affecting Activated Sludge Performance

When designing and operating activated sludge systems a number of parameters must be taken in to consideration to ensure that the biological treatment and organic removal rates operate at maximum efficiency. These parameters that were of importance include: sludge loading or So/Xo ratio; sludge age, mixed liquor suspended solids and the wastewater characteristics.

2.7.7.1 Sludge Loading or So/Xo ratio

The So/Xo ratio is a very useful parameter as it is the only form of loading which the operator has any control over. The reason being that an increase in the sludge wastage rate will cause an increase in the ratio as the mixed liquor suspended solids (MLSS) will decrease. Gray, (1989) (cited in Atkinson, 1999), states that the ratio basically describes the carbonaceous load (COD) entering the plant which is available to the active biomass. The So/Xo ratio has influences in the COD removal rates and sludge settleability.

This So/Xo ratio is the initial substrate concentration to the initial biomass concentration (So/Xo as COD/biomass). At low ratios, a high amount of biomass is supplied with a low quantity of substrate and no or negligible cell multiplication takes place during exogenous substrate removal. Gray, (1989) (cited in Atkinson, 1999), stated under these conditions complete organics oxidation takes place which results in an effluent of high quality and sludge flocculating and settling well. At higher ratios, a low amount of biomass is supplied with a higher quantity of substrate. The number of

microorganisms increases during the exogenous substrate removal, which is indicated by a large reduction of COD and biomass growth (Chudoba, *et al.*, 1992). Also at this ratio the microorganisms do not form flocs and are generally dispersed which causes settleability problems (Gray, 1989, cited in Atkinson, 1999).

2.7.7.2 Sludge Age

The performance of activated sludge system depends on the mean cell retention time (MCRT). MCRT is also referred to as solids retention time (SRT) or sludge age. When the well formed flocs settle and separate from the effluent, a portion of this settled sludge will be wasted from the system. The fraction of solids which are wasted will determine the average amount of time which the biomass will occupy the reactor. The sludge age can therefore be defined as the mass of sludge in the reactor divided by the mass of sludge wasted per day (cited in Atkinson, 1999).

2.7.7.3 Mixed Liquor Suspended Solids

The MLSS is the concentration of suspended solids, which constitutes to the resident biomass. The value of the MLSS offers the systems operator a crude measure of the biomass contained within the process (cited in Atkinson, 1999).

2.7.7.4 Wastewater Characterisation

The wastewater provides a source of substrate and nutrients for the microbial consortium of activated sludge and thus its composition must be monitored to obtain maximum process efficiency. Also in the design of the activated sludge process, the chemical characteristics of the wastewater to be treated are significant factors to be considered. It is important to determine the following

characteristics of the influent wastewater:

- Mean total influent COD
- the total nitrogen and COD ratios and total phosphorus and COD ratios
- the average minimum and maximum temperatures at which the process operates (Atkinson, 1999)
- the pH.

The carbon, nitrogen and phosphorus are required by microorganisms in balanced amounts, however in activated sludge processes this is not so easy to achieve. An estimate for the C:N:P ratio is 100:5:1 (Chemical Weekly, 2001).

2.8 FUNGAL BIOLOGICAL TREATMENT

Microbiological treatment could prove to be an effective approach in removing, or reducing a major component of high lipid containing waste effluents prior to discharge in to water systems. Reports have demonstrated that many fungi have the ability to degrade a wide variety of hazardous organic compounds (Ratledge, 1994).

2.8.1 General Characteristics of Fungi

Molds, rusts, smuts, yeasts and mushrooms are organisms that are included in the fungi kingdom (Mycota). Most of the fungi are multicellular, though some appear unicellular. Many fungi appear in a thread-like mycelial body form with chitinous walls. Fungal reproduction occurs sexually and asexually, commonly both, which results in the formation of reproductive cells called spores. Fungi are beneficially used in the production of microbial antibiotics, commercial alcohols, in bioremediation and in various food and beverage industries (Arms & Camp, 1995).

2.8.2 Fungal Classification

- Masigomycetes: this is a subdivision of fungi with motile spores or gametes. It can be further be divided into Oomycetes and Chytridiomycetes.
- Oomycetes: fungi of this group reproduce by oogonia eggs and their hyphae have no cross walls. Fungi belonging to this genus are *Achlya* and *Saprolehnia* .
- Chytridiomycetes: this division consists mainly of unicellular fungi. Asexual and sexual spores are produced in sporangia and escape as zoospores. Those belonging to this genera are *Allomyces* and Chytridiomycetes (Landecker, 1972).
- Zygomycetes: these have coenocytic hyphae, forming cross walls only between vegetative mycelium and reproductive structures. Asexual spores are formed within sporangia and sexual reproduction is by the formation of a diploid zygote nuclei encloses within a thick-walled zygospore. Most of these genera are able to utilise carbohydrates efficiently but cannot utilise polysaccharides. The fungal genera in this classification are Rhizopus, Mucor and Phycomyces (Griffin, 1994).
- Ascomycetes: these are distinguished from other fungi by formation of a sac or ascus, within which ascospores are formed (Arms & Camp, 1995). Many yeasts are considered to be Ascomycetes, however the fungi that fall within this classification are *Chaetomium sp*, *Sphaerotheca sp*, *Neurospora sp*, *Gibberella sp*, *Aspergillus sp* and *Penicillium sp*.
- Basidiomycetes: Fungi in this division are characterised by the formation of spores on differentiated hyphal tips (basidia) that are the site of sexual reproduction. Asexual spores are produced as conidia. The Basidiomycetes are classified according to the form of the basidia and their supporting structures. Examples of this division are mushrooms, puffballs, rusts, smuts, bracket fungi and coral fungi (Arms & Camp, 1995).
- Deuteromycetes: fungi in this division lack a sexual stage in their life cycle and are referred to as fungi Imperfecti. They have the ability to reproduce by conidia, which are produced in structures called conidiophores. *Aspergillus*, *Penicillum*, *Fusarium* and *Cladosporium spp* belong to this division (Griffin, 1994).

2.8.3 Fungal Growth Requirements

For the successful growth of fungi, the availability of essential growth nutrients is required. The growth nutrients for fungi are divided in to two classes, i.e., macro-nutrients and micro-nutrients. The macro-nutrients, which include carbon (C), nitrogen (N), potassium (K), phosphorus (P) and magnesium (Mg) are required in large amounts, while the micro-nutrients are required in relatively small amounts.

The atmospheric oxygen and hydrogen are obtained from water via the metabolism of organic compounds. Carbohydrates, organic acids, amino acids and lipids are utilised by fungi as carbon sources (Wainwright, 1992). Inorganic nitrogen in the form of nitrates, nitrites or ammonia and organic nitrogen in the form of amino acids are utilised by some fungi (Landecker, 1972). The presence of phosphorus stimulates fungal respiration and rapid carbohydrate metabolism, while potassium aids in growth and the presence of magnesium induces the activation of fungal enzymes. Fungal growth factors which are organic molecules that promote growth include vitamins which function as co-enzymes and sterols, amino acids and fatty acids which function as structural elements (Griffin, 1994).

The physical aspects that affect fungal growth are:

a) temperature: this parameter influences fungal metabolic rates, synthesis of vitamins, amino acids and other metabolites. Increasing temperature has a effect of increasing enzyme and chemical activity;

b) pH: fungi can grow over a broad pH range. The optimum for most fungal enzymes lie between pH 4 and pH 8.

c) moisture: many fungi require a relatively high moisture content. Growth declines or ceases during very low humidity conditions.

d) light: this parameter affect the growth rate, synthetic capacity and the formation of reproductive structures. The inhibition of fungal growth in strong light is regarded as the most widespread effect of light.

e) aeration: most fungi require aerobic oxygen for growth and biodegradative processes. Oxygen is important for cellular respiration and also facilitates fungal utilisation of various nutrients (Griffin, 1994).

2.8.4 Lipid Degradation by Fungi

Degradation of lipids by fungi is predominantly an enzymatic process. Lipases belong to the group of enzymes classified as hydrolases. In aqueous systems, they catalyze the degradation of oils and fats, which are triglycerides (Lazar & Schröder, 1992). Increased production of extracellular lipase in cultures of microorganisms growing in the presence of triglycerides and other lipids has been reported (Alford, *et al.*, 1964). These lipases are present in a variety of fungi. Those genera considered to be extremely effective in the hydrolysis of triglycerides are that of *Mucor*, *Rhizopus* and *Geotrichum* (Akhtar, *et al.*, 1980). The highest lipase activity has been reported to occur at oil-water interface in the presence of hydrophobic substrates, however the activity is affected by pH, temperature and the availability of growth nutrients (Lazar & Schröder, 1992).

The fatty acid toxicity and species of microorganism not only determines utilisation of fatty acids but also the composition and pH of the growth medium, and the chain length of the fatty acid (Devnath, 2001). Studies have shown that there are three mechanisms which explain how various microorganisms make use of organic substrates or compounds having low solubilities in water as a nutrient supply or growth substrate. These mechanisms are:

- only molecules that enter the aqueous phase by spontaneous partitioning can be utilised by the microorganisms and further degradation would depend on the rate of spontaneous partitioning;
- the microorganism excretes products that converts the substrate into droplets with sizes less than 1μm and these are then assimilated by the organism. This process is defined as Pseudosolubilisation; and

- the cells that come in to contact with the non-polar compounds, on the surface of which the population develops and the chemical at or near the point of contact with the organism passes through the cell into the cytoplasm (Alexander, 1999).

2.9 YEAST BIOLOGICAL TREATMENT

The fungi known as yeasts are organisms of very great economic importance. Certain species are used in all parts of the world for the process of baking and for the production of alcoholic beverages by fermentation, for they secrete enzymes which convert sugars into alcohol and carbon dioxide (Smith, 1969).

2.9.1 General Characteristics of Yeast

Yeasts are fungi that grow as single cells. They produce daughter cells either by budding (the *budding yeasts*)or by binary fission (the *fission yeasts*). They grow typically in moist environments where there is a plentiful supply of soluble nutrients such as sugars and amino acids. With a few exceptions, yeasts are unable to degrade polymers, such as starch and cellulose which are used by many hyphal fungi (The Microbial World, 2001).

2.9.2 Yeast Classification

There are four groups of yeasts:

- Ascomycetous: these yeasts capable of forming ascospores in asci, they are considered to be primitive ascomycetes. There are 23 genera.
- Basidiomycetous: yeasts having life cycles similar to those of the order Ustilaginales of the Basidiomycetes. There are 4 genera.

- Ballistosporogenous: yeasts in the family Sporobolomycetaceae that forcibly discharge spores by the drop excretion mechanism. There are 3 genera. Morphologically, ballistospores resemble basidiospores, but generally considered to possess an asexual rather than a sexual means of reproduction.
- Asporogenous: these are yeasts incapable of producing ascospores, ballistospores, or sporidia (some species produce asexual spores called endospores). There are 14 genera. Since sexual life cycles do not occur or have not been observed so far, these yeasts are members of the Fungi Imperfecti (Macmillan & Phaff, 1989).

2.9.3 Yeast Growth Requirements

Yeasts require essential elements from the growth environment from simple food sources which need to be available at the macro-nutrient level (approximately 10^{-3} M) in the case of C, Hydrogen (H), O, N, P, K, Mg and Sulphur (S), or at the micro-nutrient level (approximately 10^{-6} M) in the case of trace elements. Many species will grow very well in simple aqueous medium at pH 5.5, however they are quite capable of growing over wide pH range (2 - 8) (Walker, 1998).

Yeasts are chemoorganotrophic organisms, which means that they obtain carbon and energy from compounds in fixed, organic linkage. The carbon sources are from sugars such as glucose etc. Hydrogen is available from carbohydrates and other sources. The hydrogen ions are very important in yeast cell physiology since variations in both extracellular and intracellular pH can have a dramatic influence on growth and metabolism of yeast cells. Yeasts are unable to grow in complete absence of oxygen. The oxygen serves as a substrate for respiratory and other mixed-function oxidative enzymes, as well as synthesis of ergosterol and unsaturated fatty acids. Nitrogen, potassium and magnesium is obtained from ammonium, potassium and magnesium salts respectively. The phosphorus is obtained from phosphates which is used for energy transduction, nucleic acid and membrane structure. The sulphur is obtained from sulphate and methionine. Yeast growth factors include vitamins, purines and pyrimidines, nucleosides and nucleotides, amino acids,

fatty acids, sterols and miscellaneous compounds (Walker, 1998).

The physical factors that affect yeast growth are:

a) temperature: this is a very important physical parameter that influences yeast growth. Most yeast generally grow best between 20 - 30°C. Most yeasts exploited in biotechnology are mesophilic. Actual values of maximum growth temperature are not only species dependant, but also growth condition dependant.

b) water: like most organisms, yeasts need water in high concentrations for growth and metabolism. If water is absent then no enzyme activity can occur as the substrate and enzymes are all in aqueous solution. Yeasts that able to withstand low water potential conditions are said to be *osmotolerant* or *zerotolerant.*

c) pH: most yeasts grow very well between pH 4-5 and 6.5, but nearly all species are able to grow in more acidic and alkaline media (around pH 3 or pH 8, respectively) (Walker, 1998).

2.9.4 Lipid Degradation by Yeasts

Although lipolytic bacteria and yeasts have long been known, the first reports of microorganisms being cultivated on oils and fats, were those in which yeasts were grown on whole fatty fish or fish-processing wastes and subsequently on isolated fish oils (Ratledge, 1994). Van der Veen, 1994 (cited in Ratledge, 1994) stated that the first reports of yeasts being grown on oils and fats in a defined medium appears to be in an unpublished Ph.D. thesis from the Netherlands, where yeasts grew on both fatty acids and animal lard. The yeasts are in decreasing order of yield: *Endomycopsis lipolytica, Saccharomycopsis lipolytica, Candida tropicalis, Pichia vinia, C. cacoi, Torulopsis haemulonii, C. cloacae, C. parapsilosis and C. edax* which only grew on the lard substrate (Ratledge, 1994).

Koh *et al.*, (1983) (cited in Ratledge, 1994) isolated over 200 yeasts from approximately 250 samples but only a few were effective in assimilating palm oil. The reason being the working media

was at a pH of 5.5, however the optimum pH of most yeast lipases lies between 6.5 and 7.0. Therefore in order of growth of a microorganism to occur on a lipid, the culture medium had to be maintained close to the optimum pH (Ratledge, 1994).

2.10 CONCLUSIONS FROM LITERATURE REVIEW

Liquid effluent treatment methods were developed in response to the concern for public health and the adverse conditions caused by the discharged of liquid effluents to the environment (Chughtai& Ahmed, 1991). Even though there are so many physico-chemical processes for edible oil effluent treatment, the degree to which they remove or reduce the organic and FOG content in the effluent is insufficient to meet environmental standards, thereby forcing industrial workers to find alternate or subsequent treatment methods.

It is for this reason that an investigation using different types of microorganisms on degradation of edible oil effluent seems warranted to determine the effectiveness of biological treatment.

CHAPTER THREE

EDIBLE OIL EFFLUENT TREATMENT USING ACTIVATED SLUDGE

3.1 INTRODUCTION

The activated sludge process is a suspended growth system comprising of a mass of microorganisms constantly supplied with organic matter and oxygen. The microorganisms grow in flocs, and these flocs are responsible for the transformation of the organic material to new bacteria, carbon dioxide and water (Horan, 1990). The process, developed by Arden and Locket, uses fill and draw reactors, i.e., batch processes, to successfully treat wastes in a short period of time. Thereafter continuous flow reactors were designed. Even though batch reactors were ignored for over 50 years, batch treatment systems have been re-established (Droste, 1997). Batch treatability testing is used to screen the impact of industrial wastewater on the activated sludge process. It can be performed on combined plant discharges, individual waste streams or specific organic compounds. There are quite a few batch test procedures which have been developed to evaluate the activated sludge process, including:

- Batch Activated Sludge (BAS) testing
- Fed-Batch reactor (FBR) testing: the feed in a FBR is continuously added until the maximum liquid volume is reached. The reactor is then allowed to continue or be partially or completely emptied depending on the process (School of Life Sciences and Chemical Technology, 2002; Fed Batch Fermentations, 2002).

These tests measure the response of the activated sludge biomass to the waste stream or constituent of interest (Torrens *et al.*, 1999) . In this study, a combination of the fed-batch and batch process were used for the activated sludge treatment process.

Each batch test comprises of an aerated reactor containing an activated sludge biomass and the

wastewater source of interest at the desired concentration. Adjustment of pH and dissolved oxygen are monitored in order to provide proper biological treatment conditions. If the biomass is not acclimated to the wastewater, then a short acclimation period is desirable to provide exposure of the biomass to the wastewater (Torrens *et al*, 1999).

3.2 MATERIALS AND METHODS

3.2.1 Effluent Sampling

Effluent samples were collected from the edible oil industry in Pietermaritzburg, (for confidential reasons the company name would not be disclosed). The samples were collected after the deodorisation process in cleaned 25 L vessels and stored in a cold room at 4°C, until further use.

3.2.2 Effluent Characterisation

The effluent was then characterised by applying the following the tests:

1) Chemical Oxygen Demand (COD): The NOVA 60 spectroquant was used in determining the COD. Appendix 2 can be referred to for details of procedure.
2) Total Suspended Solids (TSS): Standard Method No.: 2540 D (See Appendix 3)
3) Fats, Oils and Greases (FOG): Standard Method No.: 5520 B (See Appendix 4)
4) pH: Appendix 5

3.2.3 Mixed Liquor Sampling

Sludge samples were obtained from the aerobic zone of the full-scale activated sludge system at Darvill Wastewater Works, Pietermaritzburg. Samples were collected in cleaned, dry 5 L vessels.

3.2.4 Mixed Liquor Concentration

The following tests were conducted:

1) Mixed Liquor Suspended Solids (MLSS): this test was performed in order to determine the amount of biomass, and adjusted by dilution to achieve the desired concentration which was 3000 mg/L. Standard Method No.: 2540 D (See Appendix 6)
2) Volatile Suspended Solids (VSS) also known as MLVSS : Standard Method No.: 2540 E (See Appendix 7)

3.2.5 Mixed Liquor Characterisation

Microscopic investigation of the sludge was conducted on a daily basis for the five experiments. Protozoa counts were performed on a wet mount slide and viewed under the 10x magnification. At the same time the floc structure and type were determined. Appendix 8 can be referred to for the protozoa evaluation sheet. In addition the filamentous bacteria were also identified. Appendix 9 and 10 can be referred to for the filamentous microorganisms identification sheet and the survey of the characteristics of the filamentous microorganisms respectively. Appendix 11 illustrates some images of the protozoa and filamentous organisms found during the sludge investigation.

The following staining techniques were conducted to identified filamentous bacteria:

1) *The Gram Stain*: With this staining method, the bacteria are first stained blue by means of carbolgentianviolet. Subsequently the cells are rinsed with an alcohol solution. During this treatment the cells of some bacterial genera release the dye. These bacteria are called gram negative. The gram positive ones do not release the absorbed carbolgentianviolet when rinsed with alcohol. The colorless gram negative bacteria are subsequently counter-stained with safranine. This gives them a light-red color (Eikelboom & Buijsen, 1983). Appendix 12 can be referred to for the procedure.

2) *The Neisser Stain*: Some bacteria form granules in the cell which mainly consist of polyphosphates. These granules cannot clearly be observed with a light microscope without staining. By means of this staining their color turns to blue-black. Neisser negative filaments stain light-brown to yellowish and are often hardly visible. Neisser positive filaments contain dark granules or are stained entirely grey-blue (Eikelboom & Buijsen,

1983). Appendix 13.

3) *The Polyhydroxybutyrate (PHB) Stain*: While the Gram and Neisser stains are used routinely, the PHB stain is more specialised. This staining procedure is used for the detection of intracellular storage products such as poly-β-hydroxybutyrate which also aids in the identification process (Jenkins, *et al.*, 1993). Appendix 14.

3.2.6 Experimental Set-up

The following parameters were investigated to optimise treatment i.e., (1) temperature, (2) supplementation of nutrients and (3) lack of nutrients. For the first experiment a temperature of 21°C without nutrients was conducted, the second was at the same temperature but with nutrients added to the effluent. The third and fourth experiments were exactly the same as the first two except the temperature was at 31°C. All four experiments were conducted using diluted edible oil effluent at a concentration of approximately 2000 mg/L. Experiment 5 was conducted using optimised conditions determined from the investigation of the first four experiments. However, in experiment five undiluted edible oil effluent was used which was at a concentration of approximately 3000 mg/L.

For the experiments the following experimental set-up was conducted:
A 10 L pyrex cylindrical vessel served as the reactor. A total working volume of 6 L was used. Of the six liters, 3 L of the innoculum, i.e., the sludge at approximately 3000 mg MLSS/L, was added and to this 500 mL of the diluted influent at a concentration of approximately 2000 mg COD/L was added. Everyday 500 mL of the diluted influent, at adjusted pH 7.00, was added until the total working volume (6 L) was reached, this provided a 5 day acclimatisation period. Once the 6 L mark was reached, 600 mL of sludge was wasted daily, and 600mL of influent was added to bring the volume up to 6 L. This resulted in a 10 ten day sludge age, however the experiment was left to continue for a further 10 days, resulting in a total experimental period of 20 days. For the higher temperature of 31°C, the reactor was placed in a waterbath. Figure 3.1 shows the experimental design.

For the 5th experiment the exact procedure was followed as mentioned above, except the COD of

the influent was undiluted (~ 3000 mgCOD/L).

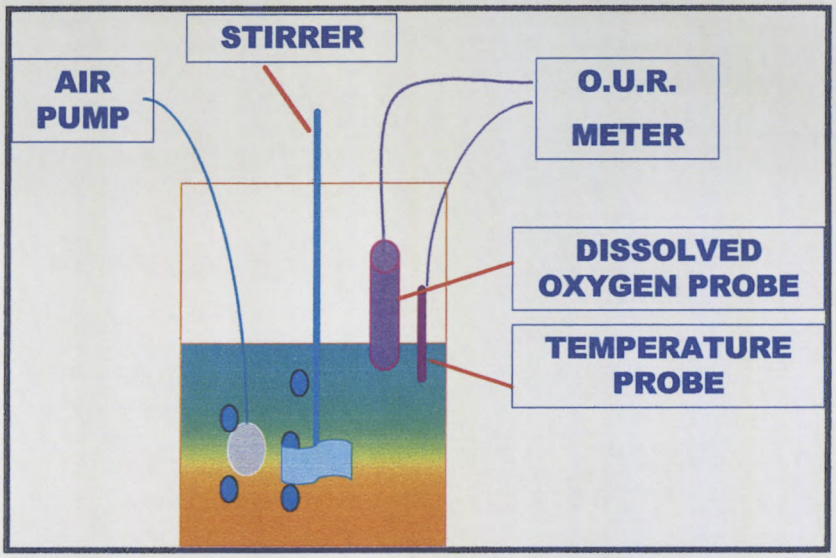

Figure 3.1: Schematic diagram of the experimental set-up

The oxygen utilisation rate (OUR) was determined for each experiment using an OUR meter. The dissolved oxygen (DO) concentration was set between 2.0 to 5.0 mg OUR/Lh^{-1}. The temperature was monitored using this meter as well. The probe was calibrated twice a week while the membrane was changed once a week.

For the experiments where nutrients were added, the C:N:P ratio of 100:5:1 was used. Therefore the total nitrogen (TN)(Appendix 15) and total phosphorus (TP)(Appendix 16) were determined. The effluent served as the sole carbon source. Ammonium chloride served as the nitrogen source and Potassium di-hydrogen orthophosphate served as the phosphate source.

3.2.7 Analysis of Effluent

From the 600 mL that was wasted, effluent was taken from here and analysed for the following parameters: COD, TSS, FOG and pH. When the MLSS was too low, wastage of sludge was stopped and the effluent withdrawn directly from the reactor, after the sludge was allowed to settle for 30 min.

3.2.8 Analysis of Sludge

For the sludge that was wasted, the following parameters were analysed for: MLSS, VSS, and sludge volume (SV30 and SVI) (Standard Method Nos.: 2710 C and 2710 D respectively)(see Appendix 17). Also from the sample the protozoa counts were determined, and the filamentous bacteria were identified using the staining techniques mentioned previously.

3.3 RESULTS

3.3.1 Experiment 1: Degradation of diluted edible oil effluent at 21°C without supplementation of nutrients.

In order to dilute the effluent the initial characteristics of the undiluted effluent was required, table 3.1 provides the information of the undiluted and diluted effluent. The initial MLSS of the sludge was 5567 mg/L, which was adjusted by dilution to provide an initial biomass concentration of 2860 mg/L. By the time the 6 L working volume was reached i.e., after the 5 day acclimatisation period, the biomass concentration was halved, which was 1430 mg/L for day 1 analysis. The TKN and TP were measured in order to determine the quantity of nutrients, if any were present in the effluent. Analyses showed that 7.1 mg TKN/L and 3.4 mg TP/L were present. The batch of undiluted effluent collected was also very acidic.

Table 3.1: Initial characteristics of the undiluted and diluted effluent for Experiment 1

PARAMETER	UNDILUTED	DILUTED
COD (mg/L)	6313	1553
FOG (mg/L)	1000	338
TSS (mg/L)	895	198
pH	~2.00	7.10

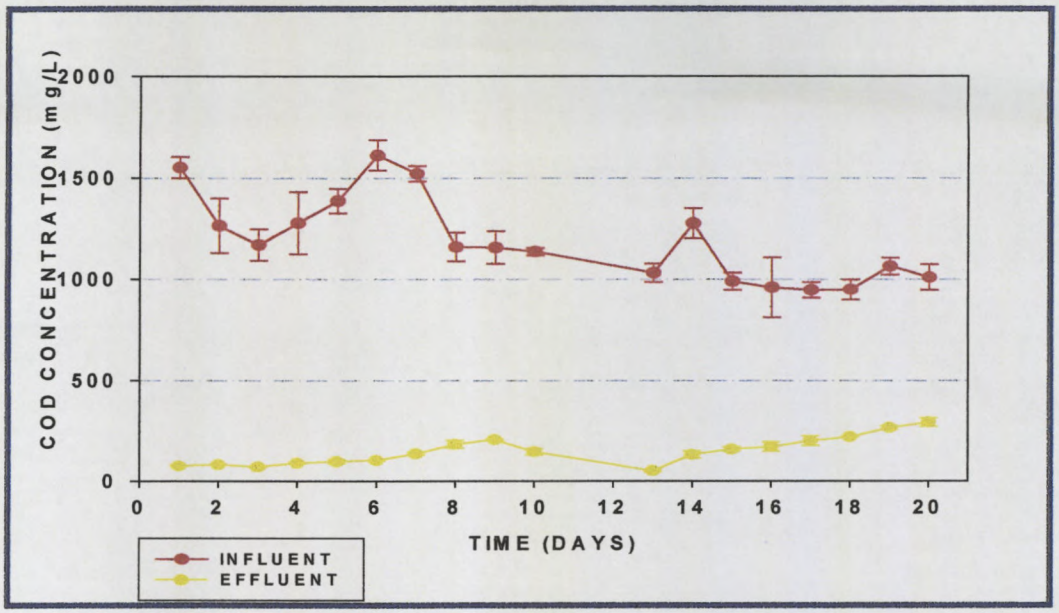

Figure 3.2: Average COD profiles of the influent and effluent for experiment 1

The organic degradation was measured in terms of the effluent COD. The effluent was taken from the supernatant of the sludge that was wasted per day. However when the MLSS became too low to waste, the effluent was removed from the reactor manually once the sludge in the reactor was allowed to settle for 30 mins. A volume of 600mL of the supernatant was removed and 600mL of influent was added. Figure 3.2 shows the COD between the influent and the effluent.

The average influent COD was 1182.42 mg/L while the effluent COD was an average of 160.74 mg/L. An average COD of 1021.68mg/L was removed in this experiment. The COD of the influent decreased with time and there were fluctuations in the concentration of the influent. A better description of the degradation rate can be seen in Figure 3.3, which shows the percentage of the organics removed. This figure showed that for the first 6 days the percentage of COD removed was above 90 %, after that the removal rate declined to the 80 percentile range, but increased after the 10th day back to the 90 percentile range. However after the 13th day the percentage rate continued to decline to the end of this experiment to a percentage removal rate in the 70 percentile range. An average percentage of 85.37 COD was removed.

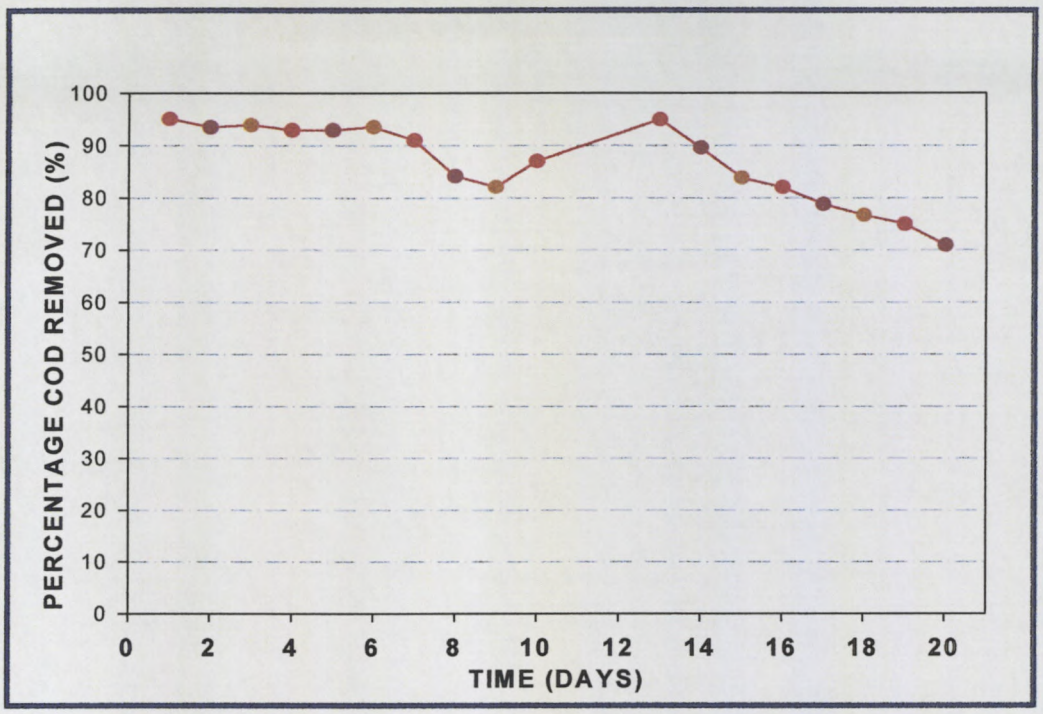

Figure 3.3: Percentage COD removed over time for experiment 1

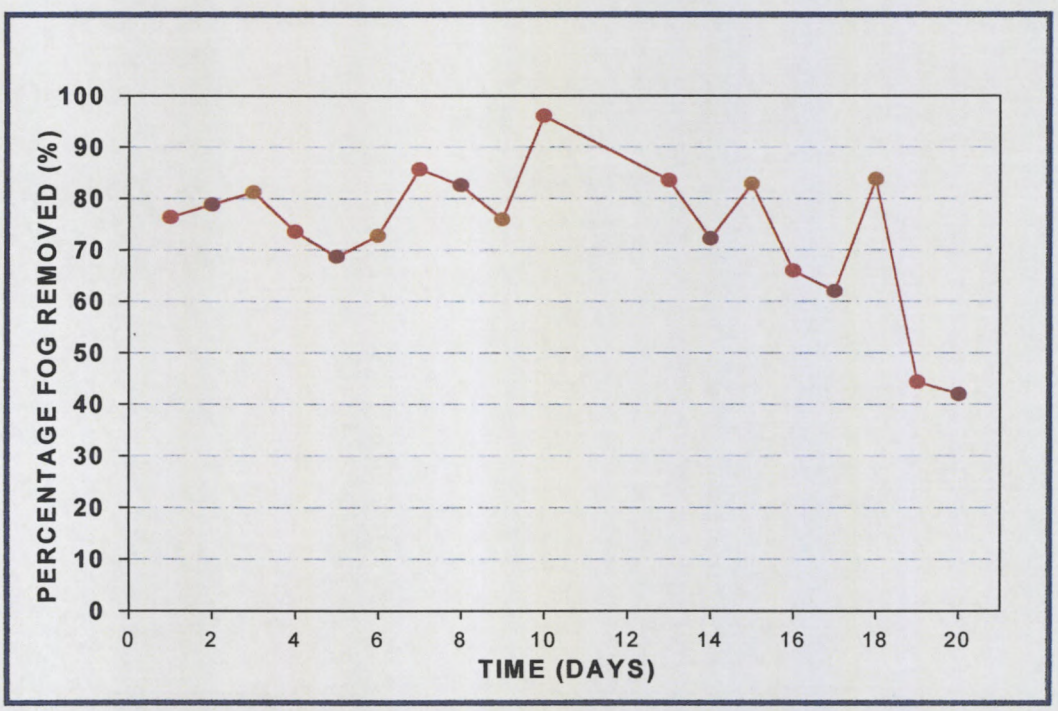

Figure 3.4: Percentage FOG removed over time for experiment 1

For the removal of FOG's in the system, shown in figure 3.4, the removal rate started with a percentage of about 75% and increased to slightly above 80%. It then declined to just below 70% at day 5 and thereafter increased, to about 87%. This pattern was witnessed throughout the 20 days, with the greatest removal at 96% on day10. Thereafter from day 18 the removal rate declined from 85 % to 41% on day 20. The average FOG removed for this experiment was 73.18%. The average influent FOG was 284.42 mg/L and the average effluent FOG was 74.53 mg/L.

The MLSS for this experiment continued to decrease with time (table3.2), hence as it reached the 10^{th} day the sludge volume was too low to waste sludge, therefore the wastage of sludge was discontinued after day 9. Although the systems MLSS concentrations decreased, the active portion or MLVSS or TSS showed an increase from an initial value of 67% to 84% from day 1 to day 9. The SVI also shown in table 3.2 indicates that the sludge had good settling properties. It started from 76 mL/g and increased to about 92 mL/g at day 5 thereafter it began to declined to about 73 mL/g.

Table 3.2: MLSS, MLVSS/MLSS, So/Xo ratios and SV30 and SVI values for days 1 to 9

DAY	MLSS (mg/L)	MLVSS/MLSS (mgVSS/mgTSS)	SV30 (mL/L)	SVI (mL/g)	So/Xo
1	1430	0.67	110	76.92	1.09
2	1506	0.71	120	79.68	0.84
3	1374	0.70	110	80.06	0.85
4	1200	0.76	100	83.33	1.07
5	976	0.81	90	92.21	1.42
6	970	0.78	80	82.47	1.66
7	994	0.78	70	70.42	1.53
8	792	0.83	60	75.76	1.46
9	682	0.84	50	73.31	1.70

The initial substrate to initial biomass (So/Xo) ratio is also shown in table 3.2. This is indicative of the initial food (So) i.e., influent, available to the biomass (Xo) in the system. There were slight fluctuations in the ratio, with day 1 being at 1.09 and then decreasing. After day 3 the ratio increased from 0.85 to being at 1.70 on day 9.

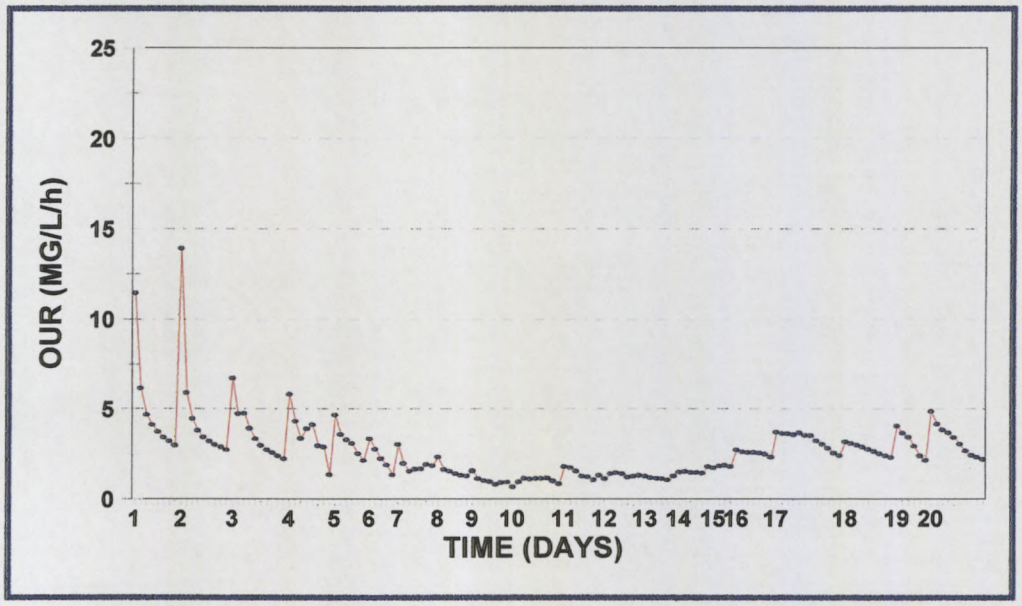

Figure 3.5: Average Oxygen Utilisation Rate over time for experiment 1

The oxygen utilisation rate (OUR) was monitored for each process. This reading shows the amount of oxygen the organisms utilised during the process. Figure 3.5 shows the OUR rate for the twenty day period. The OUR increased each time a new batch of influent was added per day. This can be seen in the sudden increase in OUR for each day, however the OUR readings declined as the influent became depleted. As the process continued and new influent added each day the concentration increased but not as much as the first few days. Towards the end of the process (days 17 - 20) the readings increased slightly. The highest OUR was about 14 mg/Lh^{-1} on day 2, thereafter all the readings remained below 10 mg/Lh^{-1}.

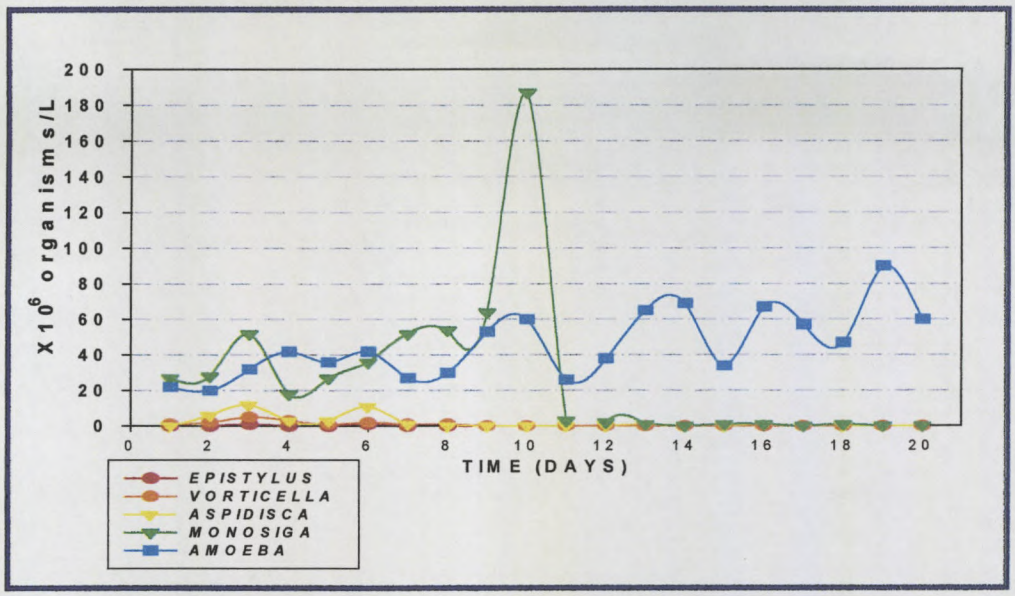

Figure 3.6: Protozoan survey over time for experiment 1

A protozoan survey was conducted for the duration of the experimental period. Figure 3.6 shows the protozoa that were present in the system during the process. The *Amoeba* and *Monosiga* proliferated throughout this process. The monosiga after the 8th day had a sudden increase in number (1.87×10^7 orgs/L and after day 10 decreased dramatically to almost zero. The *Amoeba* on the other hand had slight fluctuations but with these fluctuations increased in number and became the dominant species in the system with the highest concentration being 90 $\times 10^6$ orgs/L on day 19. Sessile ciliates such as *Vorticella* and *Epistylis* were present, but not in large numbers. The crawling ciliates present was *Aspidisca*, this as well was not present in large numbers and eventually died off towards the end of the process. Other organisms that were present in the system, but in very few numbers were *Nematodes* and *Colipidium*(a crawling protozoa). The filamentous bacteria present in the system were Type 0041, Type 0675, *Nocardia sp* and towards the end *N. limicola II.* The morphology of the floc ranged from being firm irregular, compact to weak and irregular. The diameters of the floc ranged between 10 to 100 μm.

The TSS concentration is indicative of how much suspended solids are present in the influent and effluent, represented in figure 3.7. It can be seen that initially the TSS of the effluent was low and then increased after day 7, reaching 74 mg/L on day 9. However after the 13 day the TSS value

increased once more and this continued to the last day which was 56 mg/L. The influent TSS increased to 210 mg/L on day 5 and thereafter decreased. There were slight fluctuations after day 14 but the decline continued even lower than the effluent. The final influent concentration was 26 mg/L.

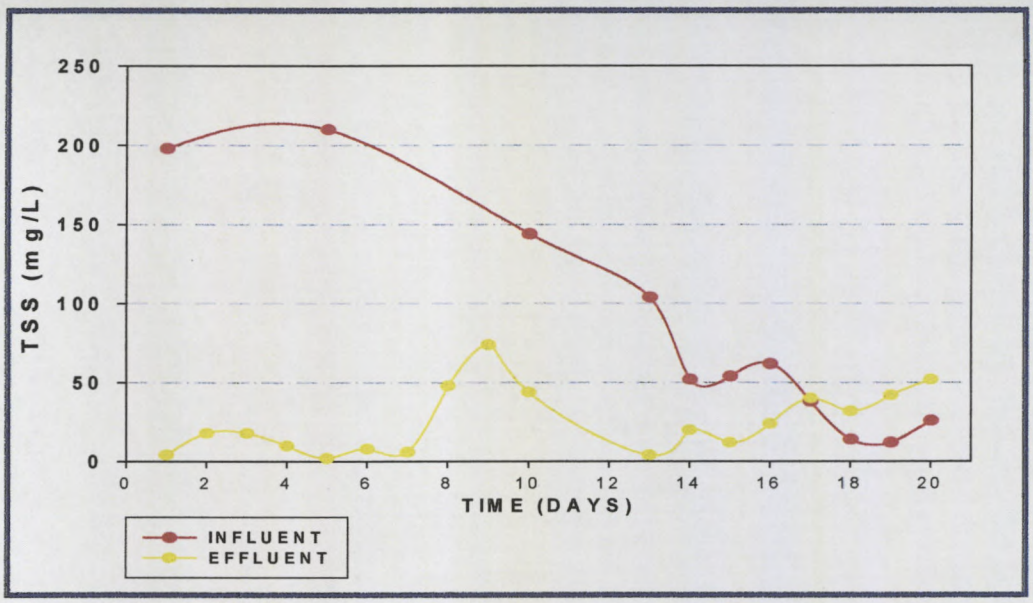

Figure 3.7: TSS profiles of the influent and effluent for experiment 1

3.3.2 Experiment 2: Degradation of diluted edible oil effluent at 21°C with supplementation of nutrients

The same protocol in experiment 1 was followed in experiment 2, except the influent was supplemented with nutrients. The supplementation was carried out according to the C:N:P ratio which was 100:5:1. The TKN and the TP of the influent were measured before dosing in order to determined the quantity of nutrients to add. Analyses showed that 3.6 mg TKN/L and 4.3 mg TP/L were present. The influent served as the carbon source, ammonium chloride (105.4 mg/L) provided the nitrogen source and potassium di-hydrogen orthophosphate (21.08 mg/L) provided the phosphate source. Fresh samples of edible oil effluent and sludge were collected for this experiment. The MLSS was diluted from 4882 mg/L to provide a biomass concentration of 2944 mg/L. After the 5

day acclimatisation period the biomass reached a concentration of 1342 mg/L. Table 3.3 shows the undiluted and diluted effluent characteristics. The undiluted batch had an initial organic concentration that was lower than the last (4000 mg COD/L) and the pH was more alkaline as compared to the very acidic batch in experiment 1. The pH of the diluted influent was maintained around 7.

Table 3.3: Initial characteristics of undiluted and diluted effluent for experiment 2

PARAMETER	UNDILUTED	DILUTED
COD (mg/L)	4000	2108
FOG (mg/L)	888	692
TSS (mg/L)	812	264
pH	~11	7

Figure 3.8 shows the influent and effluent organic concentrations. The average influent concentration was 1714.88 mg COD/L and the average effluent concentration was 210.70 mg COD/L. The process was able to remove an average organic concentration of 1504.18 mg COD/L. Even though there were fluctuations in the influent COD, the decrease in the concentration over time was not as drastic as experiment 1. The COD of the influent remained between 1500 and 2000 mg/L The percentage COD removed shown in figure 3.9 had an average organic removed of 87.66%. There was a slight decrease after day 1 but the removal rate increased and remained relatively constant throughout the rest of the 20 days. The removal rate was quite good remaining in the upper 80 percentile range.

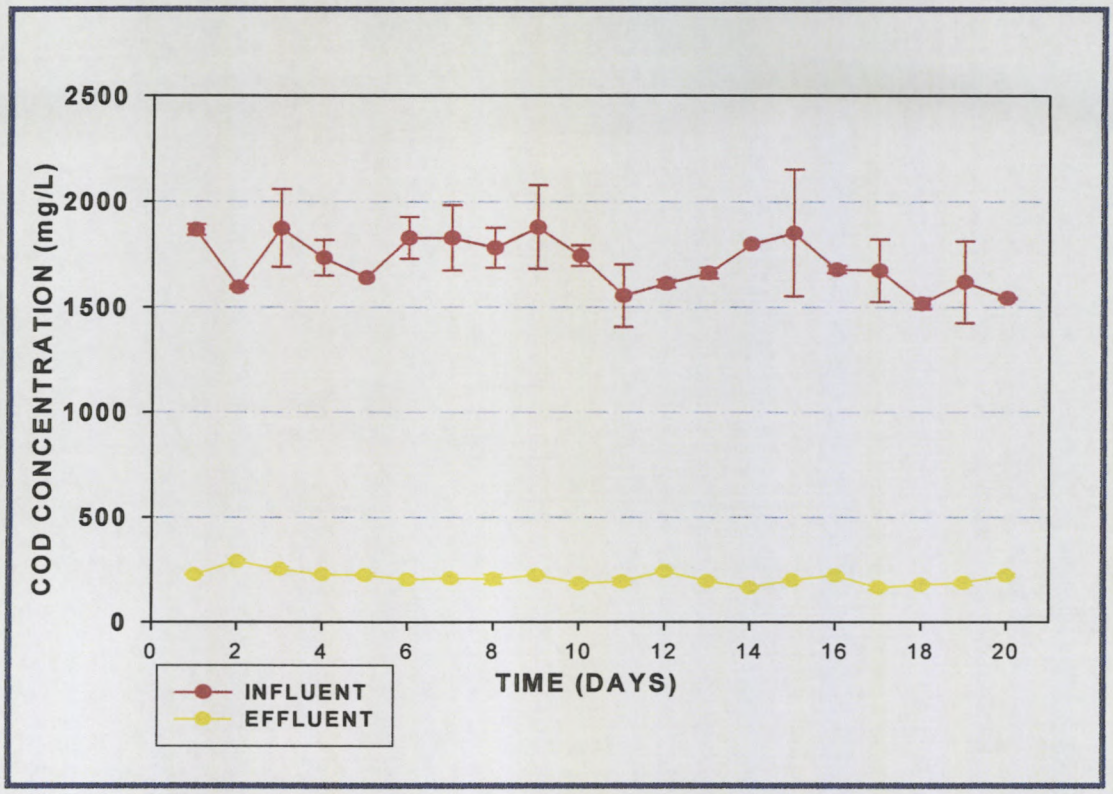

Figure 3.8: Average COD profiles of the influent and effluent for experiment 2

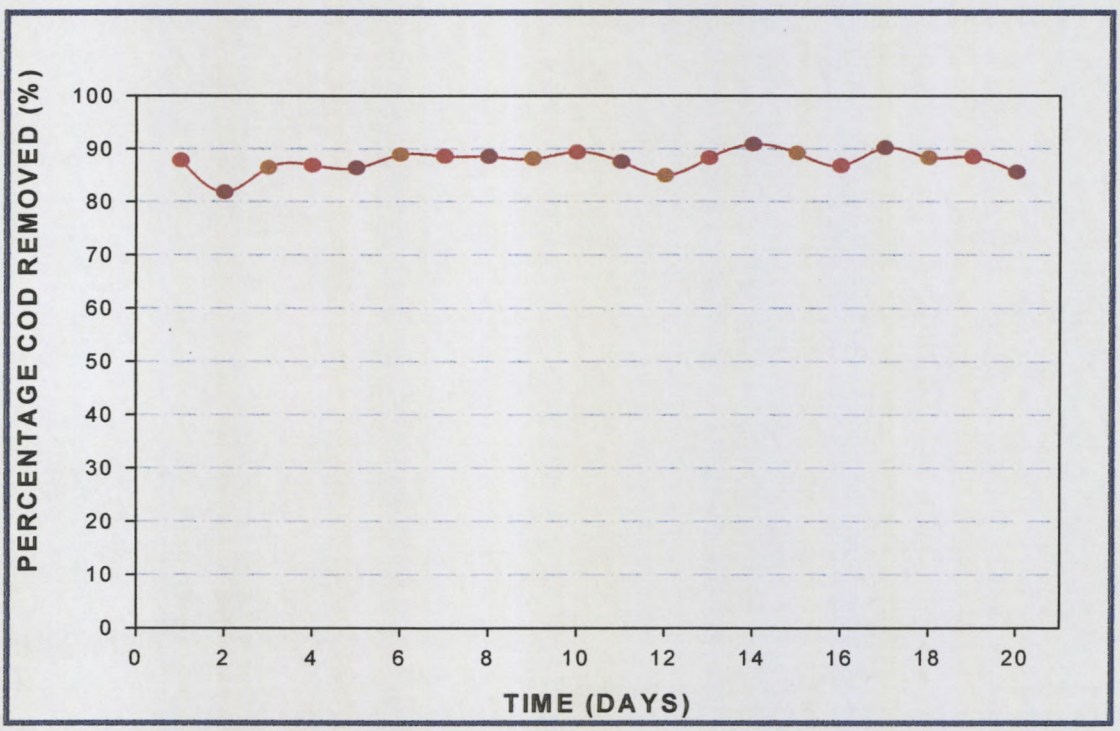

Figure 3.9: Percentage COD removed over time for experiment 2

There were a lot of fluctuations in the removal of FOG's represented in figure 3.10. The removal rate started at about 60% and increased to 86% on day 5. Thereafter the removal rate declined to 42% by day 8, but by day 10 increased to about 70%. The removal remained fairly constant for about 3 days and then increased slightly . It then declined after the 13th day to about 50%. After day 16 the percentage increased (~ 70 %), then decreased (~ 60 %) and once again increased to about 80% removal on the last day. The average FOG concentration removed by this system was 68.06 %. The average influent FOG was 389.59 mg/L and the average effluent was 129.10 mg/L

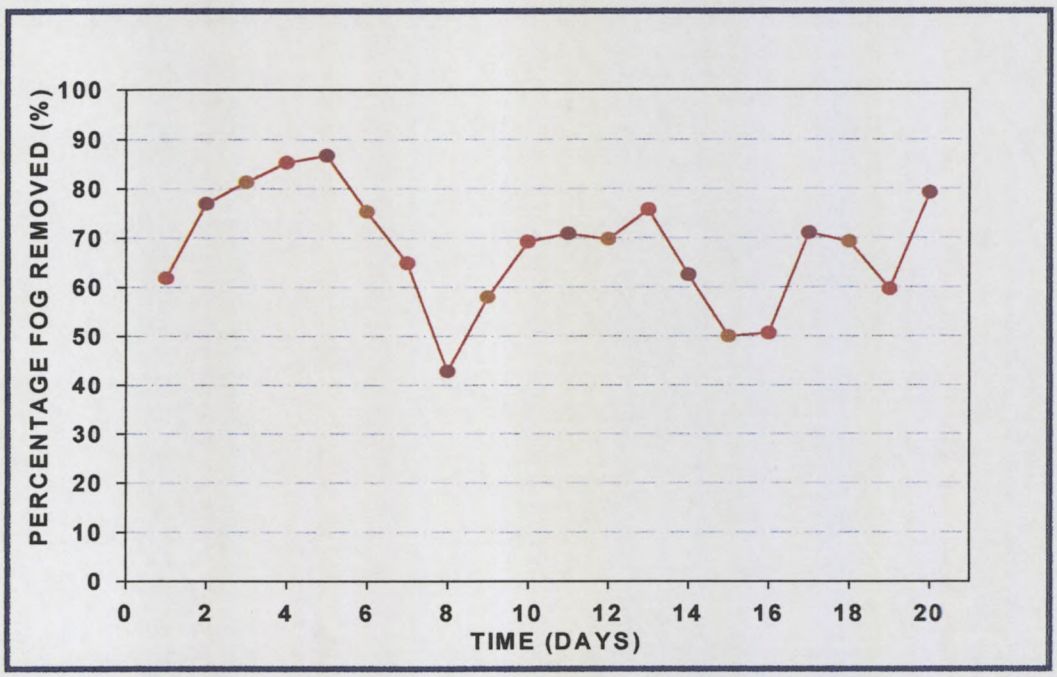

Figure 3.10: Percentage FOG removed over time for experiment 2

In this system as well, after a few days the MLSS was too low to waste, hence wastage of sludge was stopped after the 5th day. The MLSS dropped from 1342 mg/L to 1068 mg/L which is shown in table 3.4. The sludge volume reached 50 mL/L. Once again even though the MLSS concentration declined, the active biomass (i.e., MLVSS/MLSS ratio) increased slightly from 27 % on the first day to 30% on day 4 and then declined to 29% on day 5. The SVI values show that the sludge once again had good settling properties. The So/Xo ratio was also fairly constant and was between 1.1 to 1.7 for the 5 days.

Table 3.4: MLSS, MLVSS/MLSS, So/Xo ratios and SV30 and SVI values for days 1 to 5

DAY	MLSS (mg/L)	MLVSS/MLSS (mgVSS/mgTSS)	SV30 (mL/L)	SVI (mL/g)	So/Xo
1	1342	0.27	70	52.16	1.19
2	1194	0.27	60	50.25	1.57
3	1314	0.25	70	53.27	1.32
4	1368	0.30	70	51.17	1.20
5	1068	0.29	50	46.82	1.71

The OUR's for this experiment reached higher concentrations each day than the previous experiment. The rate at which the organisms utilised the influent was fairly constant throughout the process. There was no decline of the OUR readings in the overall process. Figure 3.11 shows the pattern of the readings over the 20 days. The OUR readings increased each time when a new batch of influent was added and decreased as the influent was utilised. For each day the increase reached almost the same OUR, i.e., about 20 mg/Lh^{-1}.

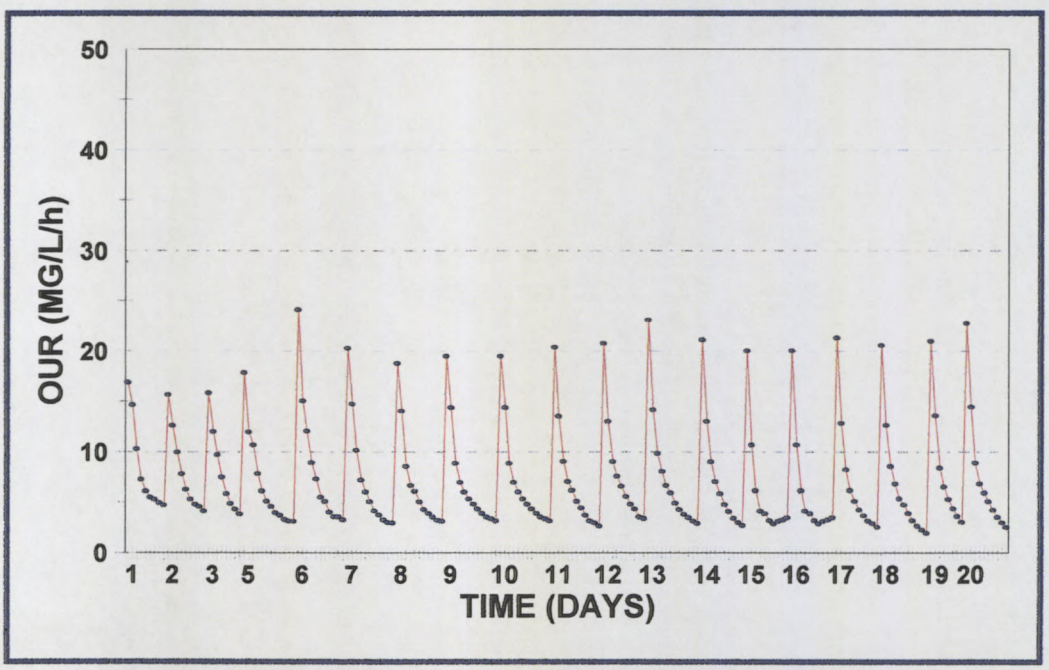

Figure 3.11: Average Oxygen Utilisation Rate over time for experiment 2

For the protozoan survey represented in figure 3.12, there were more organisms that proliferated than in the previous batch. At the beginning of the process the flagellate, *Monosiga*, proliferated, reaching a high of 6.7 x 10^7 orgs/L, but decreased in number as the process continued. Out of the three sessile ciliates (*Epistylis, Opercularia* and *Vorticella*), the *Vorticella* was one that was predominant. The number of *Vorticella* was at its highest point on day 11 (29 x 10^6 orgs/L) and thereafter declined. The *Rotifers* were also present throughout the process. This organism became the dominant one towards the end of the process with a concentration of 26 x 10^6orgs/L. Other organisms present in the system were *Bodo*, which was present between days day11 and17. The numbers were too numerous to count. *Chilodonella, Nematodes* and *Actinopoda* were also present. The filamentous bacteria present in the system were Type 0041, Type 0675 and *Nocardia*. The floc morphology maintained a firm, irregular to rounded and compact structure. The diameter of the flocs ranged between 20 to 150 µm.

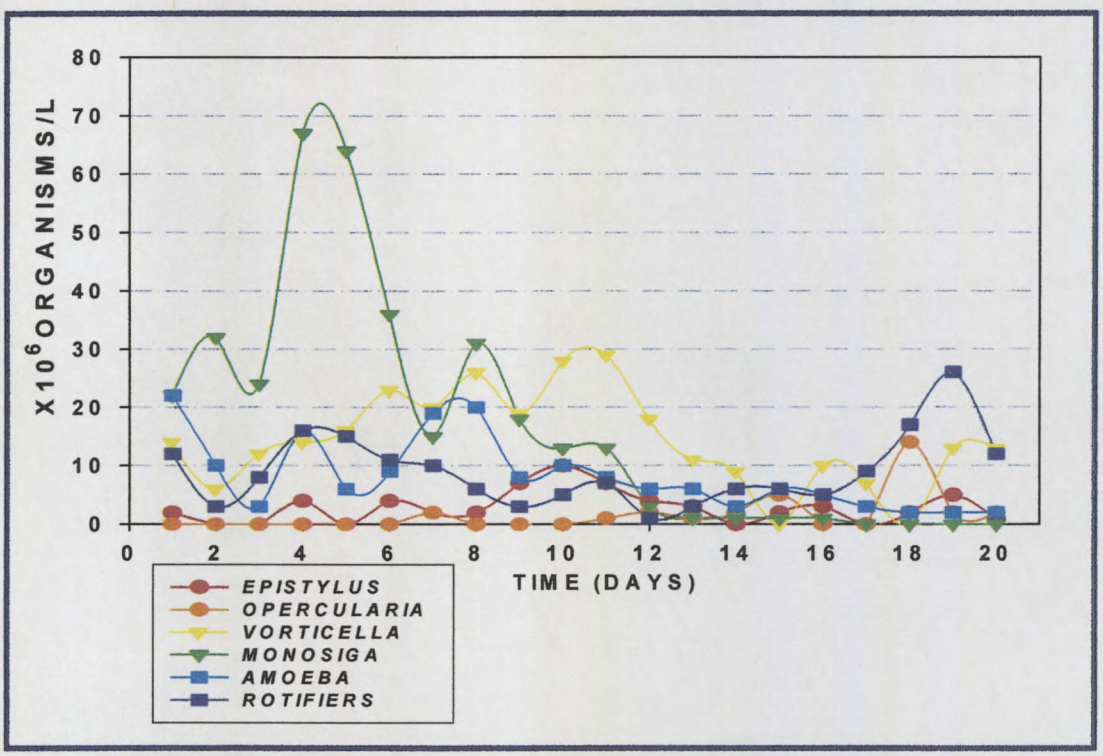

Figure 3.12: Protozoan survey over time for experiment 2

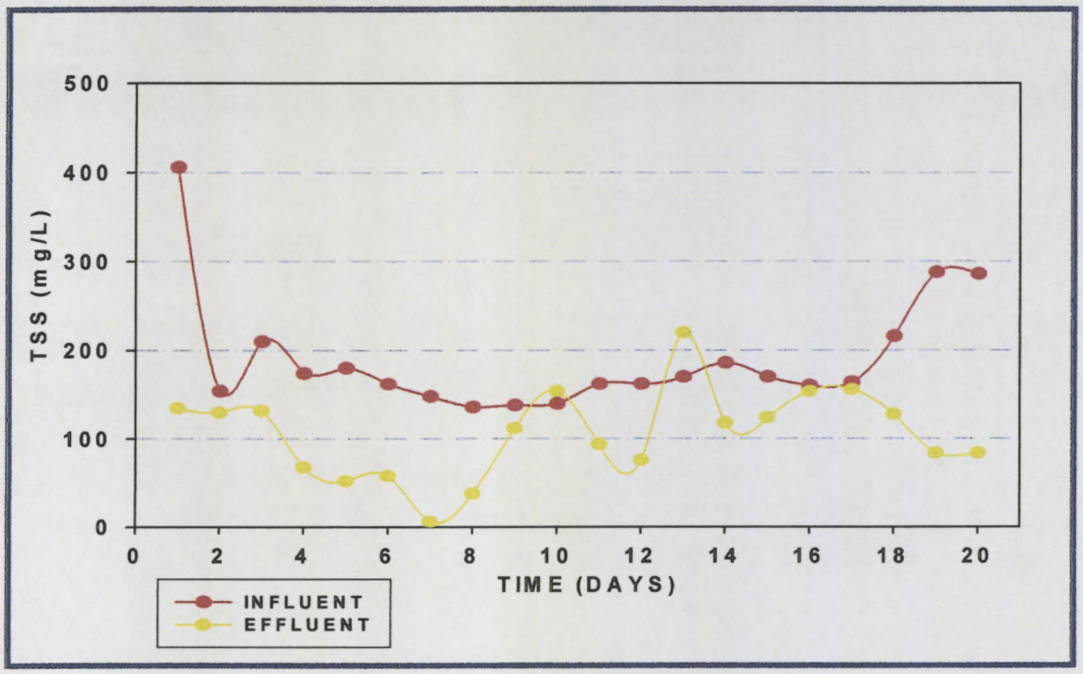

Figure 3.13: TSS profiles of the influent and effluent for experiment 2

The influent suspended solids, shown in figure3.13, showed a rapid decrease in the concentration, but towards the end increased. The effluent TSS started to decline after day 3 but after day 7 increased in concentration to approximately 160 mg/L on day 10. It decreased after day 10 and increased after day 12. The concentration fluctuated a bit, and at one point (day13) became higher than the influent concentration (210 mg/L). It then declined to <100 mg/L on days 19 and 20.

3.3.3 Experiment 3: Degradation of diluted edible oil effluent at 31°C without supplementation of nutrients

In this process the temperature of 31°C was maintained by placing the reactor in a waterbath for the entire duration of the experiment. This was the only parameter that was different, as the process followed the same protocol as the previous experiments. Once again fresh samples of edible oil effluent and sludge were collected. The MLSS was diluted from3900 mg/L to 3000 mg/L. Table 3.5 shows the characteristics of the undiluted and diluted effluent. The undiluted batch of effluent had an acidic pH and the COD was slightly lower than the previous batch. The FOG content was

higher than the previous batches. The TKN and the TP of the effluent were determined in order to see how much nutrients, if any, were present in the effluent. The analyses showed 1.9 mg TKN/L and 3.2 mg TP/L.

Table 3.5: Initial characteristics of undiluted and diluted effluent for experiment 3

PARAMETER	UNDILUTED	DILUTED
COD (mg/L)	3720	1733
FOG (mg/L)	3288	444
TSS (mg/L)	1644	274
pH	4.02	6.99

The organic concentration of the influent represented in figure 3.14, shows a decline in the COD over time. The effluent concentration after the 8th day increased (i.e., the removal rate dropped) and from day 12 remained more or less constant through the rest of the 20 days. The average influent concentration was 1193.13 mg COD/L and average effluent concentration was 205.10 mg COD/L. The average concentration removed was 988.03 mg COD/L.

The initial removal of the organic load was in the 90 percentile range for the first 8 days. The percentage organic removal is represented in figure 3.15. After the 8th day the percentage decreased to the 70 percentile range. It increased slightly after day 14, but there was another decline this time falling in the 60 percentile range at day 17. The removal rate increased thereafter but still remained in the upper 70's. The average percentage COD removed was 82.16.

For the percentage FOG removed represented in figure 3.16, the initial removal was 96 %, thereafter the declined to 61 % at day 4. After day 4 the removal rate increased to 94 % at day 7 and once again began to declined to 71 % at day 12. After day 12 the percentage decreased to 42 %. Thereafter for the rest of the process there were increases and decreases in the percentage removed. The overall percentage FOG removed was 71.75 %. The average influent FOG was 354.45 mg/L and the average effluent FOG was 98.10 mg/L.

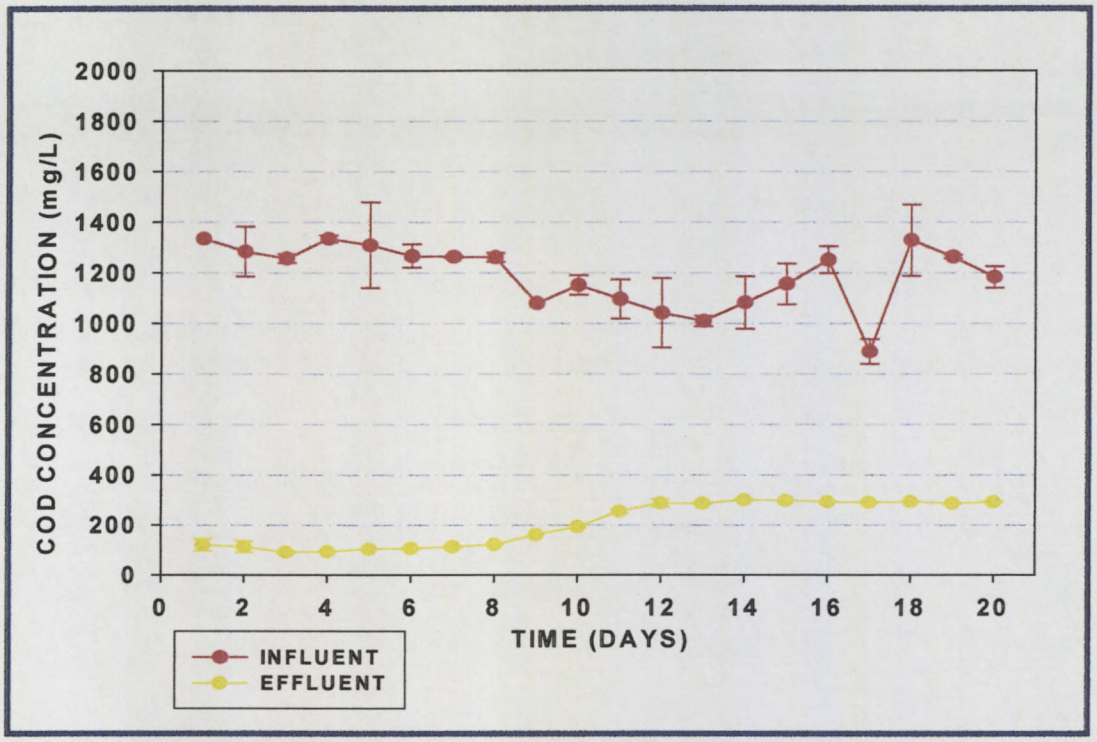

Figure 3.14: Average COD profiles of the influent and effluent for experiment 3

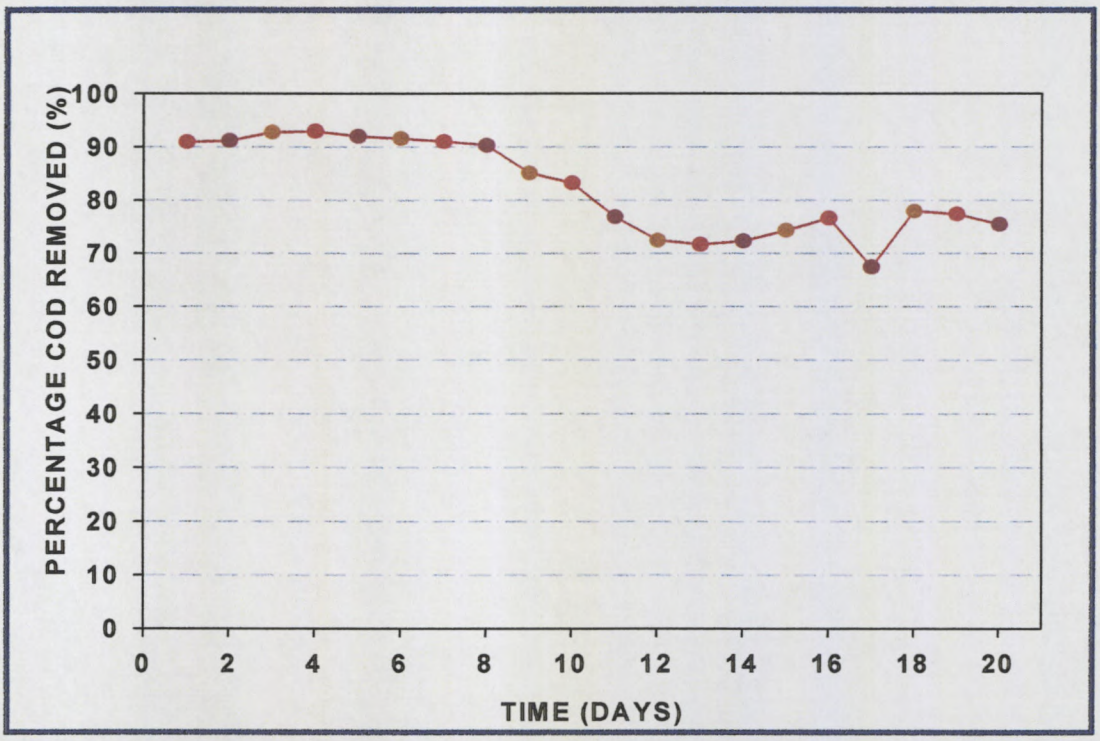

Figure 3.15: Percentage COD removed over time for experiment 3

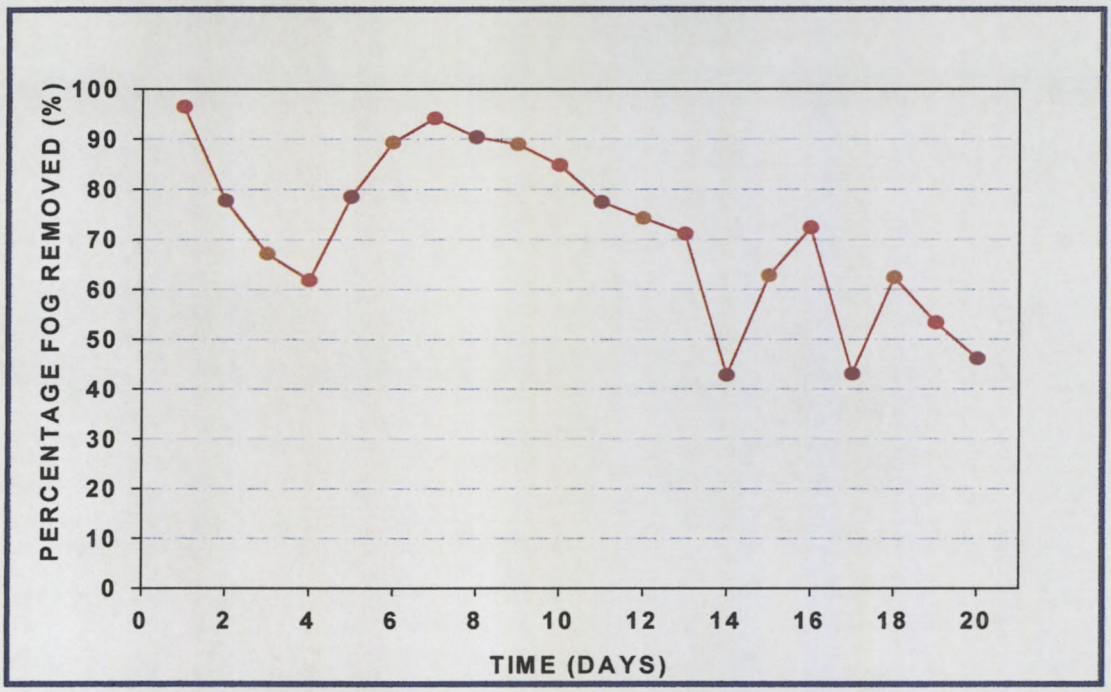

Figure 3.16: Percentage FOG removed over time for experiment 3

With reference to table 3.6 the following was observed. The MLSS concentration was determined throughout the 20 day period. The MLSS concentration followed the same trend as the previous processes, i.e., it decreased in concentration. The active biomass (MLVSS/MLSS ratio) remained relatively constant with slight declines and inclines. The average MLVSS/MLSS was 75 %. The SV30 decreased from day 1 to day 14 (180 mL/L to 70 mL/L respectively), and after day 14 started to increase to day 20 (100 mL/L). There were no major bulking problems even though the SVI were slightly above 100 mL/mg. Only on the last day the SVI was high (135.50 mL/g). The initial So/Xo ratio was low but gradually increased throughout the process, and remained below 2. The highest value it reached was on day 18 which was 1.80.

Table 3.6: MLSS, MLVSS/MLSS, So/Xo ratios and SV30 and SVI values for days 1 to 20

DAY	MLSS (mg/L)	MLVSS/MLSS (mgVSS/mgTSS)	SV30 (mL/L)	SVI (mL/g)	So/Xo
1	1768	0.65	180	101.81	0.76
2	1720	0.72	180	104.65	0.75
3	1498	0.66	170	113.48	0.84
4	1458	0.65	160	109.74	0.92
5	1274	0.72	150	117.74	1.03
6	1118	0.72	120	107.33	1.13
7	1166	0.73	120	102.92	1.08
8	878	0.71	110	125.28	1.44
9	940	0.75	90	95.74	1.15
10	840	0.77	90	107.14	1.37
11	830	0.75	70	84.34	1.32
12	758	0.75	70	92.35	1.38
13	740	0.85	70	94.59	1.36
14	714	0.78	70	98.04	1.52
15	718	0.81	80	111.42	1.61
16	704	0.89	80	113.64	1.78
17	722	0.78	80	110.80	1.23
18	738	0.81	80	108.40	1.80
19	734	0.78	90	122.62	1.72
20	738	0.77	100	135.50	1.61

The OUR is expressed in figure 3.17. As new batches of influent were added per day the OUR increased and as the influent was utilised the OUR dropped. There was a substantial increase on day 3 reaching 44 mg/Lh^{-1}. Poor oxygen utilisation was observed between days 8 and 17. Even after day 17 there were increases in the OUR but not as high as in the initial stages of this process. After day 8 the OUR's remained below 10 mg/Lh^{-1}.

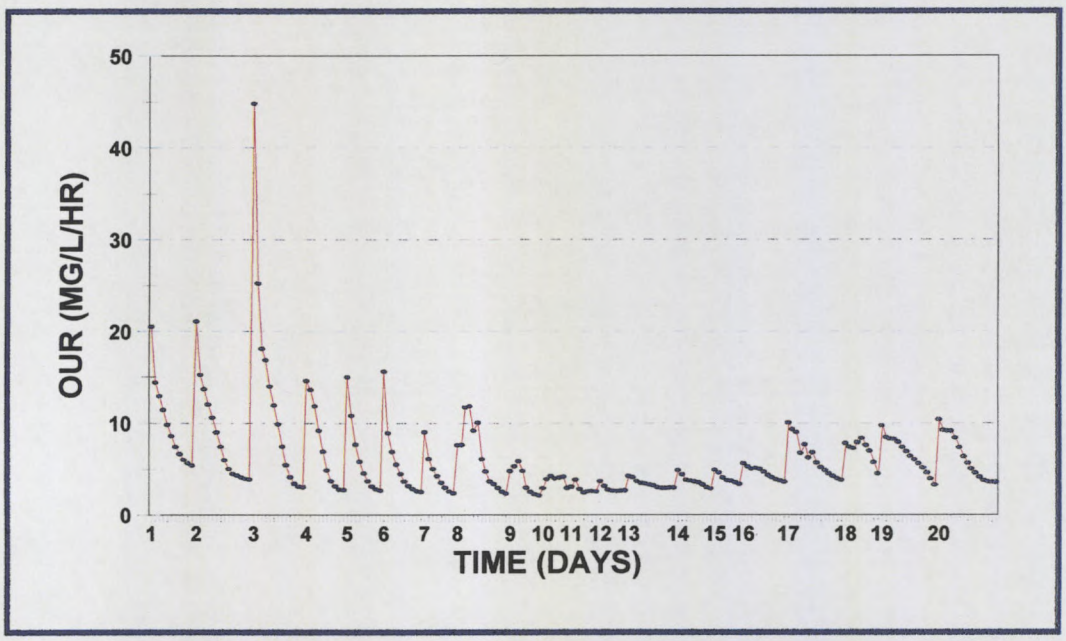

Figure 3.17: Average Oxygen Utilisation Rate over time for experiment 3

For the protozoa survey, expressed in figure 3.18, the *Aspidisca* was initially as being the predominant organism in the system (3.0 x 10^7 orgs/L) with the *Amoeba* and *Rotifer* following. However after the first day the *Aspidisca* number decreased and the *Amoeba* then became the predominant species until day 14. There were fluctuations in the *Amoeba* number during this time. The *Amoeba* reached 2.3 x 10^7 orgs/L on day 7. Thereafter for day 15 the *Rotifer* was higher than the *Amoeba* in number. The *Vorticella* increased slightly after day 2 reaching 1.0 and 1.1 x 10^7 orgs/L on days 3 and 4 respectively but not more than the *Amoeba*. The *Vorticella* number decreased after day 7 and from day 8 to 14 was not present. After day 15 the number of the *Vorticella* increased and it became the predominant species in the system to the end of the process. The

Vorticella reached 1.9 x 10^7 orgs/L on day 19. *Charchesium* and *Monosiga* were also present but in relatively low numbers. For the last two days the flagellate *Bodo* was present in large numbers (too numerous to count). The filamentous bacteria that were present in this process were, Type 0041, Type 0675 and *Nocardia*. For days 13 to 20, Type 0675 was the only filamentous bacteria present. The floc morphology contained a few firm, rounded and compact structure and many firm, irregular compact flocs. The diameters were between 50 to 150 µm.

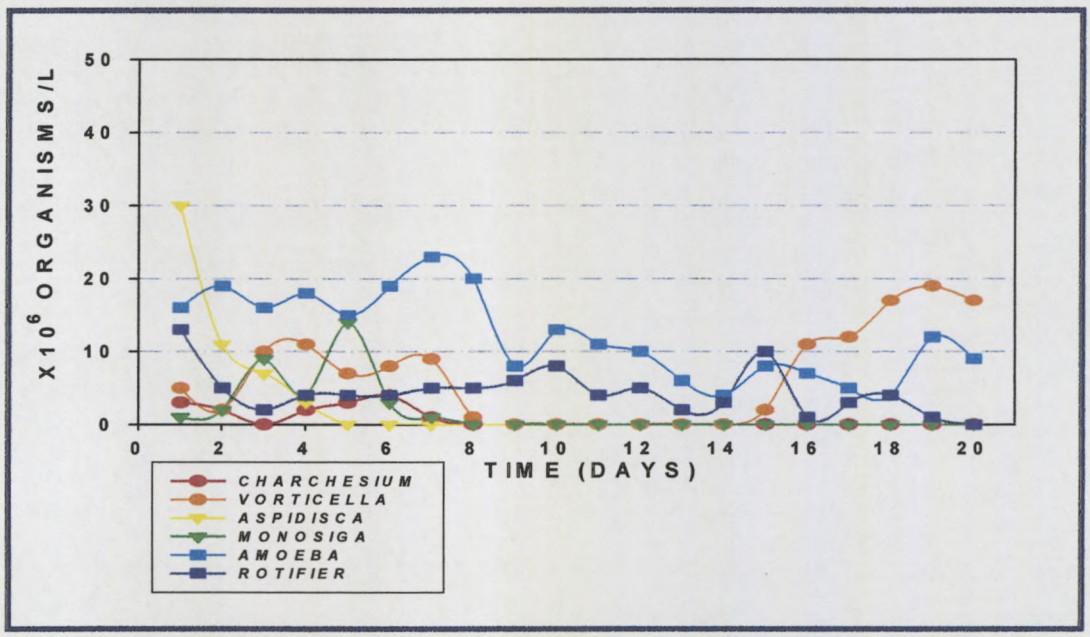

Figure 3.18: Protozoan survey over time for experiment 3

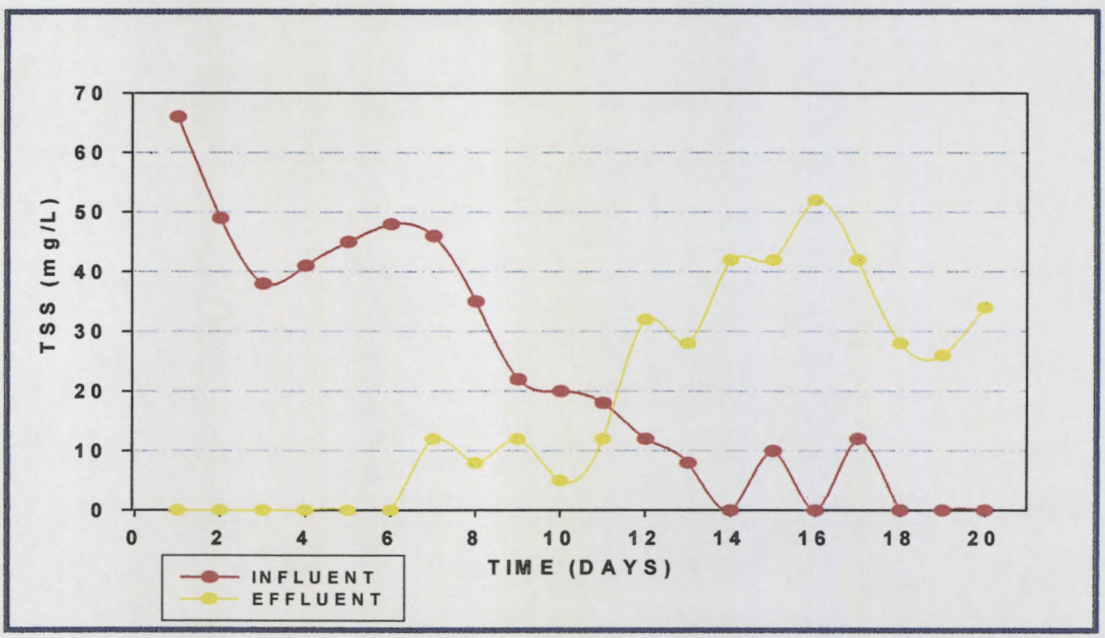

Figure 3.19: TSS profiles of the influent and effluent for experiment 3

The TSS concentration of the influent and effluent is represented in figure 3.19. The results produced differed from the other processes. The TSS concentration of the influent with time decreased quite dramatically and eventually became lower than the effluent concentration. After day 1 the influent TSS declined, and after day 3 it increased up to nearly 50 mg/L at day 6 but thereafter started to decline below 10 mg/L on day 14. A different trend was observed for the effluent TSS concentration. For the first few days the effluent TSS was 0 mg/L and eventually after the 6^{th} day increased. There were slight fluctuations, but the concentration continued to increase. After day 11 the effluent TSS became higher than the influent TSS and reached a high of 52 mg/L.

3.3.4 Experiment 4: Degradation of diluted edible oil effluent at 31°C with supplementation of nutrients.

The same protocol was followed for experiment 2. Only the temperature was maintained at 31°C in a waterbath. The nutrients were added according to the C:N:P ratio which was 100:5:1. The influent was the carbon source, ammonium chloride (126.27 mg/L) was the nitrogen source and potassium di-hydrogen orthophosphate (25.25mg/L) as the phosphate source. The MLSS was diluted from a concentration of 4445.4 mg/L to 2981 mg/L. The undiluted effluent had an alkaline pH. The FOG content was not as high as the previous batch as shown in table 3.7.

Table 3.7: Initial characteristics of the undiluted and diluted effluent for Experiment 4

PARAMETER	UNDILUTED	DILUTED
COD (mg/L)	5515	2383
FOG (mg/L)	998	676
TSS (mg/L)	684	356
pH	~11	6.96

The organic concentration of influent represented in figure 3.20, showed the same trend as the other influent samples, i.e., it decreased in organic concentration over time. The effluent concentration remained relatively constant throughout the process. The average concentration of influent was 1838 mg COD/L and the average effluent concentration was 156 mg COD/L. The average concentration removed was 1682 mg COD/L.

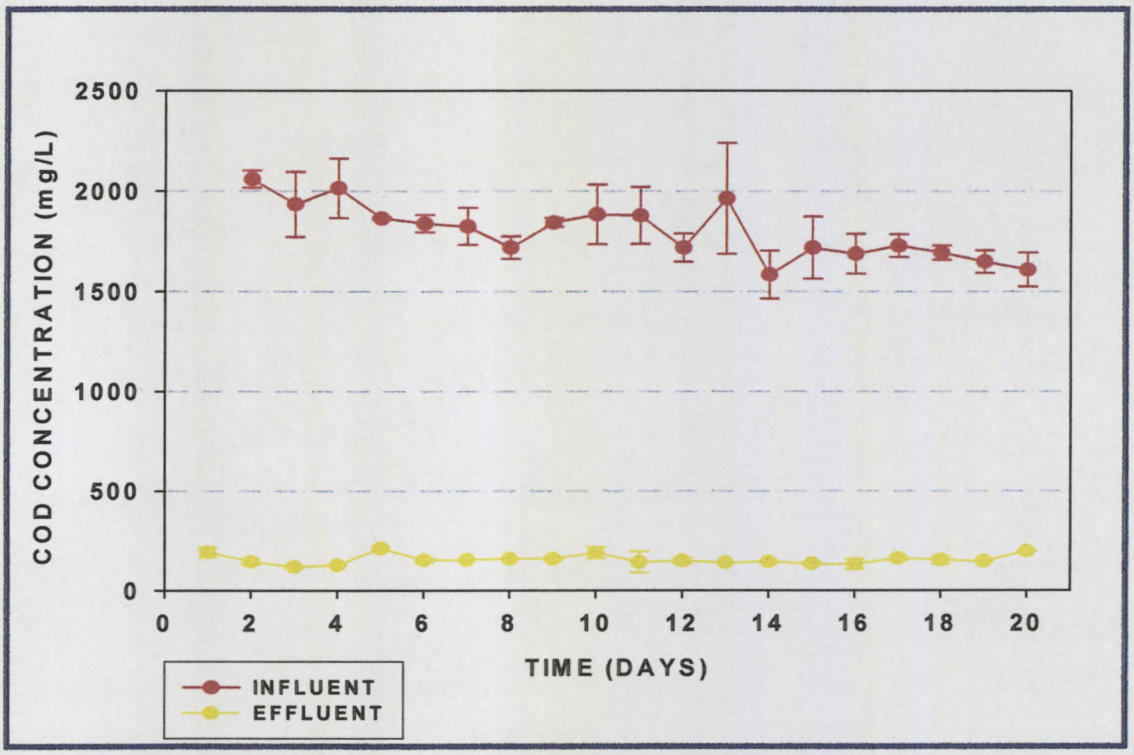

Figure 3.20: Average COD profiles of influent and effluent for experiment 4

The percentage COD removed for the duration of the process remained in the 90 percentile range. There was a slight decline at day 5 (88%) but increased thereafter and the decrease is noted once again on the last day. The average percentage COD removed was 91.44%. Figure 3.21 expresses this trend.

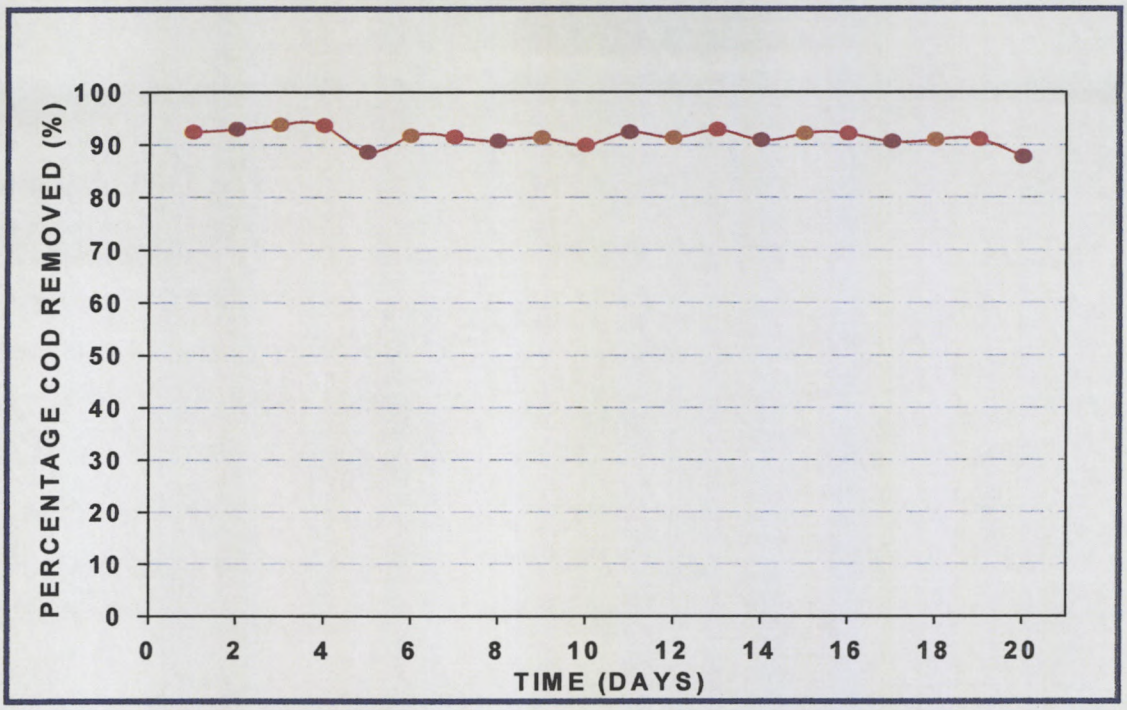

Figure 3.21: Percentage COD removed over time for experiment 4

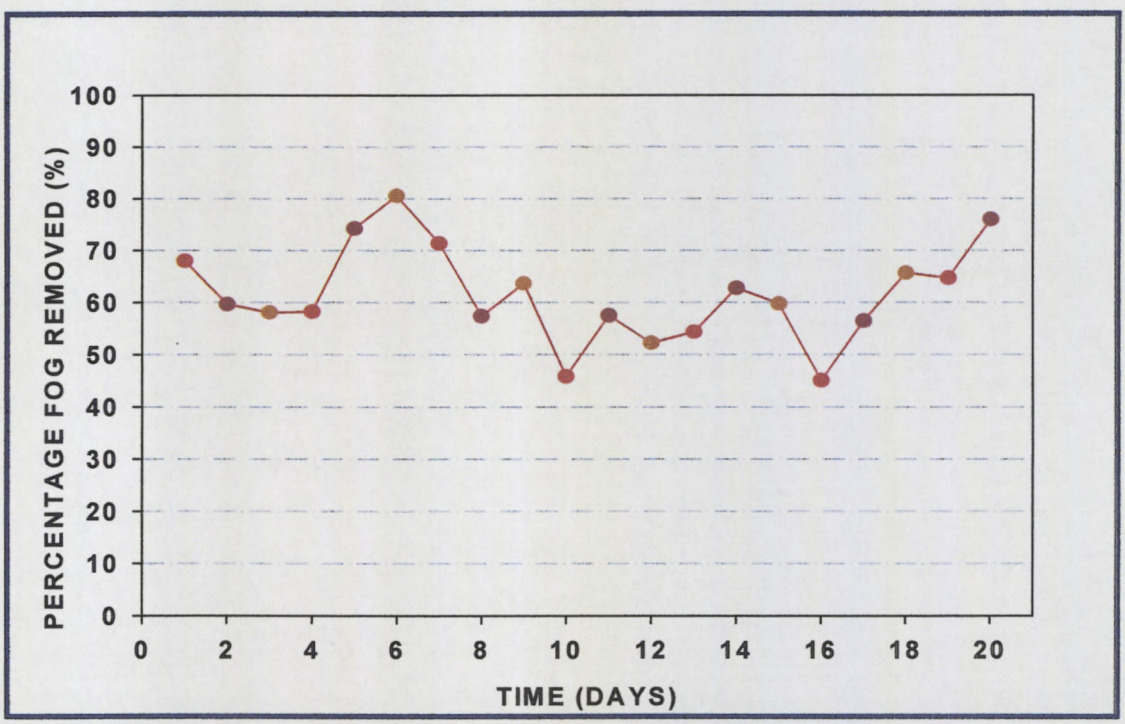

Figure 3.22: Percentage FOG removed over time for experiment 4

Figure 3.22 illustrates the FOG removed in this process. After day 1 the removal decreased and after day 4 increased. It reached the maximum removal at day 6 which was 80 % and thereafter decreased to about 59 % at day 8. The increase and decrease continued until day 16. From this day to day 20 the percentage removed increased. The average percentage FOG removed was 62.58 %. The average influent FOG was 522.30 mg/L and the average effluent FOG was 194.27 mg/L.

With reference to table 3.8, the following was observed. The MLSS concentration decreased after day 1, but after day 2 started to increase. By day 4 the MLSS was at its highest concentration, almost double the first day MLSS, with a concentration of 2804 mg/L. After day 4 the decline in concentration continued once again. However in this process there were also fluctuations, with the MLSS concentration increasing and decreasing. This can be seen on day 9 when the concentration increased up to 1076 mg/L and the thereafter began to decrease. The active biomass (MLVSS/MLSS),was constant throughout the process with an average of 74 %. The sludge volume was constant for the first three days and then increased on the 4th day and thereafter continued to decrease until the end of the process. The values of the SVI showed that the sludge was also of good settling properties. The So/Xo ratio on the other hand had various values. It started with a ratio of about 1.5 and decreased until day 4. On day 4 it was 0.72 and then increased to 1.28 on day 5 to 1.50 and 2.43 on day 6 and 7 respectively. It then decreased on day 9 and then increased to 2.04 on day 11 and to a further 2.16 on day 13. These fluctuations continued up to day 17 (2.08) and thereafter decreased to 1.72 on day 20.

Minor technical difficulty was experienced during this process with the OUR meter. OUR readings on days 3 to 5, 8 to 10 and days 12 to 14, could not be downloaded. The OUR is illustrated in figure 3.23. The same trend occurred as previously, i.e., the OUR increased when a new batch of influent was added and gradually decreased as the substrate was utilised. A sudden increase in oxygen utilised occurred on day 6, reaching a value of 54 mg/Lh^{-1}. After day 6 the OUR's were relatively constant throughout the rest of the process. The utilisation rate did not decrease in value as in the previous process. After day 6 the OUR's remained below 25 mg/lh^{-1}.

Table 3.8: MLSS, MLVSS/MLSS, So/Xo ratios and SV30 and SVI values for days 1 to 20

DAY	MLSS (mg/L)	MLVSS/MLSS (mgVSS/mgTSS)	SV30 (mL/L)	SVI (mL/g)	So/Xo
1	1598	0.72	80	50.06	1.58
2	1474	0.75	80	54.27	1.40
3	1532	0.72	80	52.22	1.26
4	2804	0.68	100	35.66	0.72
5	1456	0.73	72	49.45	1.28
6	1224	0.71	60	49.02	1.50
7	750	0.65	50	66.67	2.43
8	940	0.72	50	53.19	1.83
9	1076	0.73	60	55.76	1.71
10	958	0.80	60	62.63	1.97
11	922	0.74	60	65.08	2.04
12	922	0.74	60	65.08	1.87
13	908	0.71	50	55.07	2.16
14	900	0.72	40	44.44	1.76
15	826	0.74	40	48.43	2.08
16	880	0.78	40	45.45	1.92
17	830	0.84	40	48.19	2.08
18	884	0.79	40	45.25	1.92
19	940	0.78	40	42.55	1.76
20	938	0.75	40	42.64	1.72

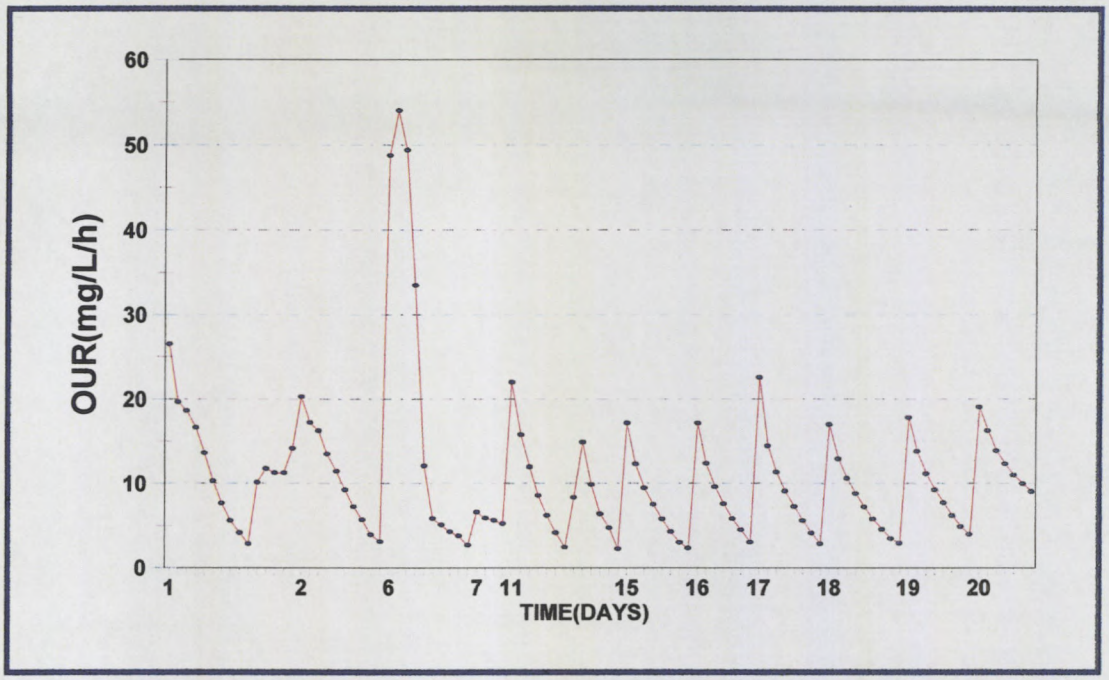

Figure 3.23: Average Oxygen Utilisation Rate over time for experiment 4

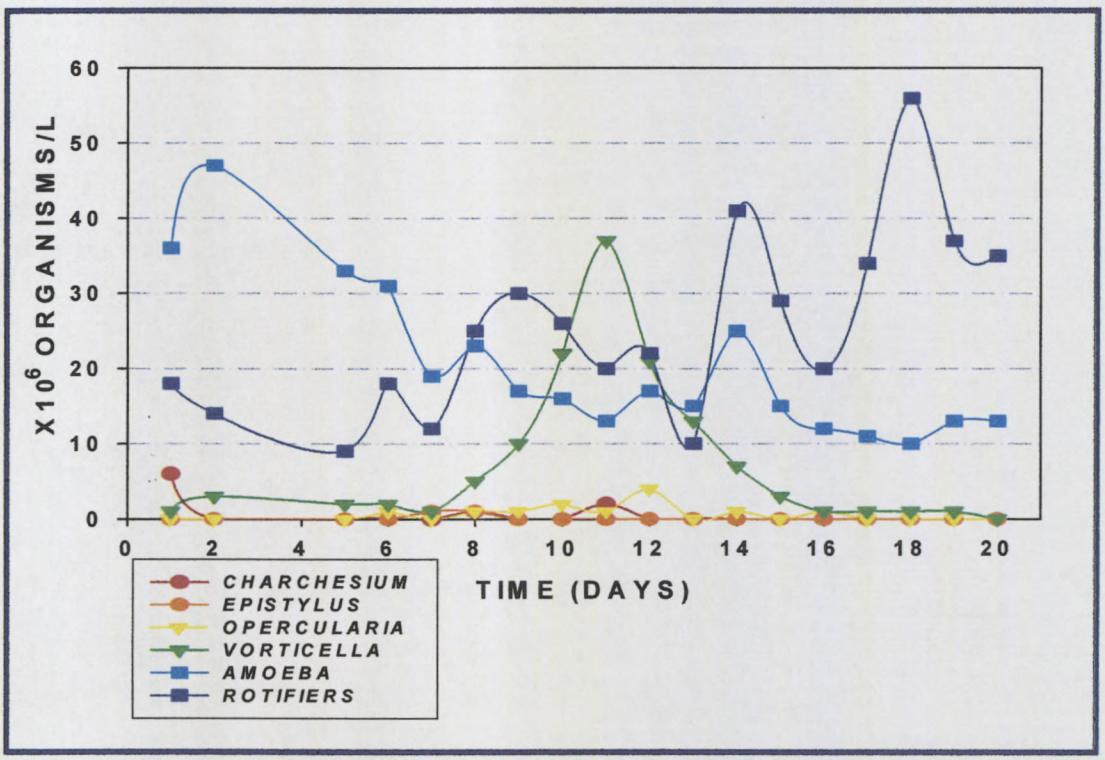

Figure 3.24: Protozoan survey over time for experiment 4

Figure 3.24 illustrates the number and type of organisms that proliferated during this process. The *Amoeba* species was dominant at the initial stages of the process, declining after day 2. The *Rotifer* was the second organism that was present but slightly lower in concentration than the *Amoeba*, however this organism increased after day 5 and reached about 30 x 10^6 orgs/L by day 8 and then declined to about 10 x 10^6 orgs/L at day 13. After day 13 the concentration increased to about 40 x 10^6 orgs/L becoming the predominant species in the system until the last day of the process. From the *Charchesium, Epistylis, Opercularia* and *Vorticella*, the latter was the one that proliferated and at one stage of the process became the predominant species, i.e., between days 10 and 12 the number of *Vorticella* increased to 37 x 10^6 org/L. The filamentous bacteria present in this system were Type 0041, Type 0675 and *Nocardia*. The floc morphology contained many firm, rounded and compact and few firm, irregular and open flocs. The floc ranged from small to medium in size.

The TSS profiles of the system are expressed in figure 3.25. The influent TSS was high and the trends differed from the previous batch, i.e., it did not go lower than the effluent TSS concentration. Influent TSS decreased after day 3 but gradually increased thereafter. The effluent did not exceed 100 mg/L and remained below that concentration for the duration of the process.

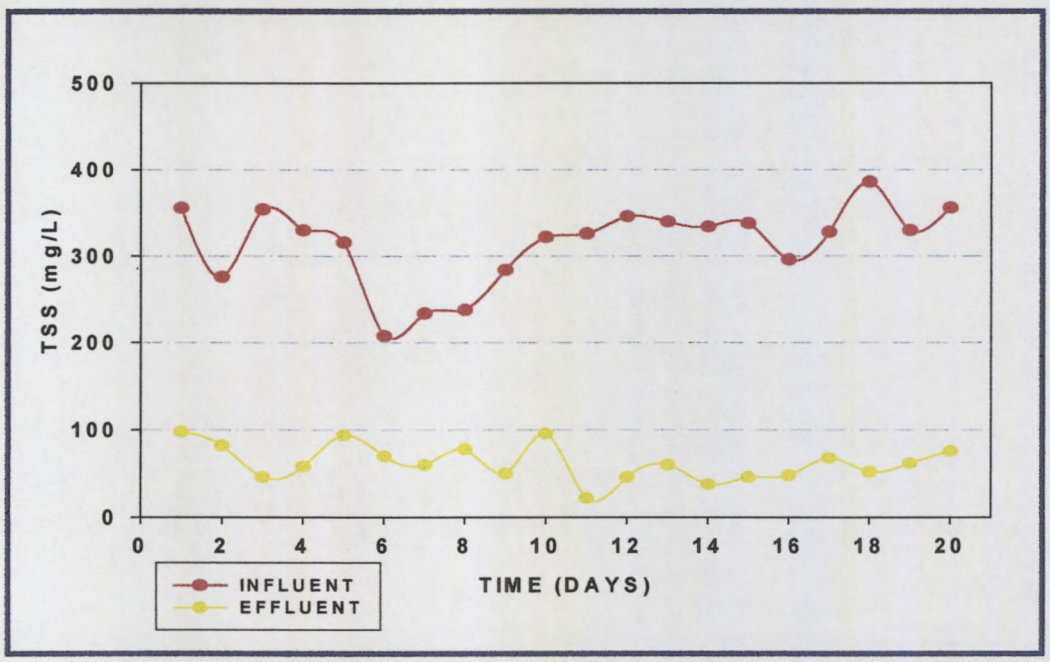

Figure 3.25: TSS profiles of the influent and effluent for experiment 4

3.3.5 Experiment 5: Degradation of undiluted (raw) edible oil effluent

Based on previous experiments the optimum conditions for degradation of the undiluted effluent was at 21°C with nutrients. Once again fresh effluent samples and sludge were collected. The MLSS was adjusted by dilution from 5579 mg/L to a biomass concentration of 3060 mg/L. After the 5 day acclimatisation period, sludge was wasted, and analyses began. The initial characteristics of the undiluted effluent is given in table 3.9. This batch had a concentration of 3400 mg COD/L. It had a very low TSS concentration, but a very high FOG content. The effluent had a high alkalinity content with a pH of 10. The pH of the influent was adjusted to 7 prior to addition in the reactor. The effluent was supplemented with nutrients according to the C:N:P ratio which was 100:5:1. The TKN and the TP were measured before dosing with the other nutrients and was found to be 7.0 and 5.5 mg/L respectively. The carbon source was from the influent, the nitrogen source was from Ammonium chloride (170 mg/L) and the phosphate source was from potassium di-hydrogen orthophosphate (34.08 mg/L).

Table 3.9: Initial characteristics of the undiluted effluent for Experiment 5

PARAMETER	UNDILUTED
COD (mg/L)	3408
FOG (mg/L)	1633.6
TSS (mg/L)	6.0
pH	10

Figure 3.26 illustrates the influent and effluent organic content for this process. The influent COD remained between 3000 and 3500 mg/L throughout the process. The average organic content of the influent was 3195.63 mg COD/L and the average effluent organic content was 147.65 mg COD/L. Hence the average organic concentration removed was 3047.98 mg COD/L.

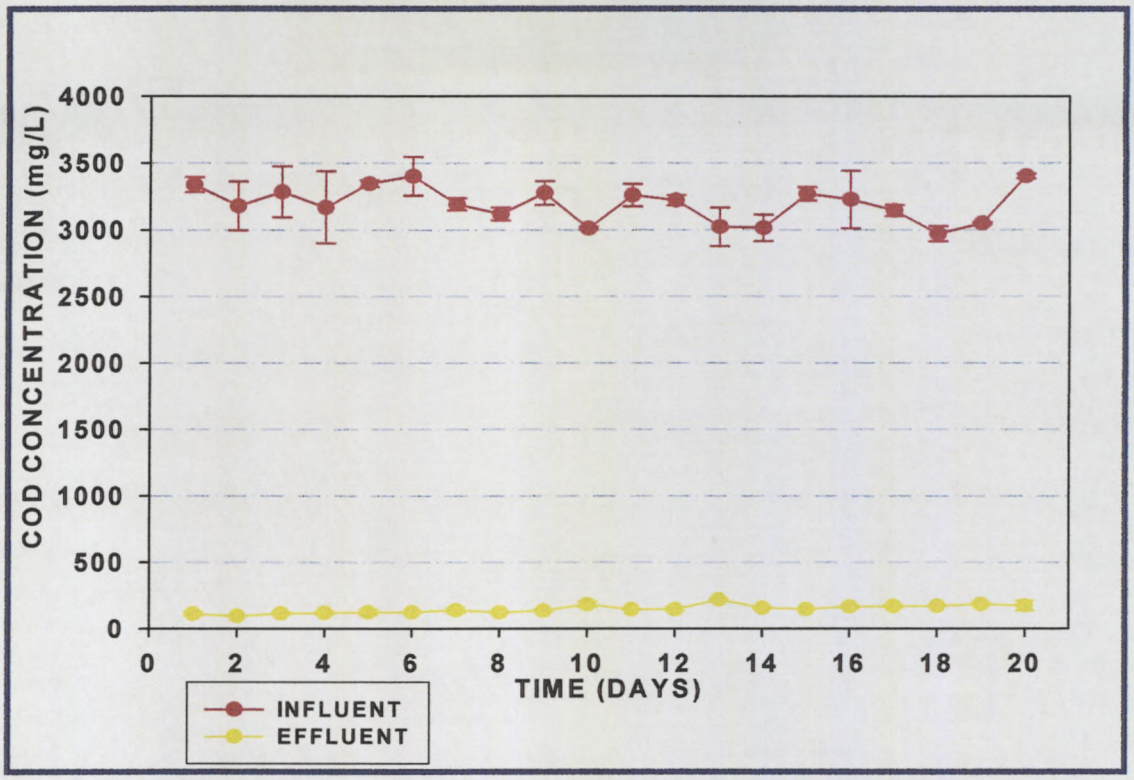

Figure 3.26: Average COD profiles of influent and effluent for experiment 5

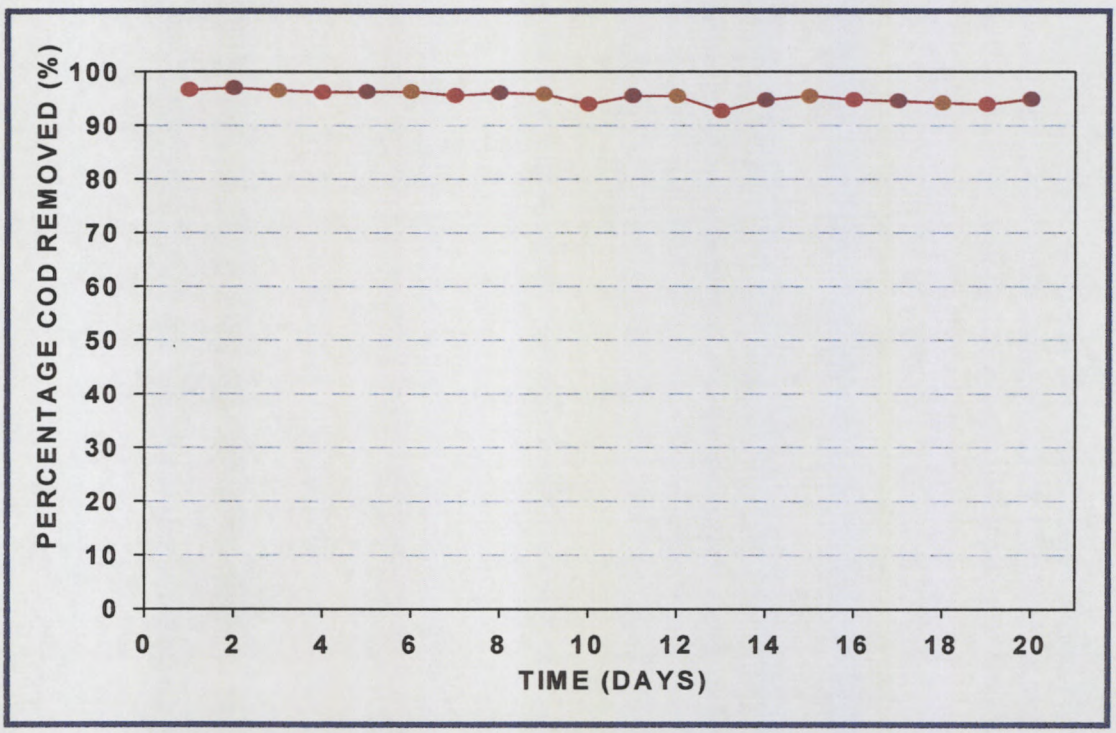

Figure 3.27: Percentage COD removed over time for experiment 5

The percentage COD removed is expressed in figure 3.27. The removal rate remained above the 90 percentile range throughout the 20 day process. There was a slight decrease at day 10 and 13, but still remaining in the lower 90 %. The average COD removed was 95.35 %.

Figure 3.28 illustrates the percentage FOG removed for this process. It can be seen that the removal for the first three days was ~ 90%. After the 5th day the percentage declined to about 69 % but increased to about 88 % at day 8. There were then decreases and increases until day 16. Thereafter the rate of FOG removed declined to 70% on day 19 and increased slightly to 75% on the last day. The average FOG removed was 81.36 %. The average influent FOG was 1148.78 mg/L and the effluent FOG was 208.94 mg/L.

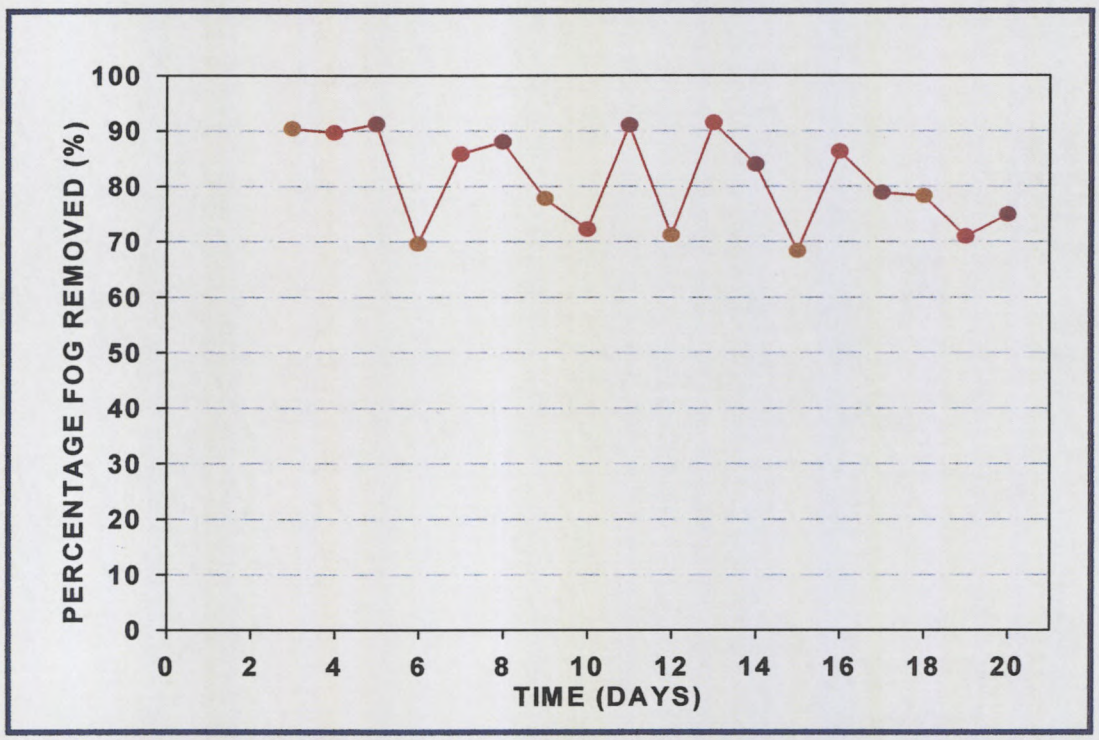

Figure 3.28: Percentage FOG removed over time for experiment 5

Table 3.10 illustrates the following. The MLSS concentration decreased from 1936 mg/L to 1146 mg/L on days1 to 7. At day 8 there was an increase to 1228 mg/L but thereafter declined to 942 mg/L on day 10. However after day 10 the concentration increased reaching 1404 mg/L on day 14. It then decreased after day 14 to 1256 mg/L. An increase was once again noted on day 20 to 1564 mg/L The active biomass i.e., MLVSS/MLSS, remained relatively constant throughout the process with an increase towards the end of the process. The average active biomass ratio was 0.88. The SV30 for the first two days were constant at 100 mL/L, but decreased after the 2nd day to 60 mg/L on days 8,9 and 10. After day 10 the concentration increased reaching 90 mL/L on day 18, but decreased to 80 mL/L on days 19 and 20. The SVI indicated once again that the sludge had good settling properties, as the concentration remained between 40 to 60 mL/g. For the So/Xo ratio a different trend occurred. The ratio was at 1.7 for day 1 but increased to over 2 for days 4 to 9. A further increase was noted on day 10 and 11 which reached 3.20 and 3.12 respectively. The ratio remained between 2 and 3 until the end of the process.

Figure 3.29 illustrates the oxygen utilised over the 20 day period. This process with undiluted effluent exhibited higher OUR's than for the previous diluted batches. The highest rate reached was in experiment 2, which was ~20 mg/Lh^{-1} whereas this batch reached a high of about 35 mg/Lh^{-1}. The OUR did not decrease over time like the batches from experiments 1 and 3. The trend was the same as experiments 2 and 4, where the readings reached more or less the same value each day when new influent was added. The only difference was that as compared to the other experiments the OUR readings were higher.

Table 3.10: MLSS, MLVSS/MLSS, So/Xo ratios and SV30 and SVI values for days 1 to 20

DAY	MLSS (mg/L)	MLVSS/MLSS (mgVSS/mgTSS)	SV30 (mL/L)	SVI (mL/g)	So/Xo
1	1963	0.85	100	51.65	1.73
2	1846	0.84	100	54.17	1.72
3	1760	0.86	80	45.45	1.87
4	1432	0.87	80	55.87	2.21
5	1274	0.85	80	62.79	2.63
6	1174	0.87	70	59.63	2.90
7	1146	0.87	70	61.08	2.79
8	1228	0.86	60	48.68	2.54
9	1098	0.86	60	54.64	2.98
10	942	0.89	60	63.69	3.20
11	1046	0.88	70	66.92	3.12
12	1096	0.93	70	93.87	2.94
13	1288	0.89	80	62.11	2.35
14	1404	0.89	80	56.98	2.15
15	1298	0.91	80	61.63	2.52
16	1316	0.89	80	60.79	2.45
18	1458	0.93	90	61.73	2.04
19	1528	0.95	80	52.36	2.00
20	1564	0.88	80	51.15	2.18

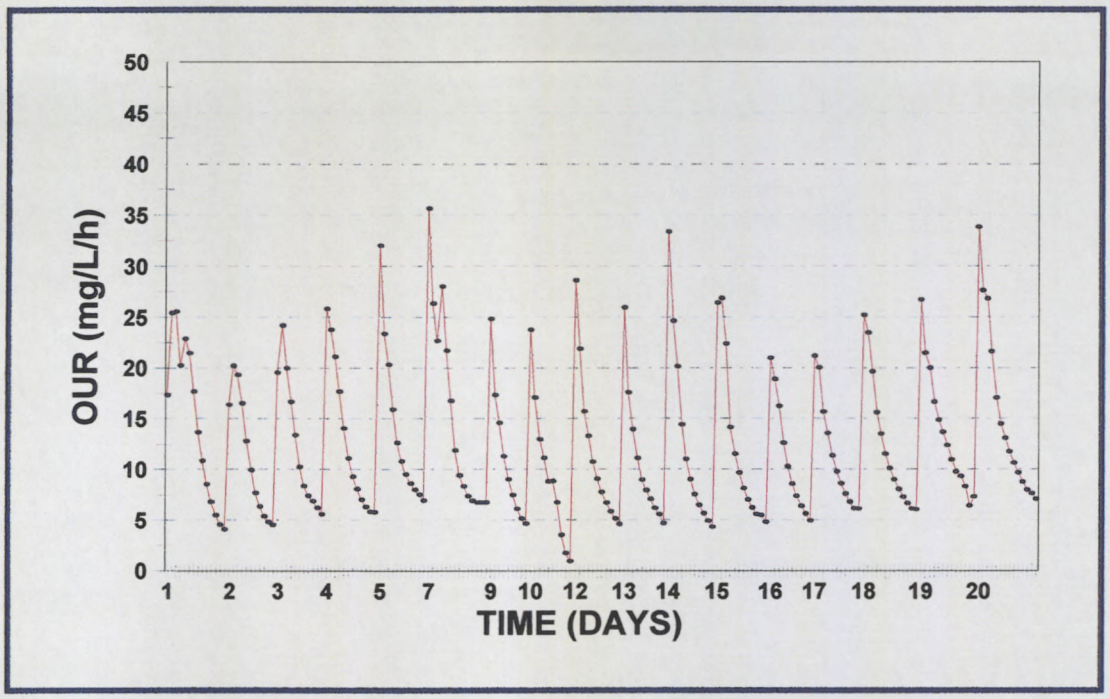

Figure 3.29: Average Oxygen Utilisation Rate over time for experiment 5

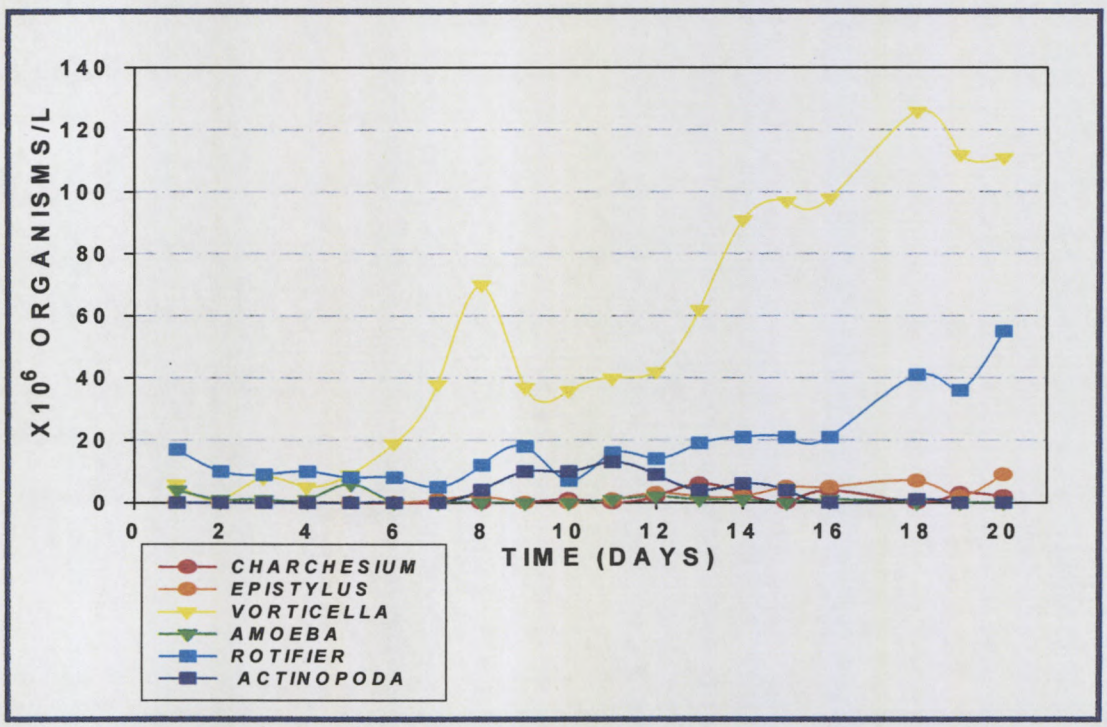

Figure 3.30: Protozoan survey over time for experiment 5

Figure 3.30 represents the protozoan survey conducted during the 20 day period. Out of the three sessile ciliates (*Charchesium, Epistylis* and *Vorticella*), *Vorticella* was the one that increased in number, becoming the dominant organism in the system. For the first few days the number was low, then gradually increased after day 5, reaching about 6.5 x 10^7 orgs/L on day 8. It then decreased after day 8 to <4.0 x 10^7 orgs/L and increased after day 9 until day 18. On day 18 it reached the highest concentration which was about 12.6 x 10^7 orgs/L. It then decreased slightly and was at 11.1 x 10^7 orgs/L on day 20. The *Amoeba* number, in this process, was very low or at times could not be detected. The *Rotifer* was the second dominant organism in the process. In the initial stages of the process the *Rotifer* was actually the dominant organism, but after day 5 when the *Vorticella* number increased it remained at approximately the same numbers. The *Rotifer* number increased after day 12 and reached about 5.5 x 10^7 orgs/L on day 20. Another organism that was present in this process was the *Actinopoda*, which increased in number between days 7 and 16, reaching its highest concentration on day 11 which was about 1.3 x 10^7 orgs/L. Other organisms that were present, but not shown on the graph were *Bodo* and *Hexamitus*. These were too numerous to count. They were present at the initial stages of the process but by day 6 all had disappeared. Also a *Nematode* was found on day 15. The filamentous bacteria found in this process were Type 0041, Type 0675, and Nocardia. Towards the end the *Nocardia* number increased. The floc morphology ranged from a moderate number of firm, rounded and compact, moderate firm, irregular, open and compact flocs and few weak, irregular flocs. The diameters range from 10 to > 150 μm.

Figure 3.31 illustrates the influent and effluent TSS concentrations. The influent TSS remained lower in concentration than the effluent TSS. The influent reached the highest concentration on day 15 which was 16 mg/L, while the effluent reached its highest concentration of 162 mg/L on day 18. The effluent had at concentration of 30 mg/L on day 1 and then increased to 80 mg/L on day 7. It then decreased to 50 mg/L on day 12. However the next day the valued increased to 90 mg/L and then decrease again to 22 mg/L on day 16. Thereafter the effluent TSS increased and a final TSS concentration of 104 mg/L was reached on day 20. The average influent TSS concentration was 2.90 mg/L and the average effluent TSS was 68.70 mg/L.

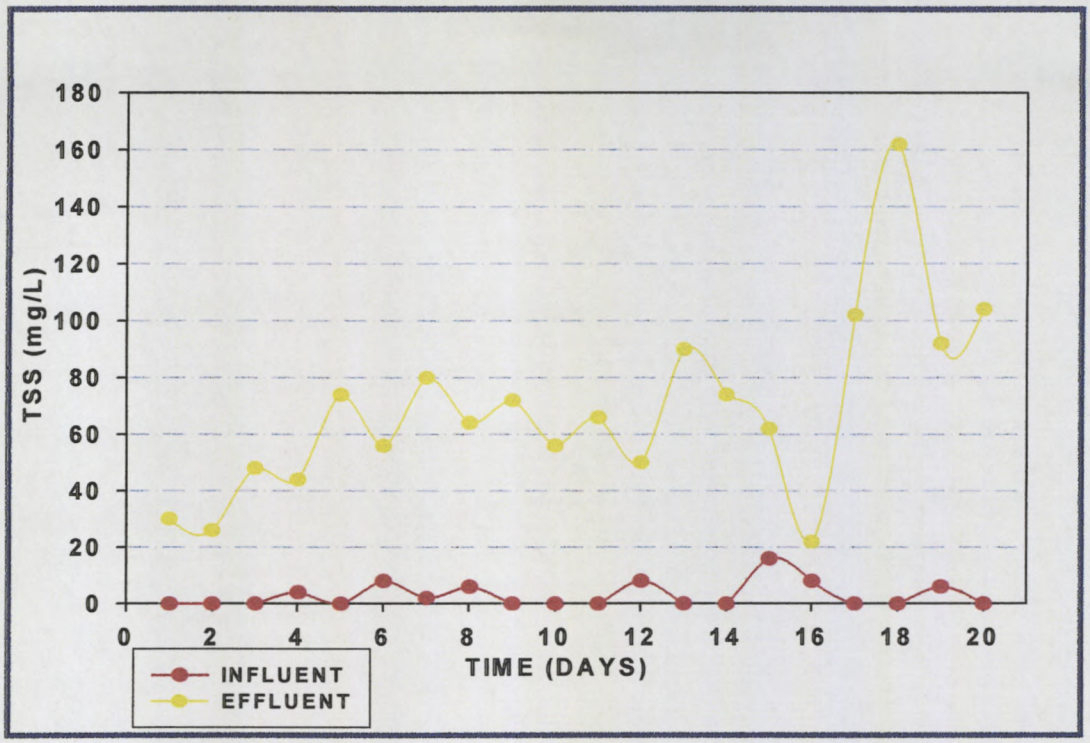

Figure 3.31: TSS profiles of the influent and effluent over time for experiment 5

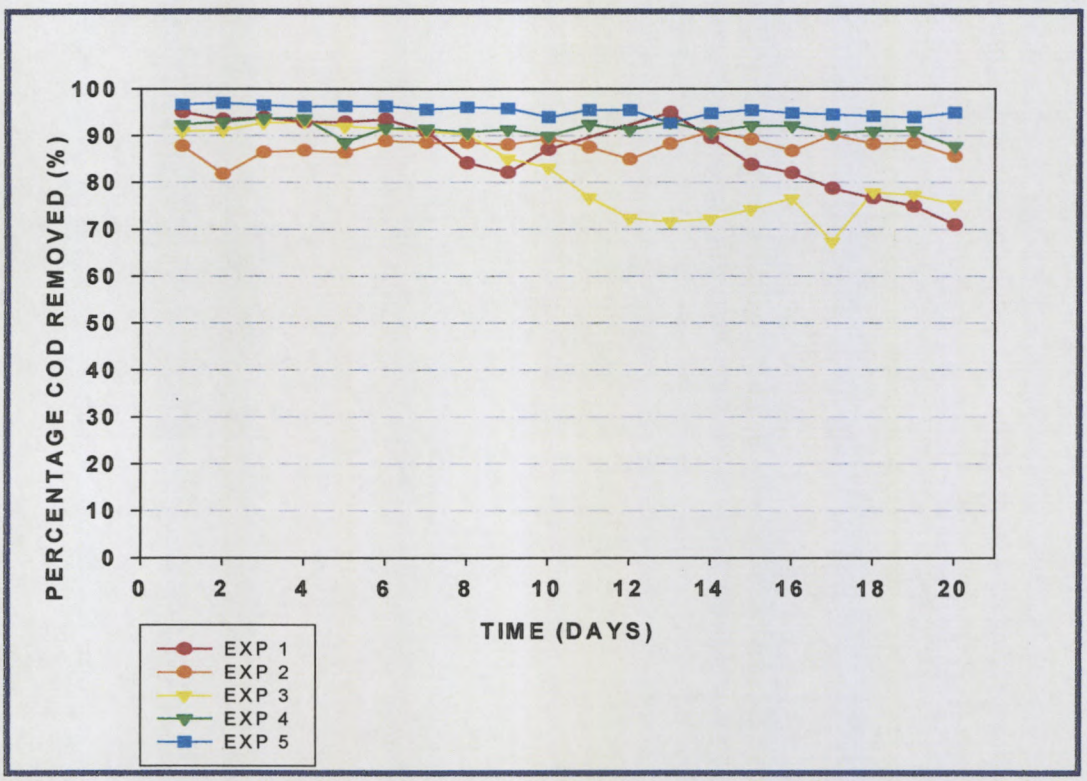

Figure 3.32: Percentage COD removed for experiments 1 to 5

Figure 3.32 compares the percentage COD removed for all the 5 experiments. The organic removal for experiments 1, 2, 3, 4 and 5, were 85.37, 87.66, 82.16, 91.44 and 95.35 % respectively.

3.4 DISCUSSION

Steffen, *et al.*, (1989), stated that the effluent an edible oil plant discharges varies considerably in quality over a 24 hr period. Appendix 18 shows the effluent quality from an edible oil processing plant (adapted from Steffen, *et al.*, 1989). The pH range was either very acidic or very alkaline. The organic content, measured in terms of COD also had a wide range from 1100 to 8990 mg/L. The soaps, oils and greases (SOG) content also varied, ranging from 80 to 1360 mg/L. The work of Mkhize, *et al.*, (2000) can also be referred to for variations in effluent quality. This variation in effluent quality had also been noted in the batches of effluent collected in this research project. Tables 3.1, 3.3, 3.5, 3.7, and 3.9 showed the characteristics of the effluent collected for each experiment. The pH, organic content, FOG content and TSS content was different for each batch. The pH ranging from 2 to 11, the COD ranging from 3000 to 6000 mg/L, the FOG ranging from 800 to 3000 mg/L and the TSS ranging from 6 to 1600 mg/L. The variation in quality was due to the process used to refine the crude oil. Either physical or chemical refining was used and this in turn changed the quality of effluent being produced. With the effluent from the industry changing in quality every 24 hr, made it very difficult to obtain an effluent with the same characteristics for the laboratory research.

The reasons for the effluent being diluted to approximately 2000 mg COD/L , was to provide similar organic content for each optimisation procedure. The other reason was to prevent shock loading of the system.

The activated sludge concentration also varied for each sample collected. All the concentrations were above 3000 mg/L and in order to keep the conditions similar for each experiment the MLSS was diluted to provide an initial biomass concentration of approximately 3000 mg/L.

Activated sludge treatment is a widely used process, treating many types of industrial effluents. However it is important to acclimatise the sludge to the type of wastewater that is being treated. Wakelin & Forster, (1997), supports this procedure. They conducted an experiment that compared the removal of FOG's from a wastewater using activated sludge with and without acclimatisation. A higher performance was achieved with acclimatised sludge as compared to the unacclimatised sludge, even though the general pattern of growth and FOG removal was similar for both the sludges. Alves *et al.*, (2001), experimental research also showed that with acclimatisation, of wastewaters containing lipids, to the sludge was beneficial as the biodegradation capacity was increased. Thus the 5 day acclimatisation period conducted for each of the five experiments in this research project.

The influent for each experiment, decreased in organic content over time. Figures 3.2, 3.8, 3.14, 3.20 and 3.24 show the influent and effluent organic content. It was thought that the influent was contaminated but the contamination checks done were negative (see Appendix 19). It was therefore acknowledged that the influent had self-degradative properties and with time decreased in organic content. There was no other explanation found for this trend.

For an activated sludge system without nutrients, the organic removal achieved by experiments 1 and 3 were substantially high, resulting in an average removal of 85.37 % (fig. 3.3) and 82.16 % (fig. 3.15) respectively. However, for both these experiments the organic removal efficiency decreased towards the latter end of the experimental period. The FOG removal for experiments 1 and 3 were 78.18 % (fig. 3.4) and 71.75 % (fig. 3.16) respectively. The efficiency removal for this parameter also decreased towards the latter end of the experimental period. A probable reason for the decreasing efficiency for the COD and FOG loads could be due to the lack of nutrients according to the C:N:P ratio of 100:5:1. Research conducted by Strynar *et al.*, (1999) ; Prendl & Nikolaveic, (2000) demonstrated that in order to have significant influence on oil and organic degradation, it is necessary to add both nitrogen and phosphorus.

The poor efficiency of COD and FOG removal can also be seen in the oxygen utilised by the microorganisms. Figure 3.5 illustrates the oxygen utilised. The system started with a high utilisation rate but as time went on, it was possible that only a few organisms managed to survive, hence

causing a decline in the amount of oxygen utilised. According to Dueholm, *et al.*, (2001), their OUR profiles showed that when the long chain fatty acid, oleic acid, was added to activated sludge as a substrate, there was an increase in the oxygen consumption, indicating that oleic acid was easily consumed in the activated sludge. One of the major components present in the effluent used was oleic acid. This can be seen in gas chromatography-mass spectrophotometer (GCMS) graph, shown in Appendix 20. This increase in oxygen consumption can be seen figure 3.5, when a new batch of influent was added each day there was an increase in the amount of oxygen utilised. Vollertsen & Jacobsen (2002), concluded that the OUR measurements are a versatile tool for characterisation of the biodegradability of the organic matter. Another possibility for the OUR peaks was that the readily biodegradable COD of the effluent was utilised immediately by organisms and thereafter the slowly biodegradable COD was utilised.

According to Curds, (1982)(as cited by Seviour, 1999), states that protozoa can be used as indicators of plant performance and thus, frequent microscopic examination of the biomass can provide a rapid, simple method for indicating sudden changes in performance. Therefore throughout the experiments the protozoa was examined microscopically. In addition to protozoa examination the filamentous bacteria was also microscopically examined by using the various staining techniques. The reason for filamentous identification was basically out of interest as to the types of filaments present in this process with activated sludge. The types of filamentous bacteria found during this investigation remained more or less the same for all five experiments. From the filaments identified which were Types 0041,Type 0675, this contributed to the few open floc structures that were present in the system. This complies to the findings by Jenkins, (1993b) as cited by Seviour, (1999).

Protozoa demonstrate a wide range of feeding modes and are capable of feeding on soluble and particulate organic material, as well as bacteria and other protozoa. They are an integral part of the food chain of wastewater treatment plants. When the wastewater treatment plant operates at steady state, the food chain also establishes itself at a steady state. When there are disturbances, such as increases or decreases in the influent COD concentration, or sudden changes in the sludge age caused by excessive solids wasting, the food chain will adjust itself to the new conditions. The changes in microbial composition of a reactor over a slowly changing range of environmental conditions is

known as a relative predominance diagram, illustrated in figure 3.33 (Horan, 1990).

When wastewater is introduced into a reactor the COD is high, and the bacteria is at the lowest number, therefore protozoa such as the *Sarcodina* might be observed. These protozoa are not very capable of competing for food and are only observed during the start-up or after recovery from toxic shock. As the numbers of bacteria increase, the Sarcodina are replaced by flagellated protozoa. The flagellates are highly mobile and are able to compete for the food supply more effectively. Due to these organisms being so active, they require a large amount of energy, they are therefore characteristic of a high-rate activated sludge reactor, where food is abundant (point A on Fig. 3.33).

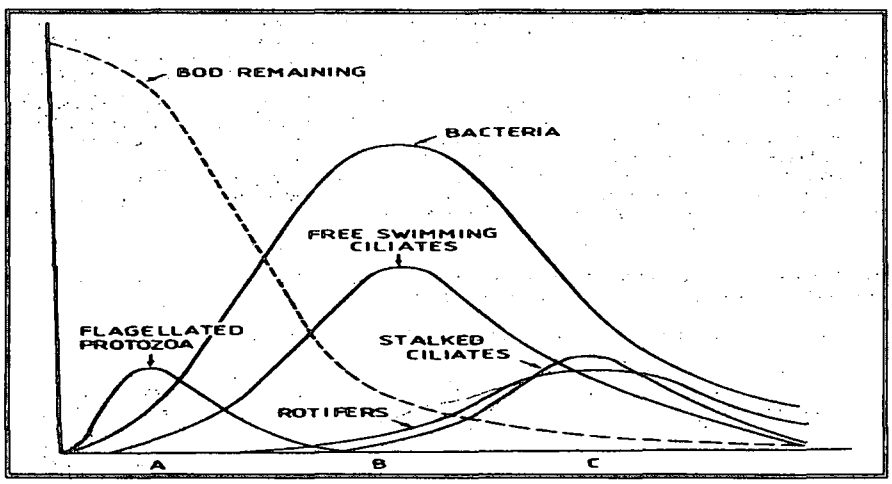

Figure 3.33: Relative predominance diagram illustrating the changes in protozoal ecology of an activated sludge as the sludge age increases and the loading rate decreases (Horan, 1990)

These also caused settling problems due to their high motility which lead to turbid effluents. With lower food supply, the free-swimming ciliates out numbered the flagellates (point B on Fig.3.33). After point B there is very little available COD, and the bacteria become the predominant protozoal food supply. Stalked ciliates and the *Rotifers*, which consume whole bacteria, start to predominant (Horan, 1990).

The relative dominance in experiment 1 (figure 3.6) portrayed similar results according to the diagram in figure3.33, up to point A, however there were no free swimming ciliates and the stalked

ciliates were present but not dominant. The flagellate protozoa was predominant right up to period B of the diagram, and thereafter the *Amoeba* (*Sarcodina*), became predominant. There were no *Rotifers*. This was probably because there were no nutrients to keep the population in balance, and towards the end of this process the organic content decreased, hence the *Sarcodina* were observed once again.

The TSS concentration of the effluent shown in figure 3.7, illustrated that between days 8 to 10 the effluent concentration increased (74 mg/L), and was turbid. During this time the *Monosiga* number's also increased, thus possibly causing the effluent to become turbid. This trend is mentioned previously by Horan, (1990), that when the flagellates are high in number, a settling problem is caused and the effluent becomes turbid. However the SVI values shown in table 3.2, illustrated that even though the TSS concentration was high at times, there was no bulking of sludge. Bitton, (1999), states that bulking sludge would produce an SVI above 150 mL/g, while an SVI below 70 mL/g indicated the predominance of pin point (small) flocs. The SVI in this process was between 70 to 100 mL/g, which indicate normal flocs were present in this experiment.

According to Chudoba, *et al.*, (1992), when the ratio of the initial substrate concentration to the initial biomass concentration, i.e., the So/Xo ratio, is sufficiently low (below 2-4), no or negligible cell multiplication takes place during the exogenic substrate removal. The So/Xo ratio in table 3.1 has a ratio between 0.8 and 1.70, thereby showing that no cell multiplication took place or that the cell multiplication was negligible. Table 3.2 also shows the MLSS concentration, as sludge was being wasted. The MLSS concentration decreased, illustrating once again that no cell multiplication took place. According to Bitton, (1999), a low ratio, indicates that the following filaments might be present i.e., *M. parvicella, Nocardia sp, H. hydrossis*, 0041, 0675, 0092, 0581, 0961 and 0803. In this process only 3 filamentous organisms were present, viz *Nocardia sp*, Type 0041 and 0675.

For experiment 3 the temperature was maintained at 31°C. Atlas and Bartha, (1993), stated that in addition to affecting survival and growth, the temperature influences the metabolic activities of microorganisms. In general, higher temperatures that do not kill microorganisms result in higher

metabolic activities. For example, an increased O_2 consumption occurs as the temperature is increased. When comparing the OUR concentrations of experiments 1 (figure 3.5) and 3 (figure 3.17), experiment 3 had a higher O_2 consumption. Although the trend was the same for the two experiments, experiment 1 had an OUR concentration less than 5 mg/Lh^{-1} after day 4 and remained below 5 mg/Lh^{-1} up to day 20, whereas experiment 3 had an OUR concentration between 10 to 15 mg/Lh^{-1}from day 4 to day 8, the concentration from day 9 to 20 remained between 5 to 10 mg/Lh^{-1} (still higher than experiment 1).

The protozoa survey, shown in figure 3.18, did not portray similar according to the relative predominance diagram. The members of *Sarcodina* group was the predominant organism until day 15. The reason for this being the COD in the system was high and the number of organisms only increased very late in the process. After day 15 the stalked ciliates (*Vorticella*) and the *Rotifers*, became predominant. The flagellate, *Monosiga*, were not predominant at any time, however *Bodo* did proliferate, but only towards the latter end of the process.

The TSS for this experiment illustrated in figure 3.19, showed an increase in the effluent TSS concentration. This was due to the numbers of bacteria increasing, causing the effluent to become turbid, hence increasing the concentration. The SVI values for experiment shown in table 3.6, indicate that no bulking of the sludge was apparent. The SVI ranged from 84 to 135 mL/g.

The So/Xo ratio shown in table 3.6 ranged from 0.75 to 1.80. As the ratio reached 1.80 on day 18, there was little cell multiplication. The MLSS and SV30 shown in table 3.6, indicated that an increase in biomass concentration occurred after day 15. The active biomass, MLVSS/MLSS ratio, remained relatively constant even as the biomass concentration increased, also represented in table 3.6. According to Droste, (1997), a typical ration of MLVSS/MLSS is in the range of 0.75 to 0.80.

Orhon, *et al.*, (1999), stated in order for the activated sludge systems to remove organic carbon, the nutritional requirements of microorganisms is the major factor controlling the efficiency of carbon reduction. The impact of this factor is practically assessed in terms of the COD:N:P ratio measured in the wastewater to be treated. Experiments 2, 4 and 5 were supplemented with nutrients according

to this ratio of 100:5:1.

In experiment 2, the overall organic degradation was 87.66 %, which is shown in figure 3.9. The removal rate was at a steady state throughout the process. Unlike in experiment 1 where the removal rate decreased towards the end. It was then deduced that the steady removal rate was due to the supplementation of nutrients as stated above by Orhon, *et al.*, (1999).

The FOG removed (68.06 %)(figure 3.10), was at a lower rate than the FOG removed in experiment 1 (73.18 %). One reason theorised was that, since the organisms had a steady nutrient supply, they possibly utilised this first, and when the nutrients became depleted, other sources of substrates were then used viz; the FOG's, whereas when no nutrients were supplied, the source of food was taken from the FOG's thus resulting in a higher FOG removal rate.

The OUR concentration shown in figure 3.11, indicated that the amount of O_2 utilised each day was also at a constant rate. This can be seen that each time the influent was added the concentration went up to 20 mg/Lh^{-1} for each day. There was no decrease in the overall amount of O_2 utilised as in experiment 1.

The protozoal ecology in this system was according to the relative predominance diagram illustrated in figure 3.33. The protozoa survey illustrated in figure 3.12, showed that *Sarcodina* (*Amoeba*) was predominant at the beginning of the process. Thereafter the flagellated protozoa (*Monosiga*) became the predominant species in the system. As it reached the middle of the process the free swimming ciliates did not proliferate as in the diagram instead the *Vorticella* became dominant and towards the end the *Rotifers* illustrated predominance in the system.

The TSS concentration represented in figure 3.13, illustrated that the effluent TSS in the initial stages of the process was high and declined until day 7. The reason for the high TSS was the *Monosig*a in the initial stages of the process were high in number and therefore affected the settling properties of the sludge. After day 7 the effluent concentration increased. This was probably due the number of bacteria increasing (as mentioned above the *Vorticella* number increased indicating an increase in

bacteria), hence also causing a turbid effluent and an increase in TSS concentration. The SVI in table 3.4 ranged from 46 to 53 mL/g, i.e., the values were below 70 mL/g. This was indicative of pin-point flocs (Bitton, 1999), which are small flocs and produces turbid supernatant with no or little filamentous organisms (Jenkins, *et al.*, 1993).

The So/Xo ratio (table 3.4) indicated that little or no cell multiplication took place as the ratios were below 2. The MLSS concentration increased after day 2 showing there was a slight increase in biomass. The SV30 show this as well with an increase in volume after day 2. The active biomass, MLVSS/MLSS, was also relatively constant, even though there was an increase in the biomass concentration.

Experiment 4 was the same as experiment 2 except the process was carried out at a temperature of 31°C. The effluent was supplemented with nutrients according to the C:N:P ratio of 100:5:1. For this process at a higher temperature and addition of nutrients resulted in an organic removal of 91.44 %. The COD removal shown in figure 3.21 had a steady removal rate. The organic degradation did not decline towards the end like in experiment 3 (figure 3.16). The steady state was due to the addition of nutrients and also the temperature had an effect on the carbon removal as well. Department of Natural Resources (2001a), stated that biochemical reactions are very temperature dependent. Lower temperatures cause such reactions to be much slower. Thus, more organisms are required to do the same job if low temperatures occur than when there are higher temperatures.

The average FOG removed (62.58 %)(Fig. 3.22) was the lowest out of all the experiments. The reason being that a constant supply of nutrients were supplied, and that the organisms did not need to utilise the FOG's in order to survive, and only when the nutrients became depleted did they utilise the FOG's as a food source.

Shown in figure 3.23 are the OUR readings . The rate utilised from day 1 to 5 and from days 7 to 11 was at a utilised at a constant rate, except on day 6 where there was a huge increase in the OUR. The reason for this increase being for that particular day the probe in the reactor, became tangled around the stirrer resulting in mixing and aeration coming to a halt. When this problem was rectified

and mixing and aeration continued the O_2 utilised became higher during that time as the biomass did not have to O_2 to survive. That was the only explanation that could be deduced for the sudden increase in oxygen utilised.

The protozoa survey, illustrated in figure 3.24, showed that at the beginning the *Sarcodina* (*Amoeba*) proliferated. However no flagellated protozoa were present in the system. Therefore this initially did not follow the relative predominance diagram in figure 3.33. However in the middle of the process the *Vorticella* became the predominant species and thereafter towards the end the *Rotifer* became predominant.

The TSS concentration shown in figure 3.25 indicated that the effluent was not very turbid as the concentration remained below 100 mg/L throughout the process. This was probably due to the fact that no flagellated protozoa were present, as they cause turbidity in the effluent. The SVI shown in table 3.8 ranged from 42 to 66 mL/g. There were no problems with bulking as the values were all below 150 mL/g. However the values were all below 70 mL/g indicating that the sludge flocs were pin-point flocs.

The So/Xo ratios indicated that there was some cell multiplication as some values went above 2. The So/Xo ratio ranged from 0.72 to 2.43. The MLSS concentration also in table 3.8, showed that there were increases in the biomass at certain times. Even though there were these increases the active biomass (MLVSS/MLSS ratio) was relatively constant (~ 0.7). This is also shown in table 3.8.

The experiments conducted at 21°C, had a good organic removal with and without nutrients, i.e., 87.66 and 85.37 % respectively. The FOG removed was higher in experiments 1 and 2 than in experiments 3 and 4. Even though experiment 4 had the highest COD removed out of the four experiments, the FOG removed was the lowest. The predominance patterns of the protozoans in experiment 2 followed the sequence in fig 3.33. It was then deduced that experiment 2 conditions i.e., 21°C with supplementation of nutrients be used to conduct experiment 5 to treat undiluted effluent.

Experiment 5 achieved the highest organic degradation. This is illustrated in figure 3.27, where the percentage COD removed throughout the process was above 90 %. The probable reason being the carbon source was higher than in previous experiments and also nutrients added according to the C:N:P ratio of 100:5:1, hence the steady removal of the COD. Rohbrecht-Buck and Sekoulov, (1990) showed they had a 79.4% COD removal rate with a discontinuous activate sludge process, a 68.3 % removal rate with a Fed-batch reactor, but with a combination of the two a 93.3 % COD removal rate was achieved.

The FOG removal achieved was also higher in experiment 5. Illustrated in figure 3.28, the percentage removed remained above 69 % throughout the process, with an average of 81.12 % removed. The rate of FOG removed was high, unlike the other experiments where the removal declined even though nutrients were added.

The oxygen utilised was at a higher concentration shown in figure 3.29. The OUR concentration throughout the process was above 20 mg/Lh^{-1} reaching a high of about 36 mg/Lh^{-1}. For experiments 1 to 4 the OUR concentration were all below 20 mg/Lh^{-1}. Since the COD of the system was higher, the organisms had higher concentrations of carbon, hence the overall metabolism increased, leading to more oxygen being utilised.

According to McGhee, (1991) and Henze et al, (1997), the presence of *Vorticella* and *Rotifer*s in a system are indicators of extremely good biological treatment and also relative stability of a treated waste. At the initial stages the *Rotifers* were the predominant organisms in the system, but thereafter the stalked ciliates took over, i.e, the *Vorticella* was the predominant organism in the system until the end of the process. The *Rotifer* was the second dominant organism. This indicated that the bacteria number could have possibly increased in the system.

The TSS concentration shown in figure 3.31, illustrated that the effluent had a higher concentration than the influent throughout the process. The effluent concentration increased with time. A probable reason being the free swimming ciliates and flagellates in the system were high in number, therefore creating a turbid effluent. This was indicated by the SVI values shown in table 3.10, which ranged

from 45 to 93 mL/g. Most of the SVI's were below 70 mL/g, indicating that the floc structure was a pin point floc which causes settling problems and creating turbid effluents.

The majority So/Xo ratio were above 2, indicating that cell multiplication took place. The ratio ranged from 1.72 to 3.12. The MLSS concentration illustrated in table 3.10 showed that there was a decrease in biomass but after a few days the concentration increased once again. The SV30 displays the same trend also shown in table 3.10. The active biomass ratio was in the upper 88 to 90 %. Even though the biomass increased the active portion remained relatively constant.

3.5 CONCLUSION

The reason for conducting experiments without nutrients, was to determine if the activated sludge can treat wastewaters without nutrients. Dosing with nutrients in industries today can be very costly, hence if a process can handle wastes without nutrients, this would save a lot of money. As shown in experiments 1 and 3, where no nutrients were added, the activated sludge degraded over 80 % of the organics present in the wastewater, but the removal capacity decreased over time. This indicated that the activated sludge can handle high organic loaded waters, without nutrients, but only up to a certain limit.

When nutrients were added, the performance of the activated sludge increased, and the removal was more consistent, as seen in experiments 2, 4 and 5. The experiments with nutrients maintained a steady removal rate, while the experiments without nutrients portrayed a decreasing efficiency towards the latter end of the experimental period. Research by Eckenfelder & Musterman, (1995), stated that deficiency in substrates such as macro- or micronutrient concentration, and/or dissolved oxygen concentration in the biological floc can promote filamentous growth and sludge bulking.

The effect of temperature was to determine whether the higher temperatures would increase or decrease the degradation efficiency. It was deduced that a higher temperature (31°C) did increase organic degradation (experiment 4), but only when supplied with nutrients. However the COD

removal capacity was still higher and steady at the lower temperature of 21°C.

It can therefore be concluded that for a semi fed-batch and batch configuration for an activated sludge treatment system the following conditions are necessary to degrade diluted and/or undiluted edible oil effluent:

- A temperature of 21°C
- A sufficient supply of nutrients is a prerequisite for a good treatment, as the aim of nutrient dosage is to supply sufficient nitrogen and phosphorus in order to ensure COD removal, however dosages in excess must be avoided.
- An acclimatisation period for the activated sludge treatment is a prerequisite especially for high organic and FOG loaded wastewaters, to ensure maximum removal efficiencies.
- Maintenance of a neutral pH for influent substrate is also a requirement for efficient performance of an activated sludge treatment process.

CHAPTER FOUR

PRETREATMENT OF EFFLUENT WITH COAGULATION FOLLOWED BY DAF AND SUBJECTING TO FUNGAL AND YEAST BIOLOGICAL TREATMENT

4.1 INTRODUCTION

Fungi have powerful degradative capabilities, which are attributed to their unique metabolic activities or enzyme systems. Fungal lipases are often produced in high concentrations that assist in the fungal degradation of various lipids (Anke,1997).

Effective activation of fungal enzymes can be stimulated by availability of various nutrient sources (e.g. carbon sources) and physical parameters (e.g. temperature, pH and oxygen requirements). Studies conducted by Ratledge, (1994), emphasized that microorganisms are capable of utilising long chain fatty acids as a sole carbon and energy source.

Yeasts also have specific enzymes which are secreted into the micro-environment in order to degrade compounds for nutrients and energy. They recognize the presence of organic compounds in their environment and respond by producing the enzyme required for the degradation of those specific compounds (Hunter, 1999). They secrete the enzyme lipase to degrade triglycerol substrates to fatty acids and glycerol. The fatty acid and glycerol are degraded into smaller components until they are eventually oxidised to carbon dioxide and water.

Methods of pretreatment are used to treat wastewater in order to improve various conditions, before subjecting to biological treatment. According to Klute, *et al.*, (1995), a widely used pretreatment method is that of coagulation followed by DAF. Coagulation refers to all the reactions and mechanisms that result in particle aggregation in the water being treated, including in situ coagulant formation and physical interparticle contacts. The physical process of producing interparticle

contacts is termed flocculation (Letterman, *et al.*, 1999). Process variables in the coagulation process are type and concentration of inorganic coagulants (Al^{3+}, Fe^{3+}, PAC), type and concentration of the organic flocculation aid, mixing conditions during the dosing phase of the chemicals, time interval between dosing of the different chemicals and turbulent flow conditions during floc formation (Klute, *et al.*, 1995 and Kalinkov &Monzo, 1996).

Dissolved air flotation has been successfully applied in treatment systems of several wastewater types and in the clarification of certain drinking waters. The flotation process of aqueous dispersions is, as a basic idea, the phase separation through the use of low density agents, normally gas bubbles, which adhering to the dispersed particles surface, increase the buoyant forces, enabling the separation (Marchioretto & Reali, 2001).

4.2 MATERIALS AND METHODS

4.2.1 Effluent Sampling

Effluent samples were collected from the edible oil industry in cleaned 25 L vessels, and stored in a cold room at 4°C, until further use.

4.2.2 Effluent Characterisation

Effluent was characterised for the following:

1) The COD (Appendix 2),
2) pH (Appendix 5)
3) FOG (Appendix 4)

4.2.3 Coagulation and Flocculation Using the Standard Jar Test Method (Coagulation Test) (Pryor & Freese, 1998)

Ferric chloride was the coagulant chosen to perform the coagulation. The coagulant was chosen

from previous research by Thomson, (2001), who used various coagulants at different concentrations and pH and selected the best concentration from the analyses conducted. Thomson, (2001), found that the ferric chloride was the most efficient coagulant to use at a concentration of 10 ppm and a pH of 5.5. Amongst the coagulants used besides ferric chloride were Aluminum sulphate (Alum), various commercially available, polymeric coagulants: DMDAAC/PAC - Z553D, DMDAAC/PAC - LP526, PA/PAC - 735 and PA - U5000. The preparation of the ferric chloride can be found in Appendix 21.

- A conical flask, containing 800 mL of the pH adjusted effluent, was placed on a magnetic stirrer and the sample was stirred at 300 revolutions per minute (rpm).
- The coagulant was added (at a concentration of 10 ppm), and stirring continued at 300 rpm for two minutes.
- The stirring speed was then reduced to 40 rpm for fifteen mins.
- The 800 mL sample was transferred from the conical flask to the DAF unit where it was allowed to settle for fifteen mins
- Following settling, a sample was taken from the supernatant above the settled flocs, for COD analysis. The method for COD is detailed in Appendix 2.

4.2.4 Dissolved Air Flotation subsequent to Coagulation and Flocculation

- The pressure cylinder was filled with water up to the halfway mark and the lid was closed
- The inlet valve on the pressure cylinder was opened to allow synthetic air to enter the water in the cylinder (dissolved air). The inlet valve was closed when a reading of 400 kPa (4 Bar) was obtained on the pressure gauge.
- The pressure cylinder was then shaken vigorously to allow an even distribution of air throughout the water.
- The outlet valve on the pressure cylinder was opened, allowing tiny air bubbles to enter the DAF unit containing the settled, 800 mL coagulated and flocculated effluent sample. The outlet valve was closed when the level of liquid in the DAF unit reached 1000 mL.

- ❑ The sample in the DAF unit was allowed to stand for approximately five minutes to allow the bubble-floc agglomerates to rise to the surface of the sample to form the float.
- ❑ A sample was taken, from the liquid below the float, for COD and GC analysis.

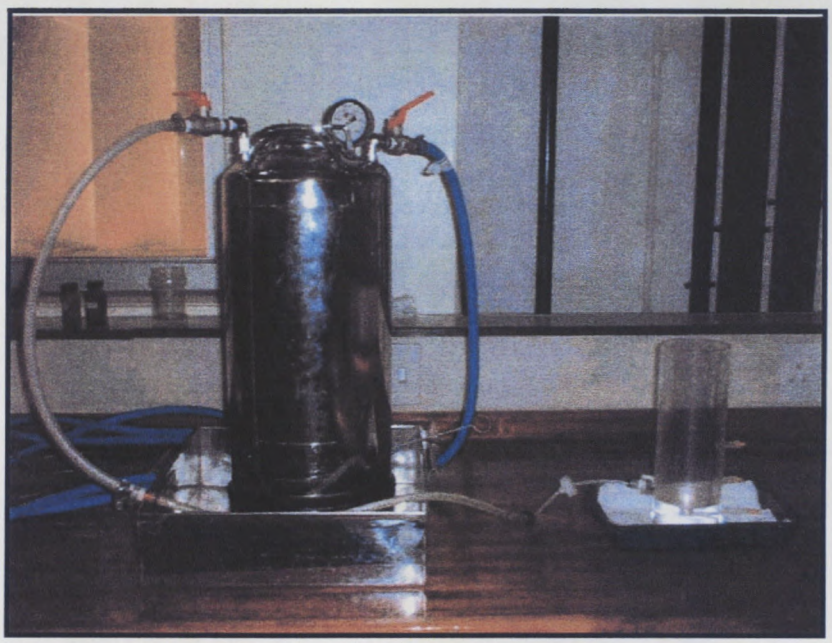

Figure 4.1: Dissolved Air Flotation (DAF) test apparatus

4.2.5 Sub-culturing of Fungal and Yeast Mono-Cultures

The fungal cultures were isolated from soil samples from the edible oil industry and identified by Devnath, (2001). The samples were then sub-cultured every month on Sabouraud Dextrose Agar (SDA) plates (Appendix 22). These plates were then incubated at 25°C for 48 hours or until growth occurred. They were then preserved in the refrigerator until used or until next sub-culture. The fungal isolates were identified up to genus level. Isolate 1 was *Alternaria sp*, isolate 2 was a *Mucor sp* 1 and isolate 3 was also of *Mucor sp* 2.

The yeast isolates were obtained also from the soil samples from the industry and identified by Reddy, (2001). The samples were then sub-cultured on Malt Extract Agar (MEA) plates (Appendix 23) and incubated at 25°C for 48 hours or until growth occurred. The isolates were then preserved

in the refrigerator until used or until next sub-culture. For further information on culture, storage, isolation and identification of yeasts works by Campbell, (1991), can be referred to. Isolate 1 was identified to species level while isolate 2 identified to genus level. Isolate 1 was the *Rhodospridium sp* and isolate 2 was *Candida succiphila.*

4.2.6 Experimental Set-up

Two liter conical flasks were used as batch reactors with a total working volume of 1 L. The following batch test were carried out:

a) Diluted effluent (to ~ 2000 mg COD/L without nutrients at 21 and 31°C

b) Coagulation and DAF of effluent diluted with double strength nutrient solution at 31°C to a COD of ~ 2000 mg/L.

See Appendix 24 for composition of double strength nutrient solution.

The fungal batch tests were at pH 5 and the yeast batch tests were conducted at a pH of 8. Following pH adjustment, each batch flask was inoculated with the respective isolates from the agar plates and incubated at either 21 or 31°C for 7 days. An identical set of batch flasks were made but not inoculated, these served as controls for batch test (a), i.e., effluent without nutrients at 21 and 31°C. For the pretreated batch process, the controls were: (1) diluted effluent (~2000 mg/L) no pretreatment, (2) diluted effluent with pretreatment and (3) diluted effluent, no pretreatment, inoculated with respective isolates.

All batch tests were carried out on a shaker, operating at 115 rpm.

4.2.7 Analysis of Samples

The flasks were removed from the shaker and allowed to stand for a few minutes to allow the biomass to settle. Samples of 20 mL were aseptically removed and the COD and pH were then determined. For the batch tests with the pretreatment, GC analysis (Appendix 25) as well as the COD and pH were determined, however due to strict financial budgets, samples for GC analysis were taken only on days 1, 2, 3, 6 and 7 for fungal batch tests and on days 1 and 3 for yeast batch tests.

4.3 RESULTS

The pH of the medium for the fungal and yeasts isolates were adapted from the works of Devnath, (2001) and Reddy (2001) respectively, who optimised the pH conditions for these specific cultures, hence the pH of 5 for fungal isloates and pH 8 for yeast isolates.

4.3.1 Fungal Batch Tests

4.3.1.1 Degradation of effluent using fungal isolates without nutrients at 21° and 31°C.

The mean COD profiles are shown in figures 4.2 and 4.3 for batch tests at 21 and 31°C respectively. The COD illustrates the degradation capabilities for the three fungal isolates at the two different temperatures without nutrients.

Figure 4.2: The diluted effluent had an initial COD of 2260 mg/L. The control COD declined from 2260 mg/L to 2110 mg/L on the last day. The *Alternaria sp* reduced the organic load from 2260 mg/L to 2075 mg/L on day 7. *Mucor sp* 1 reduced the COD from 2260 to 2087.5 mg/L, while *Mucor sp* 2 reduce the COD from 2260 mg/L to 2042.5 mg/L. The COD of the control was reduced by 6.64 %, COD of *Alternaria sp* had a reduction of 8.19 %, *Mucor sp* 1 by 7.63 and *Mucor sp* 2 had the highest COD removed which was 9.62 %.

Figure 4.3: For this batch the effluent had an initial concentration of 2360 mg/L. The control declined from a concentration of 2360 to 2010 mg/L. *Alternaria sp* reduced the concentration from the initial concentration of 2360 mg/L to 2075 mg/L. *Mucor sp* 1 degraded the effluent from a concentration of 2360 to 1970 mg/L, while *Mucor sp* 2 degraded the effluent to 1935 mg/L. For these batches the percentage COD removed was higher than the previous batch. The control had a reduction of 14.83 %, *Alternaria sp* had a reduction of 12.08 %, *Mucor sp* 1was 16.53 %, while *Mucor sp* 2 had a 18.01 % reduction.

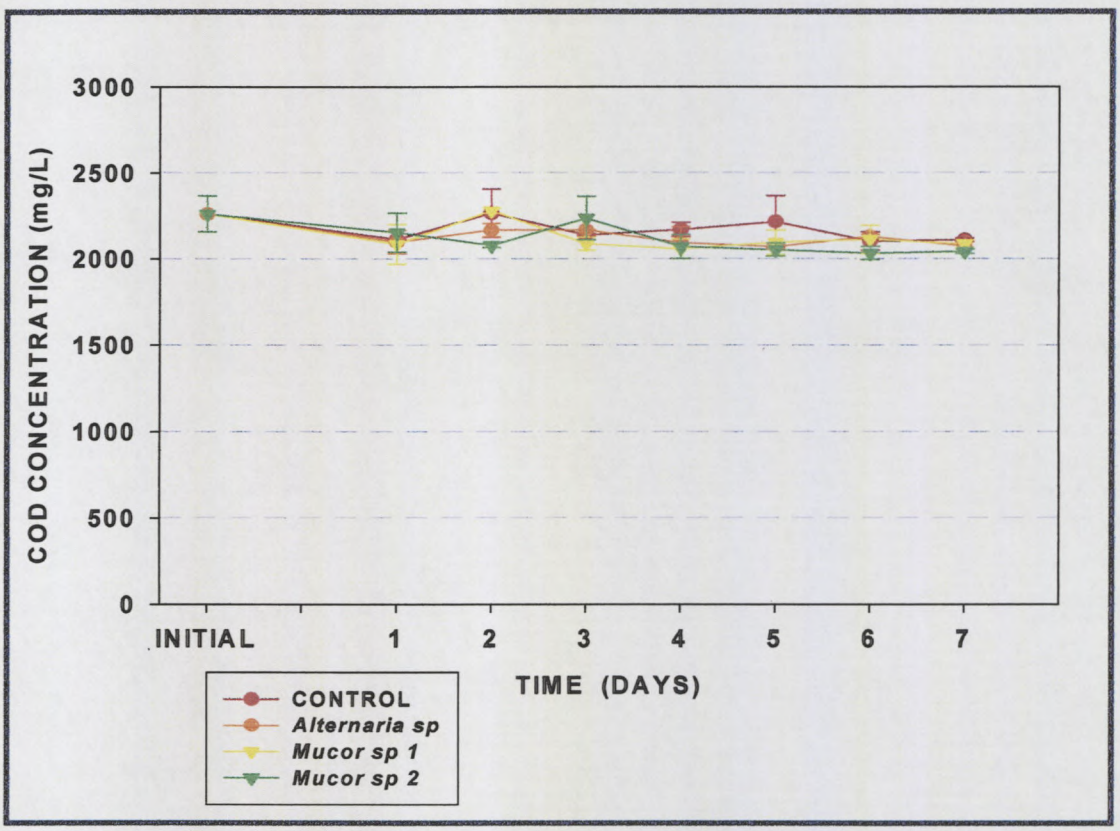

Figure 4.2: Mean COD profiles for control, *Alternaria sp, Mucor sp* 1, and *Mucor sp* 2 at 21°C without nutrients

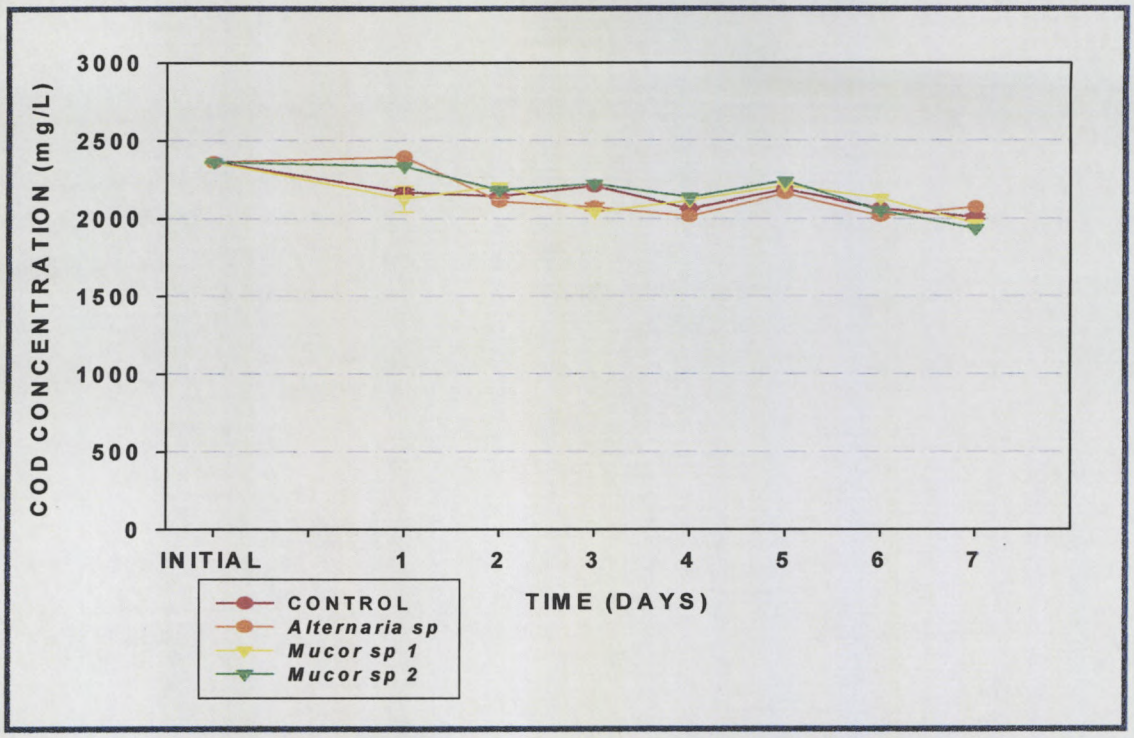

Figure 4.3: Mean COD profiles for control, *Alternaria sp*, *Mucor sp*1and *Mucor sp* 2 at 31°C without nutrients

Table 4.1: FOG concentration removed by isolates 1, 2 and 3 at 21 and 31°C without nutrients.

	21°C	31°C
INITIAL	1280	1280
CONTROL	523	593
Alternaria sp	533	403
***Mucor sp* 1**	777	570
***Mucor sp* 2**	743	1280

Table 4.1 shows the FOG's removed for the batches without nutrients. At a the higher temperature of 31° a greater FOG concentration was removed for the *Alternaria* and *Mucor sp* 1, whereas *Mucor sp* 2 had no FOG's removed at all.

4.3.1.2 Pretreatment with Coagulation followed by DAF and subjecting to fungal treatment

For these batch tests the effluent was diluted with the double strength nutrient solution and all tests carried out at a temperature of 31°C in a shaker incubator. Figure 4.3 illustrates the control and the three isolates without pretreatment. The initial concentration of the effluent was 1785 mg COD/L. The control was reduced to 1516.5 mg/L by day 7. *Alternaria sp* reduced the organic content from the initial concentration to 655 mg/L, *Mucor sp* 1 reduced the concentration to 815 mg/L and *Mucor sp* 2 to 1422.5 mg/L on the last day. This was evident that *Alternaria sp* managed to reduce the highest organic concentration with a percentage of 63.31, while *Mucor sp* 1 removed a percentage of 54.34 % and *Mucor sp* 2 a mere 20.31 %.

However after pretreatment of the effluent with coagulation coupled with DAF, the control had the lowest concentration illustrated in figure 4.5. After coagulation and DAF the initial COD of the effluent from 1785 mg/L was reduced to 1375 mg/L. Hence from 1375 mg COD/L the pretreated control had a further decline to 817.5 mg/L, the *Alternaria sp* from the initial concentration of 1375 declined to 915 mg/L, *Mucor sp* 1 reduced the organic load to 985 mg/L and *Mucor sp* 2 to 1135 mg/L. The overall COD removed after pretreatment and biological treatment was 54.20 % for the control, 48.74 % for the *Alternaria sp*, 44.82 % for *Mucor sp* 1 and 36.41 % for *Mucor sp* 2.

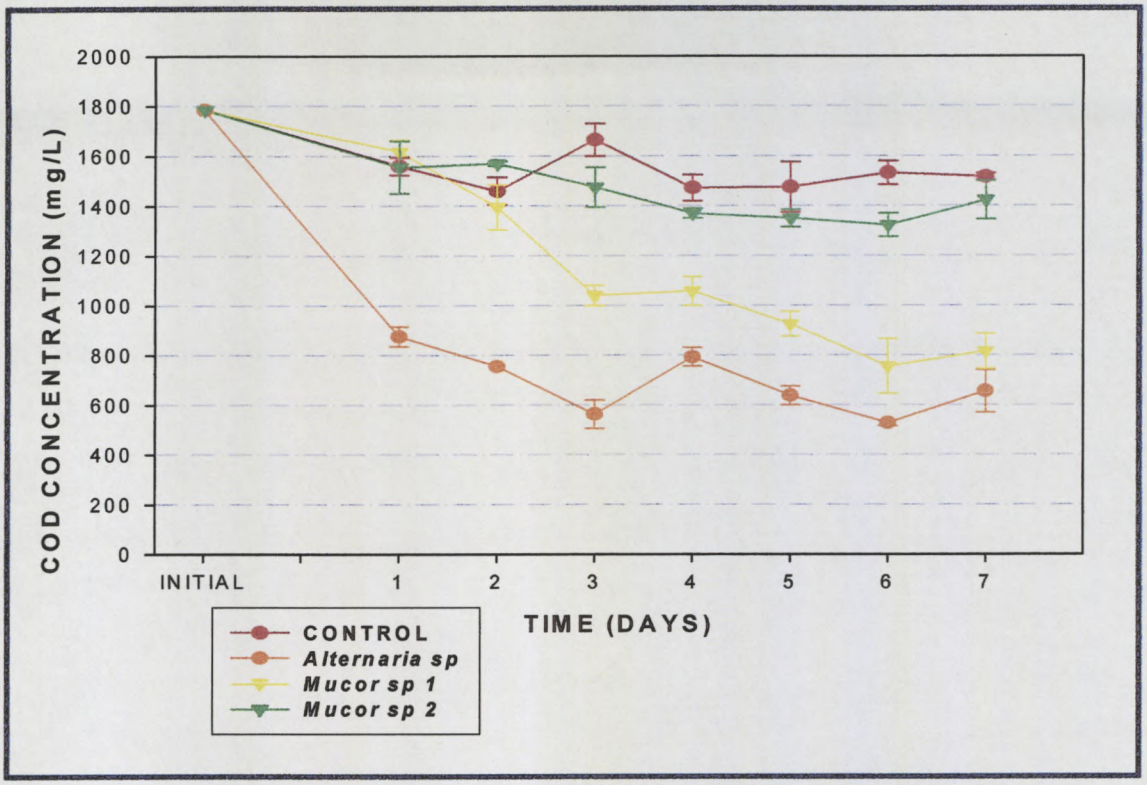

Figure 4.4: Mean COD profiles of control, *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2 without pretreatment

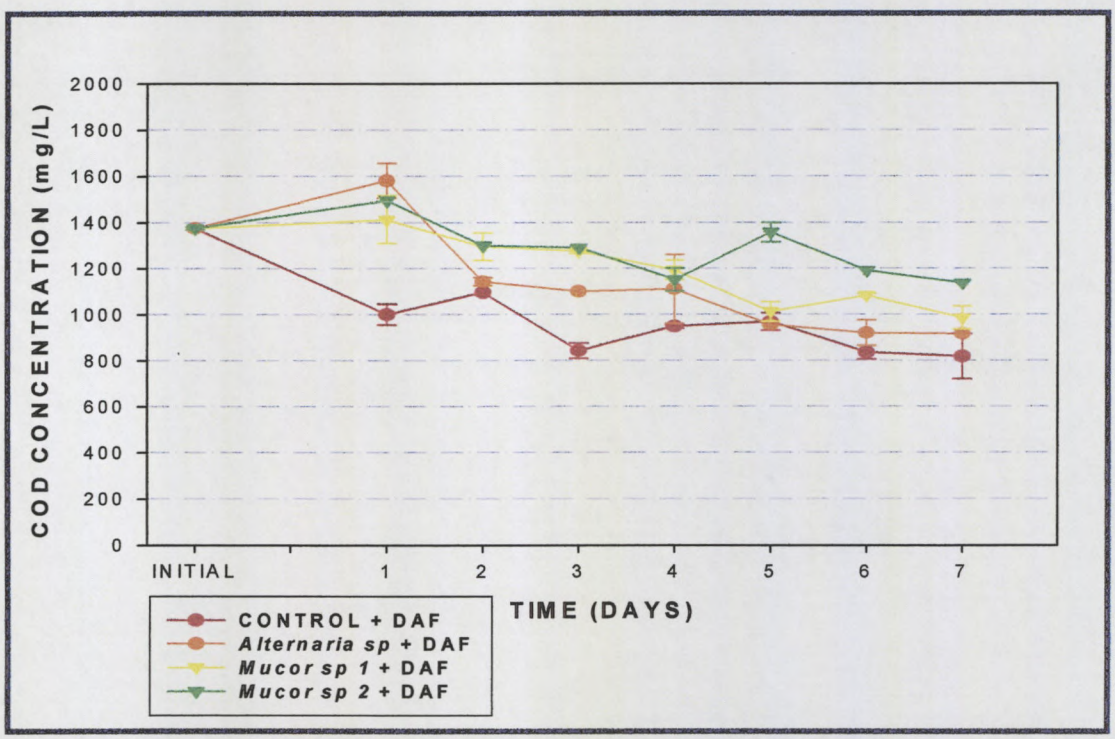

Figure 4.5: Mean COD profiles of pretreated samples with control, *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2

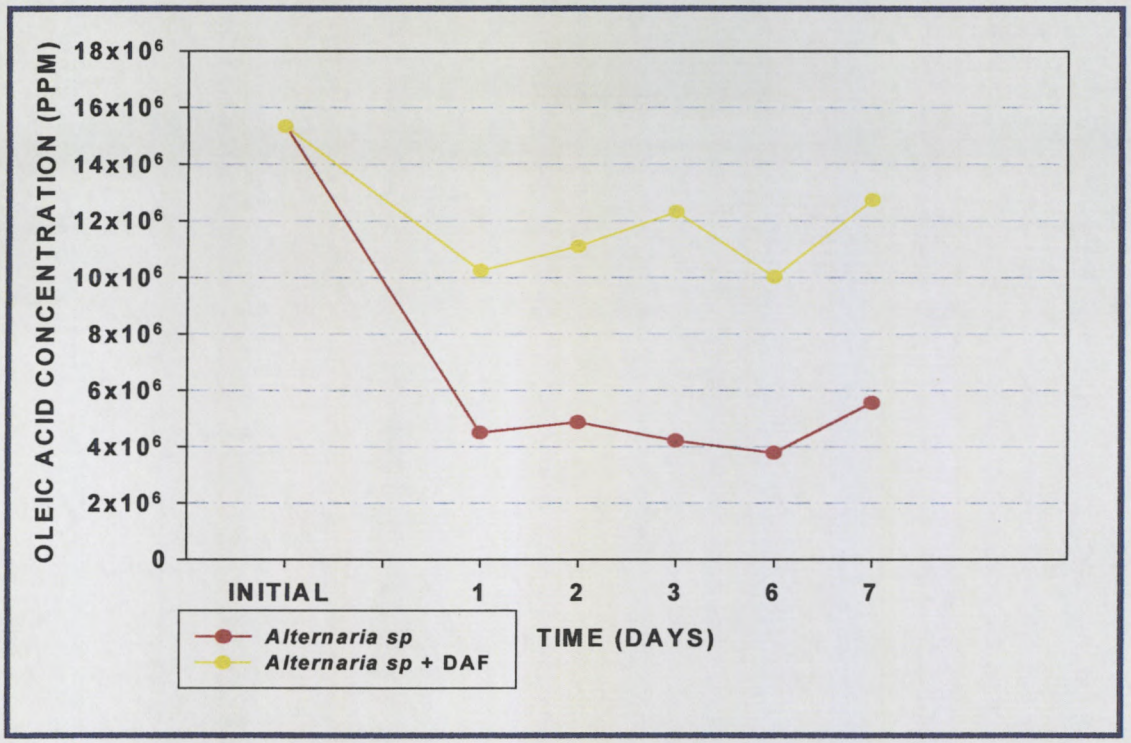

Figure 4.6: The degradation of oleic acid by *Alternaria sp* with and without pretreatment

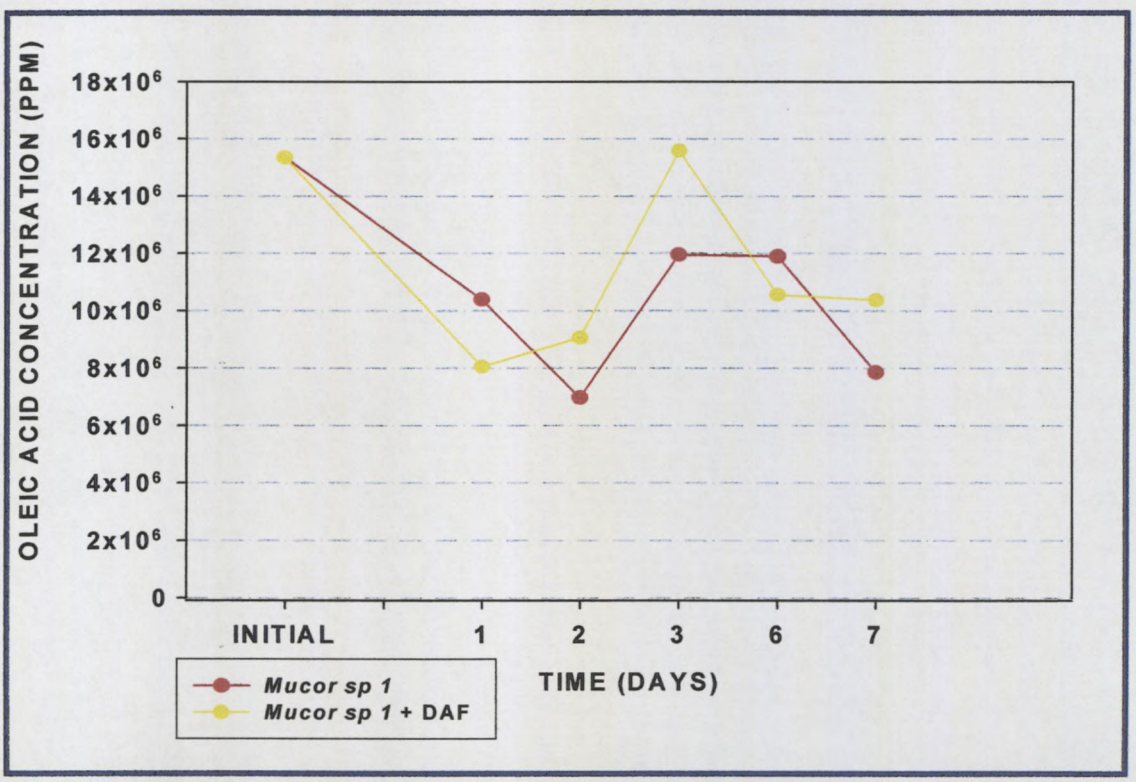

Figure 4.7: The degradation of oleic acid by *Mucor sp1* with and without pretreatment

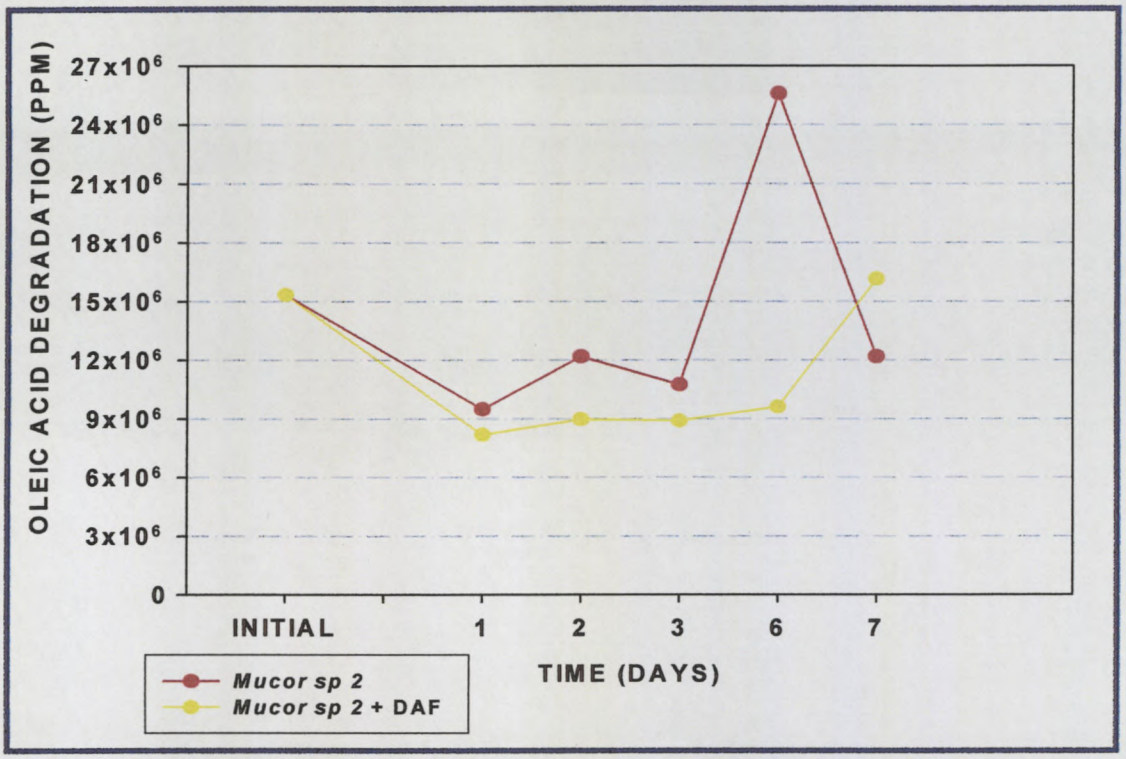

Figure 4.8: The degradation of oleic acid by *Mucor sp* 2with and without pretreatment

Figures 4.6 to 4.8compares the oleic acid degradation analysed on the GC for each fungal isolate with and without the pretreatment methods.

Alternaria sp without the pretreatment (fig. 4.6) degraded a higher oleic acid concentration than the batch with pretreatment. The concentration was reduced from 1.53 x 10^7 ppm to 5.7 x 10^6 ppm, for the batch test without pretreatment, whereas the batch with pretreatment, was reduced to 1.27 x 10^7 ppm by day 7 from the initial concentration.

For *Mucor sp* 1, (fig.4.7), the batch with the pretreatment had a higher removal efficiency, but after day 1 the oleic acid concentration increased to almost the same concentration as the initial control. After day 3 the concentration decreased to 1.20 x 10^7 ppm. However, the pretreated batch with *Mucor sp* 1 removed a higher concentration of oleic acid. GC analysis showed the concentration on day 7 was 7.85 x 10^6 ppm.

Figure 4.8, compares the oleic acid degradation for *Mucor sp* 2 with and without pretreatment. The pretreated batch with *Mucor sp* 2 had a higher oleic acid removal from days 1 to 6, but at day 7 the

concentration increased to 2.1 x 10^7 ppm which was higher than the initial concentration. *Mucor sp* 2 without the pretreatment had an unusually high increase in concentration on day 6 which was about 3 x 10^7 ppm, but decreased to 1.5 x 10^7 ppm on day 7.

Figure 4.9: Batch tests with cultures 1: *Alternaria sp*, 2: *Mucor sp* 1 and 3: *Mucor sp* 2 without pretreatment

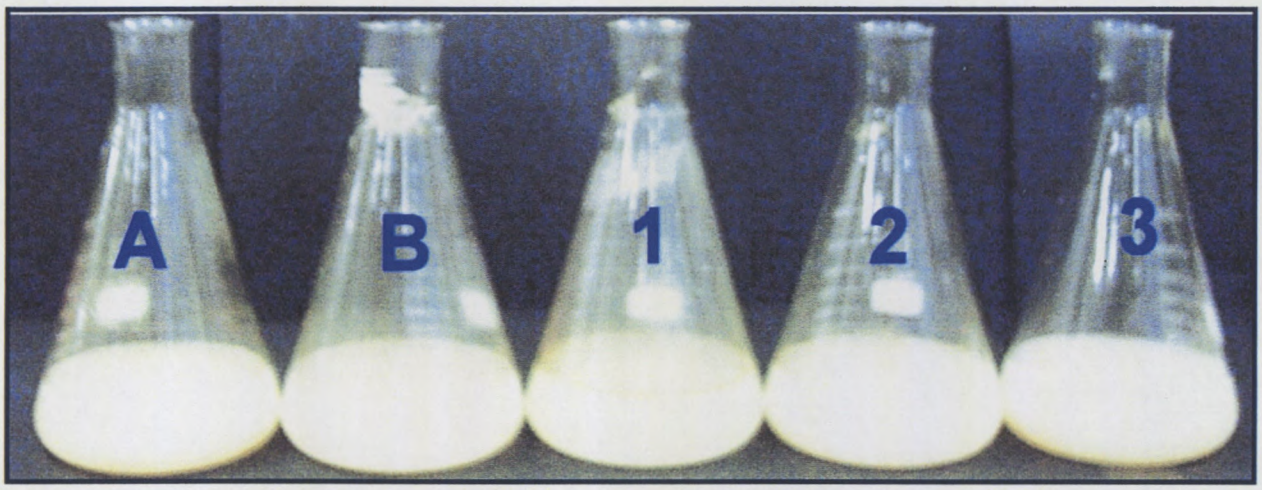

Figure 4.10: Batch tests with A: Control, B: Control with DAF, and cultures 1: *Alternaria sp*, 2: *Mucor sp* 1, and 3: *Mucor sp* 2 with pretreatment

Figures 4.9 and 4.10 illustrates the flasks of the batch tests with and without pretreatment respectively. It can be seen in fig 4.9 that there was a color change in the effluent during the 7 day process, with the white turbid color becoming transparent as seen in flask 1 (*Alternaria sp*). There was a slight color change for *Mucor sp* 1in flask 2, but no color change in flask 3 occurred. Figure 4.10 shows the controls as well as the flasks that were pretreated. Flask A is the control with no pretreatment and no fungal isolates, flask B is the control that was pretreated but not inoculated with the isolates, and flasks 1, 2, and 3 are the fungal isolates *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2 respectively. It can be seen there was no visible change in the color of the effluent, except for the *Alternaria sp*, where there was a slight color change. Also on the surface of the effluent oil droplets were visible after the 7 day period, this was not present in the initial stages of the process. Although there was not much change in the physical appearance, there were changes in the organic and lipid content.

4.3.2 Yeast Batch Tests

4.3.2.1 Degradation of effluent using yeast isolates without nutrients at 21 and 31°C

The mean COD profiles are shown in figures 4.11 and 4.12 for batch test at 21 and 31°C respectively. The COD illustrated the degradation capabilities for the two yeast cultures at the two different temperatures without nutrients.

Figure 4.11: The diluted effluent had an initial concentration of 2035 mg/L. The control organic content of the control decreased from 2035 mg/L to 1770 mg/L in the middle of the batch test, and after day 3 increased to 2010 mg/L on day 7. *Rhodosporidium sp* on the other hand decreased in organic content to 1950 mg/L on the first day and thereafter there was no removal of COD as the concentration increased to 2092 mg/L on day 4. The concentration then decreased to 1987.5mg/L on the last day. *Candida sp*, also exhibited these increases and decreases of COD. *Candida sp* reached a final concentration of 2075 mg/L by day 7.

Figure 4.12: With the higher temperature there was no degradation of the organic content by the two isolates. The initial concentration was 2035 mg/L. Both the *Rhodosporidium sp* and *Candida sp*, exhibited increased COD values, with *Rhodosporidium sp* having a final concentration of 2072 mg/L and *Candida sp*, 2115mg/L. The control however decreased in concentration, having a final organic content of 2005 mg/L.

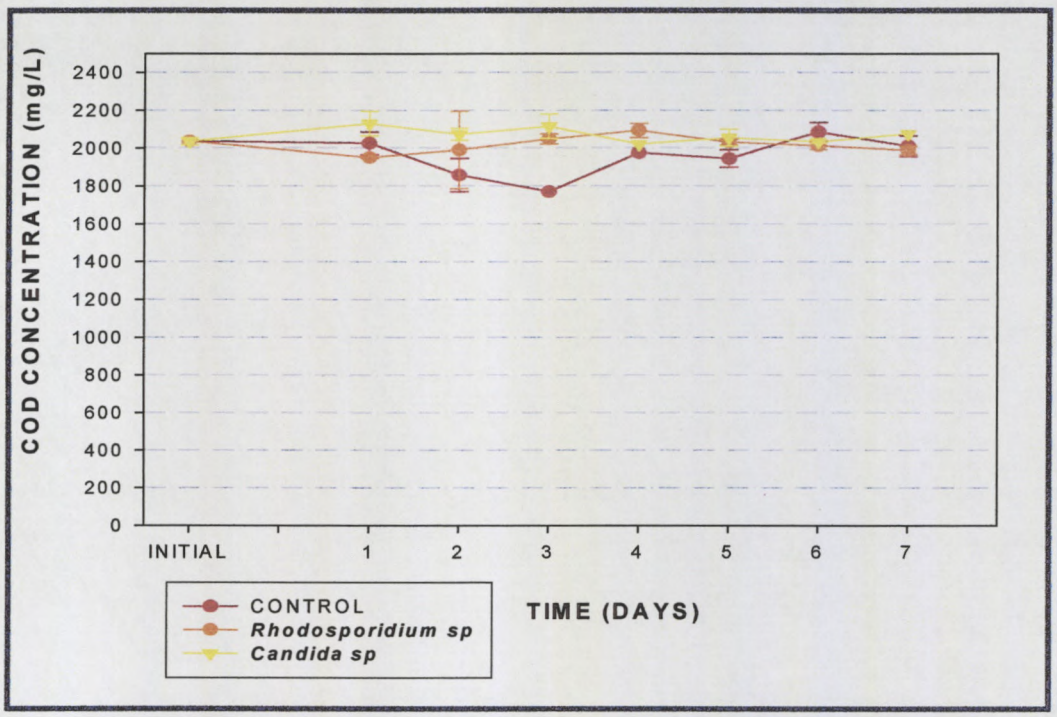

Figure 4.11: Mean COD profiles of effluent for control, *Rhodosporidium sp* and *Candida sp* at 21°C without nutrients

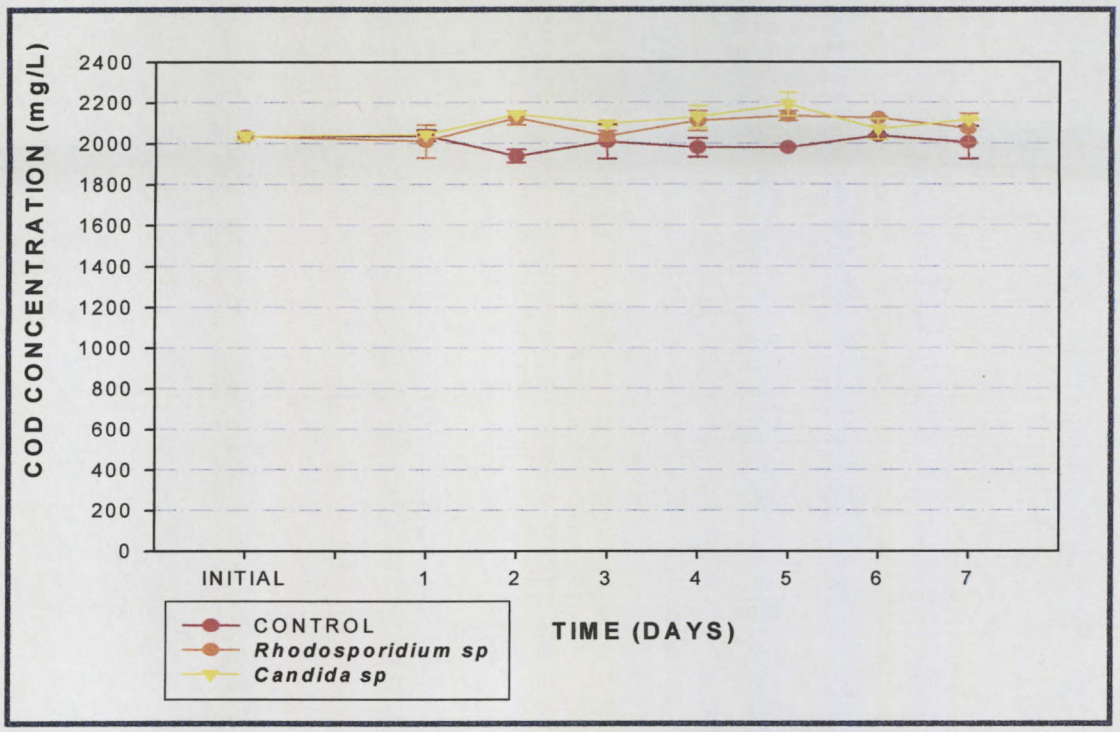

Figure 4.12: Mean COD profiles of effluent for control, *Rhodosporidium sp* and *Candida sp* at 31°C without nutrients

Table 4.2: FOG concentration removed by the two isolates at 21 and 31°C without nutrients

	21°C	31°C
INITIAL	1090	1090
CONTROL	580	550
Rhodosporidium sp.	266.67	250
Candida sp.	636.67	460

Table 4.2 shows the FOG's removed during the batch tests without nutrients. At the higher temperature the FOG removal was higher than the temperature at 21°C. The *Rhodosporidium sp* had substantial removal rates at both the temperatures, however the *Candida sp* removed more FOG's at 31 than at 21°C.

4.3.2.2 Pretreatment with Coagulation followed by DAF and subjecting to yeast biological treatment

These batch tests were carried out the same as for the fungal batch tests i.e., at 31°C for seven days in a shaker incubator. Figure 4.12 illustrates the controls for this batch test, which were the control and *Rhodosporidium sp* and *Candida sp* without pretreatment. The initial concentration of the effluent was 2330 mg/L. The control had a reduction in organic content from 2330 mg/L to 1915 mg/L, while the *Rhodosporidium sp* had a final COD of 1935 mg/L. *Candida sp* had a final concentration of 1780 mg/L. Hence the *Rhodosporidium sp* managed to reduce the organic content by 16.95 %, while *Candida sp* had a reduction by 23.61 %, and the control had a reduction by 17.81 %.

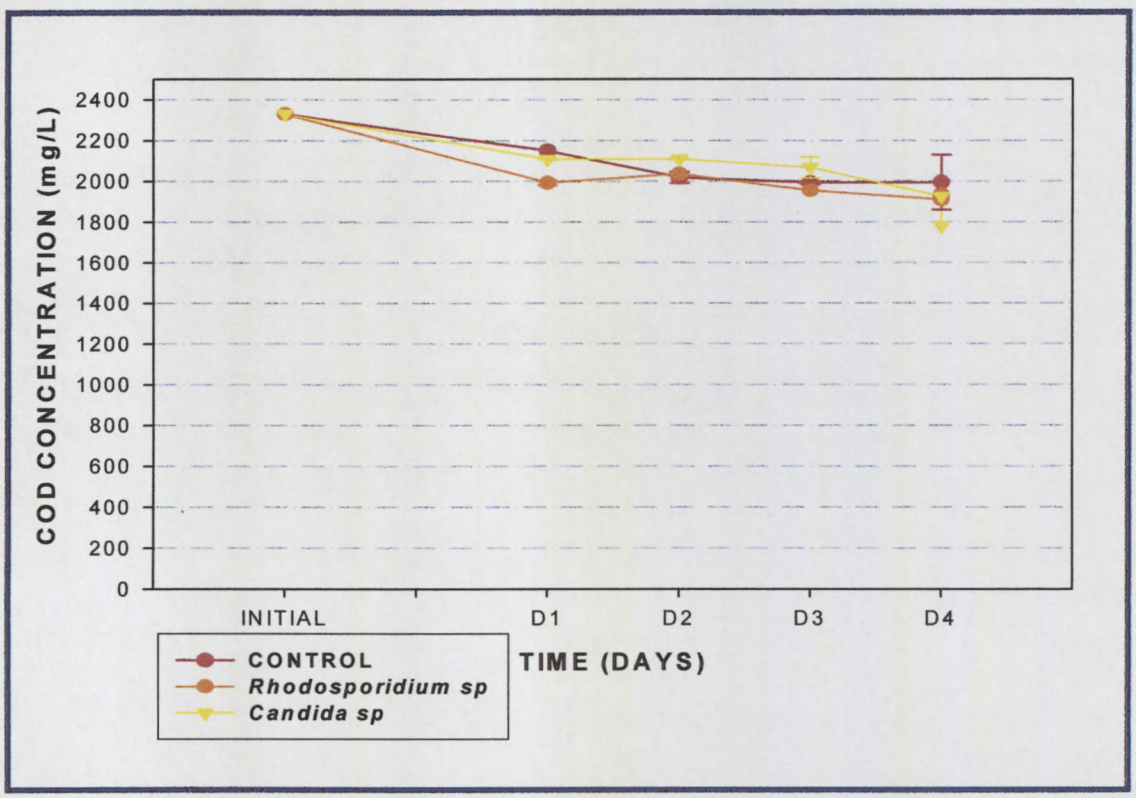

Figure 4.13: Mean COD profiles for the control, *Rhodosporidium sp* and *Candida sp* without pretreatment

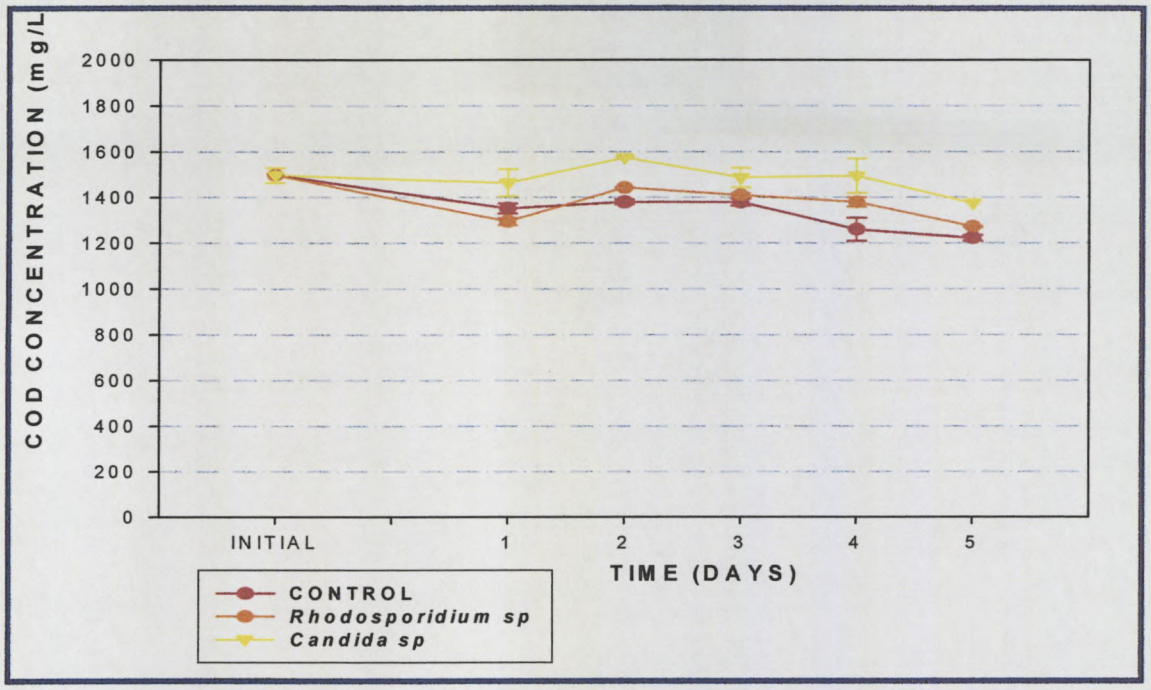

Figure 4.14: Mean COD profiles for pretreated samples with control, *Rhodosporidium sp* and *Candida sp*

However after pretreatment of the effluent with coagulation coupled with DAF, the control had the lowest organic concentration at the end of the batch test, illustrated in figure 4.14. After pretreatment the COD of the effluent declined from 2330 to 1497.5 mg/L, hence the control had a further decline after the 7 day batch test from 1497.5 to 1222.5 mg/L. *Rhodosporidium sp* had a COD reduction from 1497.5 to 1272.5 mg/L, while *Candida sp* was 1375 mg/L on the last day. The overall COD reduced after pretreatment and biological treatment was 47.53 % for the control, 45.39 % for the *Rhodosporidium sp* and the *Candida sp* had a COD removal of 40.98 %.

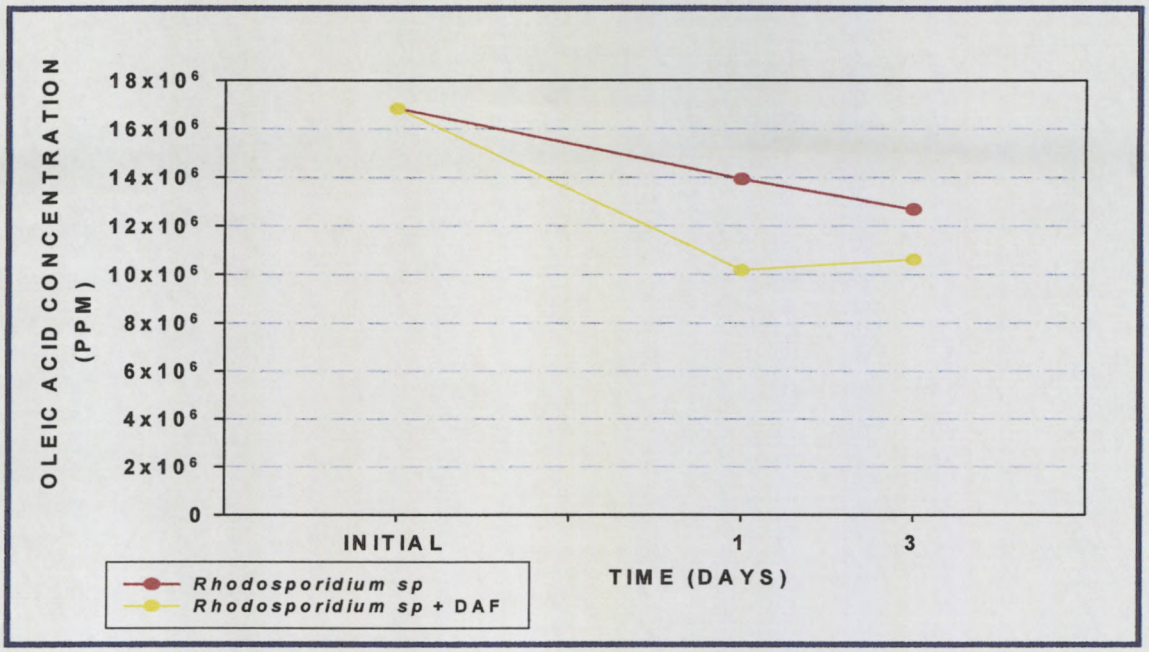

Figure 4.15: The degradation of oleic acid by *Rhodosporidium sp* with and without pretreatment

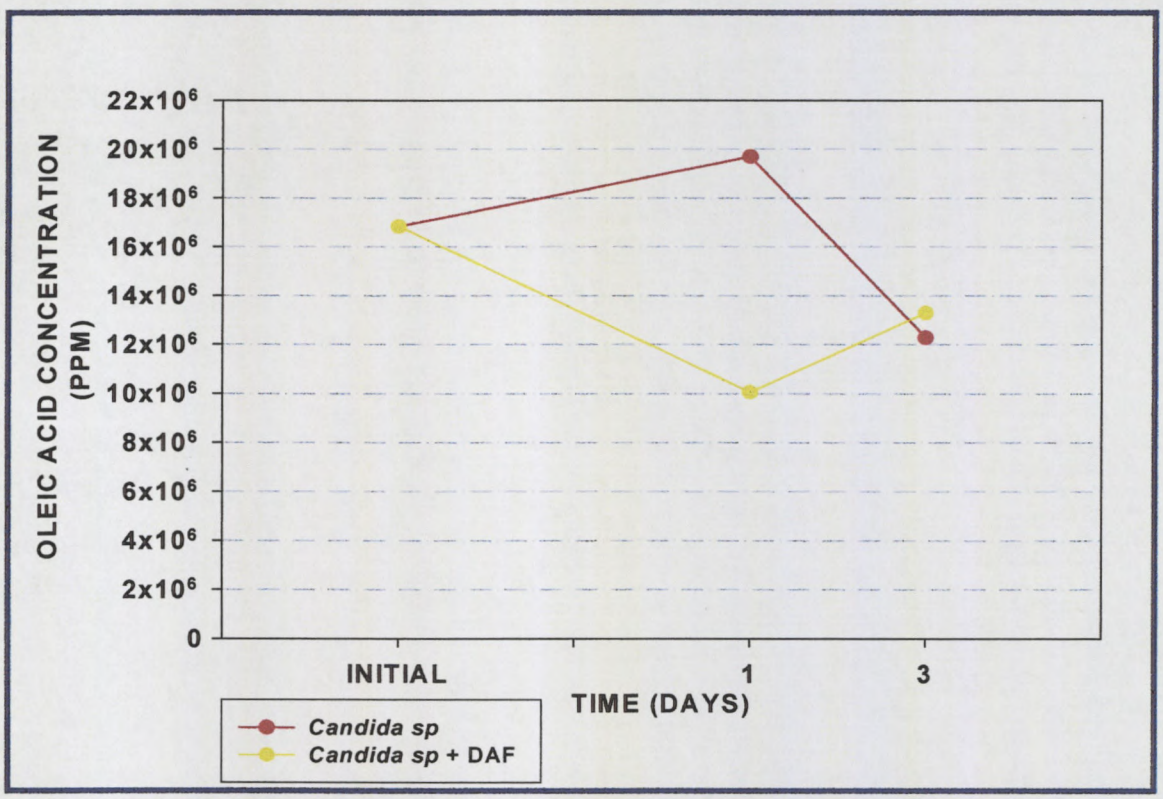

Figure 4.16: The degradation of oleic acid by *Candida sp* with and without pretreatment

Figure 4.15 and 4.16 compares the oleic acid degradation for each yeast culture with and without pretreatment respectively.

Rhodosporidium sp with pretreatment (fig.4.15) degraded a higher oleic acid concentration than the batch without the pretreatment. The concentration was reduced from an initial concentration of 1.68 x 10^7 ppm to 1.06 x 10^7 ppm. For the batch without pretreatment, the concentration was reduced but not significantly, which was 1.27 x 10^7 ppm on day 3.

For *Candida sp* the batch with pretreatment (fig. 4.16), had a higher concentration removed at day 1, which was 1.01 x 10^7 ppm. After day 1 the concentration increased to 1.33 x 10^7 ppm. The batch without pretreatment increased in concentration at day 1 which was 1.97 x 10^7 ppm, but thereafter decreased, removing a higher concentration which was 1.23 x 10^7 ppm by day 7.

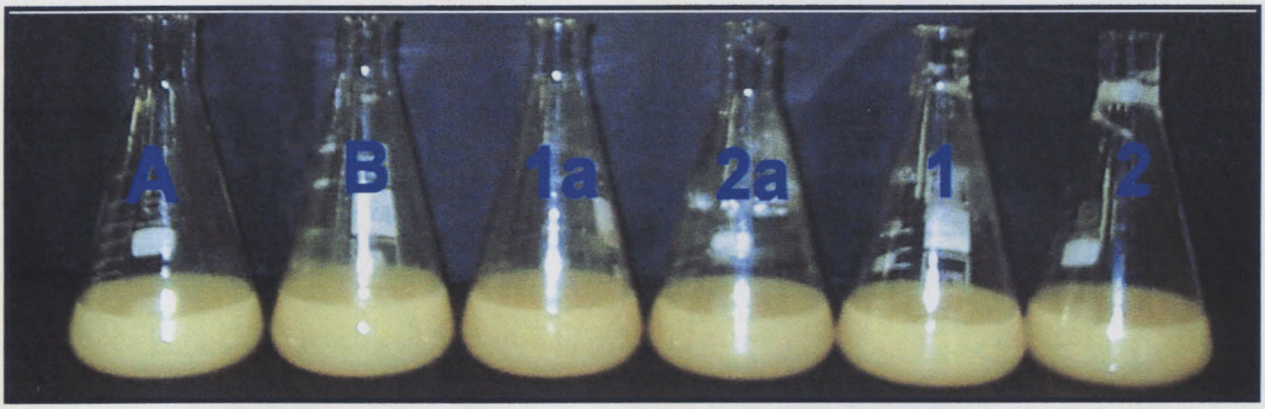

Figure 4.17: Batch tests showing flask A: control, B: control with pretreatment, 1a: *Rhodospridium sp* control, 2a: *Candida succiphila* control, 1: *Rhodosporidium sp* with pretreatment, 2: *Candida succiphila* with pretreatment

Figure 4.17 shows the batch tests with controls and cultures. The yeast cultures did not cause a color change in the effluent, like the fungal cultures did. The effluent remained as the white to yellowish color it originally was.

4.4 DISCUSSION

Microorganisms utilise various industrial pollutants or natural occurring substances as sources of carbon, nitrogen, phosphorus, sulphur or other elements that may be required by the cells to sustain growth. However organic compounds such as carbonaceous material that are oxidised by microorganisms to provide energy for efficient growth is of upmost importance (Alexander, 1999). Lipids (characterised as either fats and long chain fatty acids, oils and greases) are considered as important organic constituents of various wastewaters, as they contribute to 30 to 40 % of the total organic matter (expressed as Chemical Oxygen Demand - COD), (Dueholm *et al*, 2001). Studies have shown that various microorganisms (i.e molds and yeasts) are capable of effectively using long chain fatty acids as sole carbon sources of carbon and energy, and that if the fatty acids are present along with water insoluble substrates (i.e, vegetable oils); then partitioning of the fatty acids between the aqueous and oil phases will diminish the toxic effects of the fatty acids (Ratledge, 1994).

Figures 4.2, 4.3, illustrated batch tests for fungal isolates and figures 4.11 and 4.12 illustrated batch tests for yeast isolates at 21 and 31°C, respectively without nutrients. These batch tests determine the degradative capabilities of these isolates with just the diluted effluent at the two different temperatures. For the fungal isolates at 21°C (fig. 4.2), the organic concentrations were reduced but not effectively enough, i.e., only a maximum of ~10% removal. This showed that the organisms were capable of utilising the effluent without nutrients. For the higher temperature of 31°C (fig. 4.3), the isolates exhibited higher organic removal rates. At both these temperature *Mucor sp* 2 removed the highest concentration of COD. However at 31°C the removal efficiency was increased thereby indicating that temperature effects the degradative capabilities of the organisms.

However when looking at the FOG removed (table 4.1), it can be seen that at the higher temperature *Mucor sp* 2 could utilise the FOG's present, whereas the *Alternaria sp* had the lowest COD removed at 31°C but the highest FOG utilisation, thus the Alternaria sp was capable of utilising the FOG more easily than the other cultures. This corresponds to the findings of Devnath, (2001), where studies show that the *Alternaria sp* was more efficient in utilising the long chain fatty acids in sunflower oil

effluent, than both the *Mucor sp* at the temperature of 31°C. Once again with an increase in the temperature, the lipid degradation was more efficient in the *Alternaria sp*, than for the two *Mucor sp.*

For yeast batch tests (fig. 4.11 and 4.12), at 21 and 31°C, respectively, without nutrients, the COD removal was inefficient. In both the batch tests the control exhibited a reduction in COD, whereas both the isolates at the two temperatures increased in COD. A probable reason being the growth may have been toxic to the these isolates which resulted in lysis of the cells, releasing the internal organic content causing an increase in the COD.

However, table 4.2 indicated that even though the COD increased, FOG utilisation occurred. This could be a possibility that FOG is a small component of the COD, or that due to the lack of alternate nutrients, the organisms had to utilise the FOG as a food source.

The concept of integrating DAF in to an overall treatment plant considers that what is done ahead of flotation with coagulant addition and flocculation (i.e., pretreatment) affects flotation performance. Favorable conditions for bubble attachment to particles requires coagulation conditions that reduce particle charge and produce hydrophobic particles. Coagulant dosages and pH conditions that satisfy these criteria depend on coagulant type and raw water quality characteristics, particularly, particle concentration and type of natural organic matter (Edzwald, 1995). Hence coagulation concentration and pH for efficient coagulation was adapted from the research of Thomson, (2001), who optimised pH and coagulation concentration for DAF.

Thomson, (2001), found that a $FeCl_3$ was the most efficient coagulant. A number of studies support this finding, that ferric chloride was the most effective coagulant during physico-chemical treatment. Eroglu *et al* (1990) and Mutl & Polasek, (1996), found that ferric chloride was the most efficient reagent for the removal of organic matter. However Eroglu *et al* (1990) also stated that even though a coagulant can reduce organic matter, it still does not meet standard discharge requirements. Therefore pretreatment using coagulation and DAF was conducted followed by biological treatment

with the fungal and yeast cultures.

Figures 4.4 and 4.5 shows the COD concentration of the fungal batch tests without and with pretreatment respectively, while figures 4.6, 4.7 and 4.8 compares the oleic acid degradation for *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2 respectively with and without pretreatment.

When comparing figures 4.4 and 4.5, it can be seen that the batch test without pretreatment had a higher COD removed as compared to the batch test with pretreatment. The coagulant and DAF reduced the organic content by 23 % and following fungal treatment further COD reductions were noted. These reductions were still not as efficient as in the batch without pretreatment except for *Mucor sp* 2, which had a higher COD reduction after pretreatment. However for both the batch tests the *Alternaria sp* was the isolate removed the highest organic content. For the pretreated batch tests all three isolates had an increase in the COD on day 1, this could be due to the some of the cells in the batch lysing due to shock loading. After day 1 all the concentrations declined. However for the pretreated batch, the control decreased in organic content more than the fungal isolates. The higher concentration in the batch tests with the cultures could be due to the fact that the cells of the cultures added to the organic content, hence the higher concentration than the control. The reason for this could be the cells were multiplying, whereas with the batch without pretreatment there was no multiplying of the isolates, the organisms utilised the organic content for survival, but not for growth (however the biomass concentrations were not determined in this study).

For the oleic acid degradation, determined on a GC for quantitative analysis of the fatty acid in the effluent, the *Alternaria sp* (fig 4 .6) without pretreatment degraded a higher concentration of oleic acid than the batch with the pretreatment. Even though the pretreated batch degraded the oleic acid, it could not degrade it was not efficient as the batch without pretreatment. The reason for this is still being looked into but one probable reason could be that the coagulant and DAF removed essential components from the effluent, which prevented this organism from utilising more of the oleic acid.

For *Mucor sp*1 (fig. 4.7) only on day 1 and 6 the oleic acid degradation was more efficient in the

pretreated batch test than the batch test without pretreatment. However towards the latter end of the process the oleic acid concentration increased in both the batch tests. This was probably due the organism releasing some of the lipids back in to the solution, as they could no longer utilise it, or the nutrients became depleted causing some of the organisms cells to lyse, releasing the internal lipid content in to the environment.

For *Mucor sp* 2, (fig 4.8), the pretreated batch was more efficient in the removal of oleic acid than the batch without pretreatment. This could be due to the pretreatment creating conditions which made it possible for the *Mucor sp* 2 to utilise the fatty acid. Also in these batch tests the oleic acid concentration increased towards the end. This was probably due to cells lysing and releasing internal lipids in to solution. This can also be seen in figure 4.10, where the oil droplets can be seen on the surface of the effluent for all three cultures.

From all three isolates the *Alternaria sp* degraded the highest concentration of oleic acid. This indicated the ability of this isolate to utilise the available long chain fatty acids as a source of carbon and energy for efficient growth. Possibly attributing to this unique capability was that *Alternaria sp* has a strong lipase producing enzyme system that has a high degree of specificity for unsaturated fatty acids in an aqueous system, as sunflower oil is known to constitute significantly large amounts of unsaturated fatty acids. Also lipases reveal their highest activity at an oil-water interface or areas were a greater exposure of the fatty acid to the microbe or water is evident (Ratledge, 1994).

Figure 4.13 and 4.14 illustrated the organic concentration for the yeast batch tests without and with pretreatment respectively. For the batch test without pretreatment (fig. 4.12), the *Candida sp* removed a higher organic content than the *Rhodosporidium sp* but in the pretreated batch test (fig. 4.14), the *Rhodosporidium sp* remove a higher concentration than the *Candida sp*.. The coagulant and DAF reduced the organic content by 35 %, and after yeast treatment there were further COD reductions. However for the yeast batch tests the pretreated batch test removed a higher organic content than the batch without pretreatment. A probable reason for the yeast cultures being able to utilise the effluent after pretreatment was that the coagulant and DAF managed to remove some

components that were toxic to the organisms and therefore allowed then to utilise the effluent.

Figure 4.15 and 4.16 illustrated the oleic acid degradation, determined by GC analysis for the *Rhodosporidium sp* and *Candida sp*, respectively, with and without pretreatment. For both the isolates the pretreated batch degraded a higher concentration of oleic acid. This was probably due to the fatty acid being in a smaller concentration after the pretreatment, therefore organisms were able to utilise it. The *Rhodosporidium sp* removed a higher concentration than the *Candida sp.* The *Candida sp* (fig 4.16) had an increase in the oleic acid concentration for the batch without pretreatment. This could be due to some of the cells lysing due to shock loading therefore causing some of the cells releasing some of their internal lipids in to the solution.

However for the yeast isolates, the pretreatment aided in organic and fatty acid removal as compared to the batches without pretreatment. Another reason for poor removal in the batch tests without pretreatment could have been caused by organisms being in the lag phase, due to slower growth rates (growth rates were not monitored in this study). Wakelin & Forster (1997), observed that poor FOG was removed when the activated sludge was in the lag phase.

There was also no noticeable change in the color of the effluent as compared to the fungi which are shown in figure 4.17. This showed that the neither the pretreatment nor the yeast isolates were effective enough to cause a change in the physical characteristics of the effluent.

For all the batch tests it was noted that the controls were also reduced in organic content, thereby indicating the effluent did have self degradative capabilities.

4.5 CONCLUSION

For the fungal batch tests, the *Alternaria sp* exhibited higher lipid and organic removal efficiencies than the *Mucor sp* 1 and *Mucor sp* 2.

Pretreatment with $FeCl_3$ and DAF reduced the organic content, but is not required for certain isolates like the *Alternaria sp*, as this isolate has the ability to utilise the organic and lipid content without the aid of pretreatment. However the pretreatment does aid certain species like the *Mucor sp* 2, where the organic and lipid degradation were enhanced, after pretreatment.

For yeast batch tests, the pretreatment definitely enhanced the yeast isolates ability to degrade the effluent more efficiently. Without pretreatment there was no or negligible organic degradation.

The *Rhodosporidium sp* yielded higher removal rates under these conditions for degradation of oil effluent, i.e., pH 8 at 31°C, pretreatment with 10 ppm $FeCl_3$ and DAF. Research by Reddy (2001), noted that as the pH increased the FOG removal increased. Studies by Jeffery *et al.*, (1999), showed that a pH of 8, appears to support both emulsification of oil and its cleavage by fungal lipase (yeast is unicellular fungi) activity as well as their utilization for cell growth. Also increase in pH, could alone be responsible for most of the enhanced sunflower oil utilization and biomass growth.

At the lower temperature of 21°C, there was more COD than FOG degradation. Therefore an increase in the temperature increases both the organic but more the lipid degradation.

In this research study the results obtained for the oleic acid degradation by the yeast isolates, no conclusive statements can be made, as the degradation capabilities were not monitored for the total duration of the experimental period.

It can therefore be concluded that :

- coagulation and DAF can enhance biological treatment of edible oil effluent but only for

certain organisms and under optimised conditions.

– certain organisms are capable of utilising the edible oil effluent without pretreatment, but only when optimised conditions exist.

CHAPTER FIVE

PRETREATMENT OF EFFLUENT USING OZONATION AND SUBJECTING TO FUNGAL AND YEAST BIOLOGICAL TREATMENT

5.1 INTRODUCTION

Complete removal of wastewater pollutants can be hardly achieved by the adoption of single treatment methods. Combination of chemical and biological treatment is often the way to optimise the overall process. The first treatment, if properly chosen, will facilitate the second one, thus leading to a much more effective treatment of the waste (Andreozzi *et al.*, 1998).

Biological treatment of wastewater is usually a cost effective means of removing biodegradable organic pollutants to satisfy legal limits. However at times the removal efficiency is not sufficient for biologically recalcitrant compounds. A combination of ozonation followed by biological treatment can dramatically increase the removal efficiency for such organic chemicals (Carini, *et al.*, 2001).

Ozone is a strong oxidizing gas that reacts with most organic and many inorganic molecules. It is produced when oxygen molecules separate, collide with other oxygen atoms, and form a molecule consisting of three oxygen atoms (Spellman, 1999). Oxidation of organic contaminants with ozone can overcome the limitations of the biological processes (Bonez *et al.*, 1999).

Ozone demand can be expressed in a number of ways and is dependent upon the contact time, contactor design, transfer efficiency, and applied ozone dose, as well as a number of other parameters such as pH, temperature and the concentration and nature of organic matter present in the water (Freese, *et al.*, 1998).

5.2 MATERIALS AND METHODS

Effluent sampling and characterisation, as well as sample analysis was carried out the same way as in chapter 4.

5.2.1 Experimental Design for Ozonation

- An ozonator system was set up according to Pryor & Freese (1998).
- The Ozonox ozonator was attached to a fish tank air pump, which allowed the ozone to be introduced into the sample at a steady rate. Gas exiting the sample was fed through two potassium iodide (KI) traps that were connected in series.
- Stopcocks were used to regulate the direction of ozone flow, which allowed for the use of alternate samples.

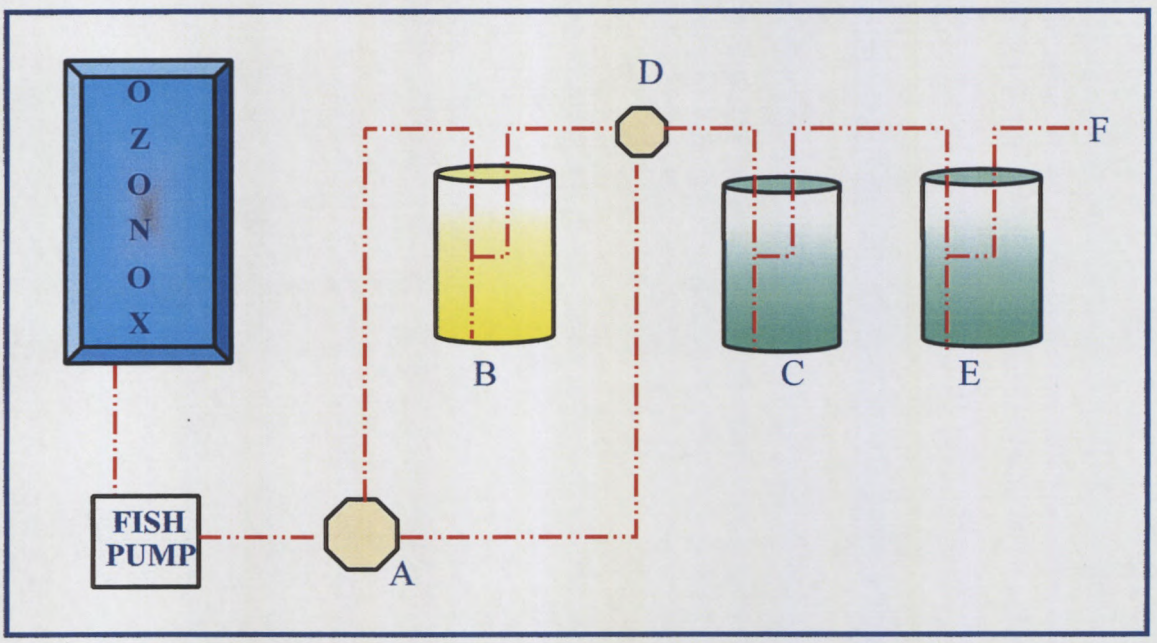

Figure 5.1: A schematic diagram of the developed ozonator system. A & D: Stop Cocks, B: Sample Bottle, C & E: Traps, F: Outlet pipe

5.2.2 Preparation of a calibration curve

- A standard sodium thiosusphate solution ($Na_2S_2O_3.5H_2O$) was prepared according to the Iodometric Method I (Appendix 26)
- This standard solution was used to determined the amount of ozone produced by the ozonator over a specific time period.
- The sample bottle and traps were filled with 400 mL and 800 mL of KI solution respectively
- The sample was ozonated for 1 minute, inducing a colour change from a clear to yellow
- The fully ozonated sample was titrated against the $Na_2S_2O_3.5H_2O$ until the sample turned from yellow to clear
- The amount of $Na_2S_2O_3.5H_2O$ used was recorded
- The procedure was repeated at various intervals from 2 - 10 minutes
- The results were recorded and inserted in to the following equation :

$$\text{Concentration } O_3 \text{ produced} = \frac{\text{vol } (Na_2S_2O_3.5H_2O) \times 24000 \times \text{Normality } (Na_2S_2O_3.5H_2O)}{\text{Vol sample}}$$

- The results were recorded and the points plotted and the calibration curve formulated

5.2.3 Ozonation Procedure

- The pH of the sample was adjusted using a base, sodium hydroxide (NaOH), or an acid, hydrochloric acid (HCl). For the fungi the pH was adjusted to 5, and for yeast the pH was adjusted to 8.
- The sample was transferred to the sample bottle of the system and KI solution was filled in the traps. After filling the sample bottle with the required amount, the bottle was connected to the two KI containing traps.
- The sample was ozonated for 3 minutes, thereafter, residual ozone was calculated using the Indigo Colorimetric Method (Appendix 27).

- ❑ Total ozone was then calculated

As soon as ozonation was complete, the samples were transferred to the 2 L conical flasks, serving as the batch reactors. The flasks were then inoculated with the respective cultures and placed in the shaker incubator at 31°C, at 115 rpm for 7 days. The residual ozone calculation can be found in Appendix 28.

5.3 RESULTS

5.3.1 Fungal Batch Tests

Figure 5.2 illustrates a control and the effluent inoculated with the fungal isolates, without ozonation. The initial COD was 1785 mg/L and at the end of the 7 day experimental period, all flasks had a reduction in COD concentration. The control was reduced to 1516 mg/L, the *Alternaria sp* reduced the COD to 655 mg/L, *Mucor sp* 1 to 815 mg/L and *Mucor sp* 2 to 1422.5 mg/L. The percentage COD removed was 15.07 % for the control, 63.31 % for the *Alternaria sp*, 54.34 % for *Mucor sp* 1 and 21.31 % for *Mucor sp* 2.

However with ozonation (fig. 5.3), the organic content was reduced further for the control, *Alternaria sp* and *Mucor sp* 2. *Mucor sp* 1 did not perform as efficiently as the previous batch test. The control was reduced from an initial COD of 1840 mg/L to 545 mg/L by day 7, *Alternaria sp* reduced the COD to 520 mg/L. It was noted for the *Alternaria sp* the COD was more or less at a constant value from day 2 to day 7, i.e., the COD remained between 595 to 520 mg/L. For *Mucor sp* 1, which did not perform very well, the COD was only reduced to 1107 mg/L, while *Mucor sp* 2 reduced the COD to 580 mg/L. The percentage COD removed was 70.38 % for the control, 71.39 % for the *Alternaria sp*, 39.81 % for *Mucor sp* 1 and 68.48 % for *Mucor sp* 2.

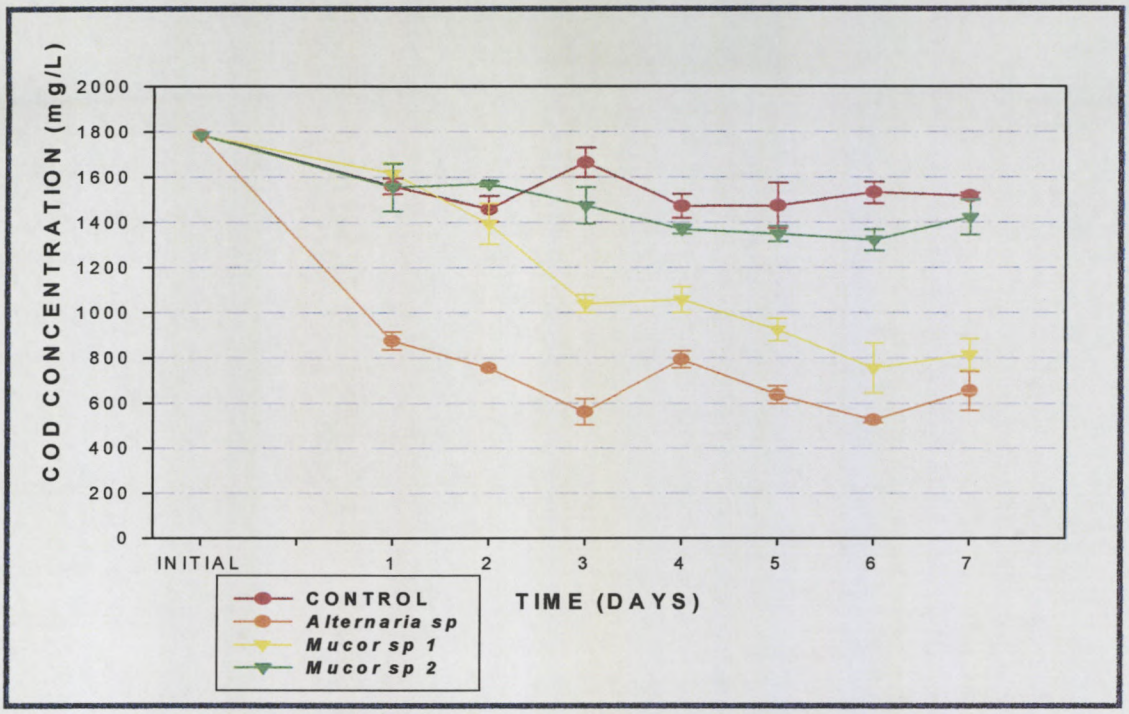

Figure 5.2: Mean COD profiles of control, *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2 without ozone

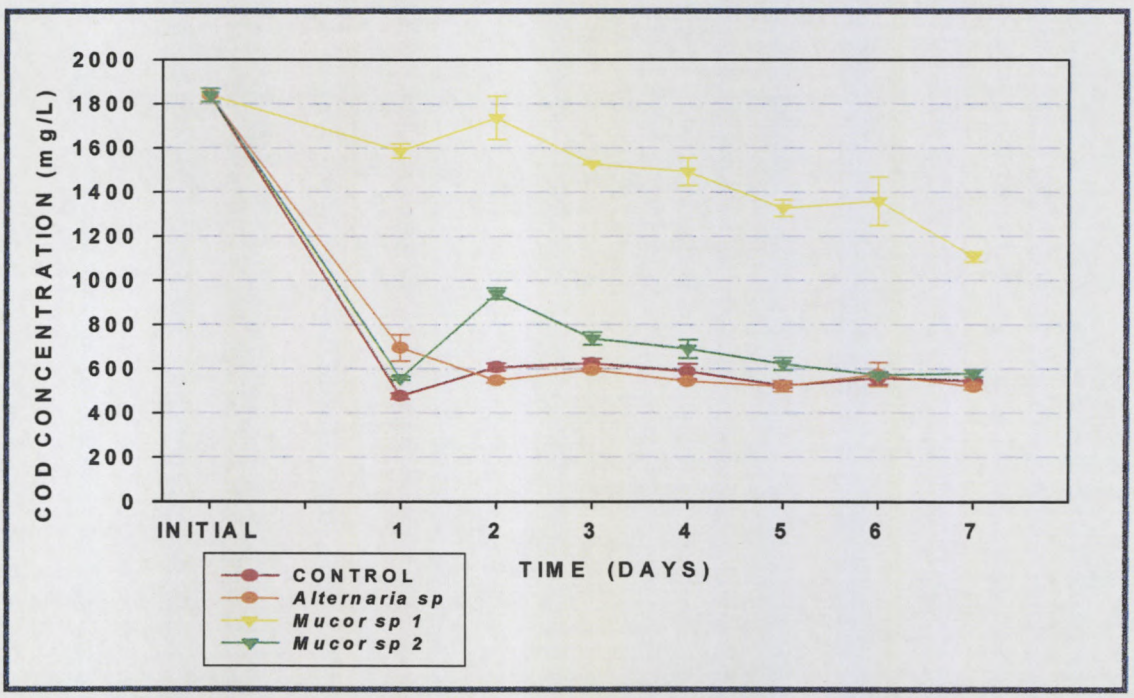

Figure 5.3: Mean COD profiles of ozonated batch tests with control, *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2

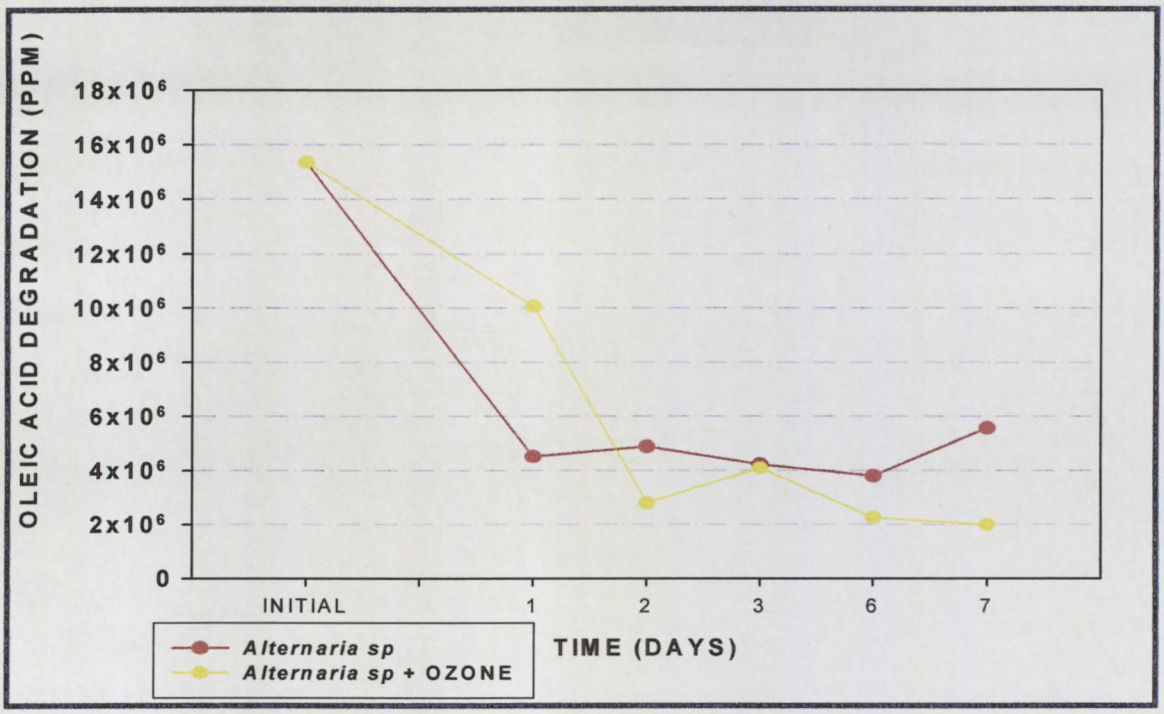

Figure 5.4: The degradation of oleic acid by *Alternaria sp* with and without ozone

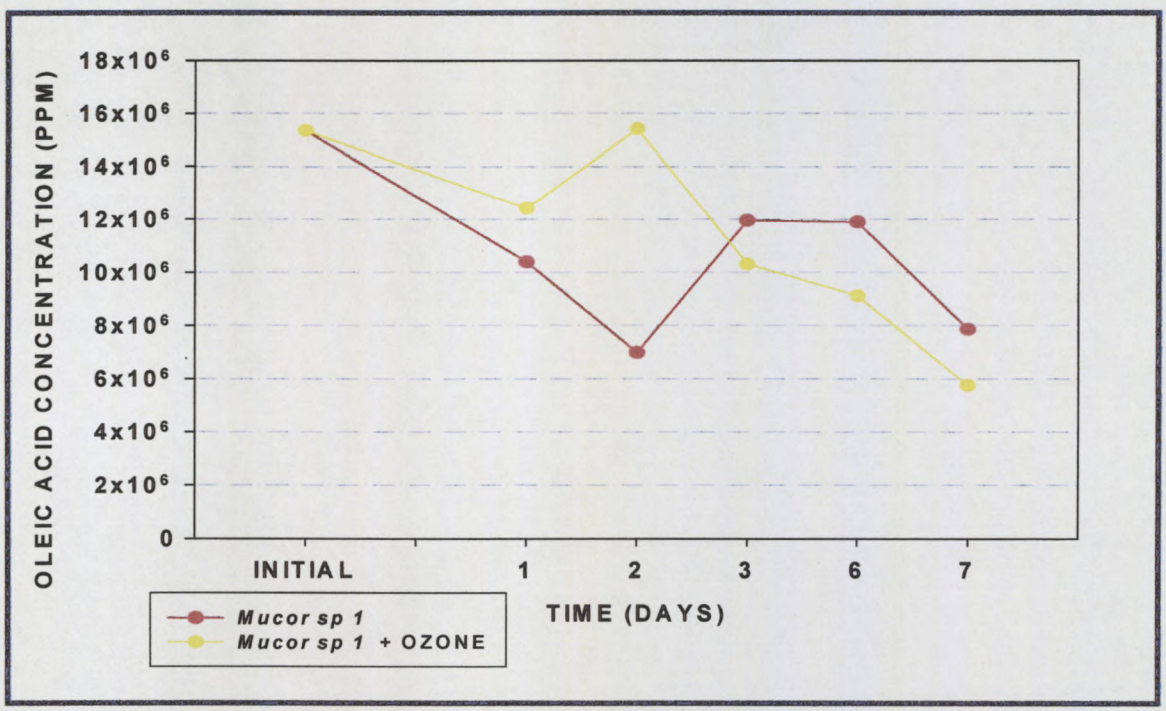

Figure 5.5: The degradation of oleic acid by *Mucor sp* 1 with and without ozone

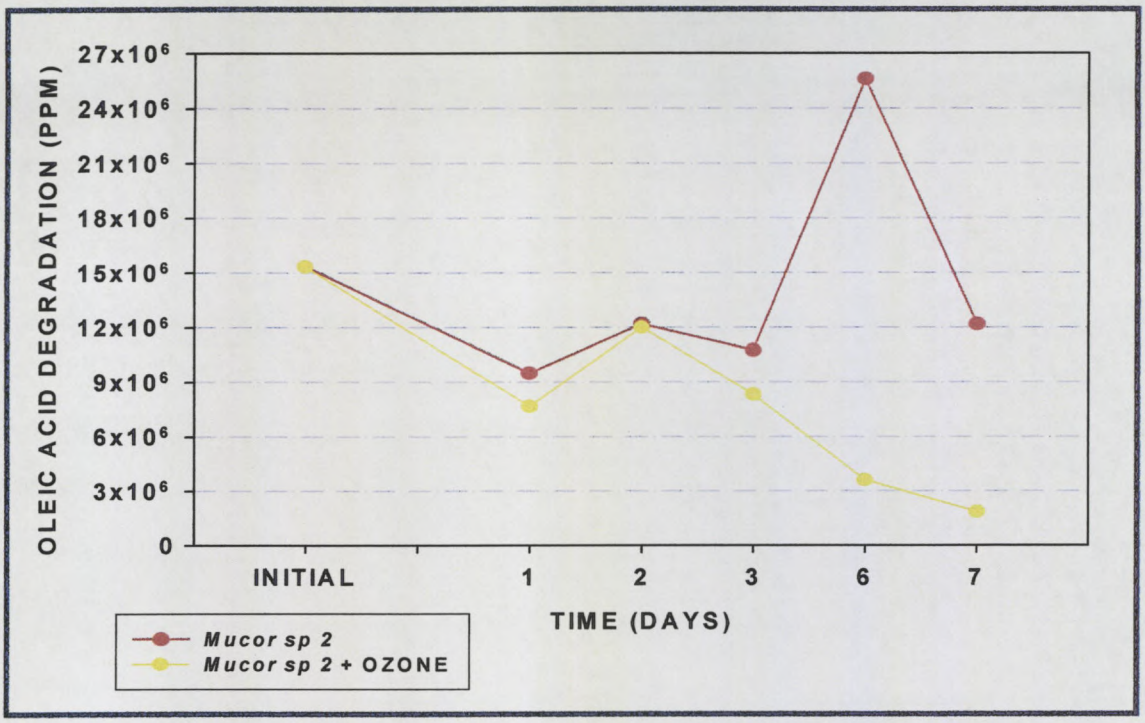

Figure 5.6: The degradation of oleic acid by *Mucor sp* 2 with and without ozone

Figures 5.4, 5.5 and 5.6 illustrates the oleic acid degradation with and without pretreatment for the *Alternaria sp*, *Mucor sp* 1 and *Mucor sp* 2 respectively.

The *Alternaria sp* in figure 5.4 maintained higher degradation capabilities of the oleic acid with pretreatment of the ozone from day 2 onwards. On day 1 the isolate without ozone removed a higher concentration than the pretreated batch. The oleic acid concentration was reduced from 1.53 x 10^7 ppm to 5.57 x 10^6 ppm for the batch without ozonation, while the oleic acid concentration of the pretreated batch was reduced to 1.98 x 10^6 ppm.

The *Mucor sp* 1 (fig. 5.5) removed a higher oleic acid concentration in the batch test without ozonation on days 1 and 2, but thereafter the efficiency declined. The batch test with ozone had an increase in oleic acid concentration on day 2, but thereafter the removal rate increased and from day 3 to 7 the concentration removed was higher than the batch test without ozone. For the batch test without ozone, the oleic acid concentration was reduced from 1.53 x 10^7 ppm to 7.85 x 10^6 ppm and

the pretreated batch was reduced in oleic concentration from 1.53 x 10^7 ppm to 5.74 x 10^6 ppm.

Mucor sp 2, (fig. 5.6), had a higher oleic acid degradation in the ozonated batch throughout the 7 day experimental period. There was a slight increase in concentration on day 2 but thereafter the concentration continued to decline to day 7. The concentration was reduced from 1.53 x 10^7 ppm to 1.86 x 10^6 ppm. The batch without pretreatment declined in concentration but at day 6 had an increase in oleic acid concentration and decreased on day 7. The concentration on day 6 was higher than the initial concentration. The oleic acid concentration for this batch test was reduced from 1.53 x 10^7 ppm to 1.21 x 10^7 ppm by day 7.

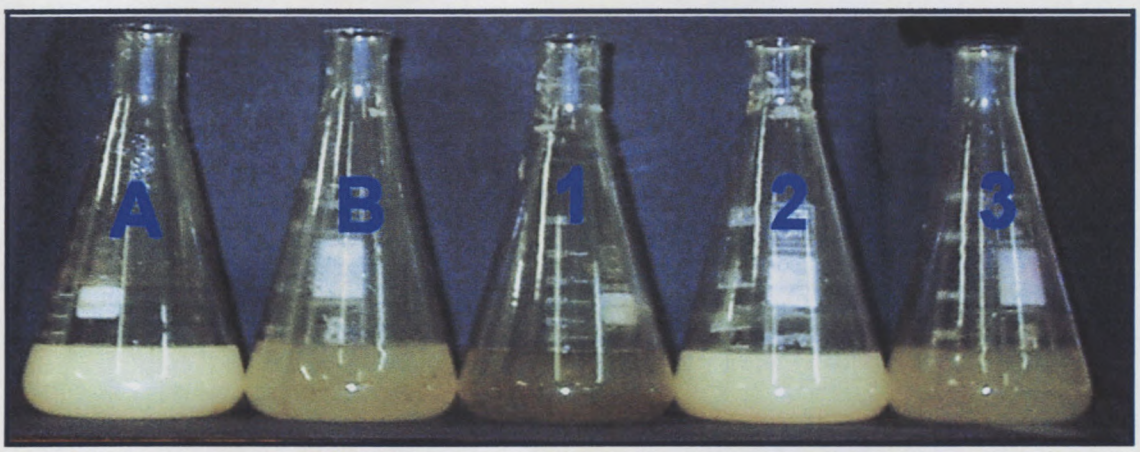

Figure 5.7: Batch tests showing A: control; B: control with ozone; 1: *Alternaria sp* with ozone; 2: *Mucor sp* 1 with ozone; 3: *Mucor sp* 2 with ozone after 7days

Figure 5.7 shows the control without ozone, the control with ozone and the three fungal isolates with ozone. It can be seen that for the flasks B, 1 and 3 the color of the effluent changed from the normal white to yellowish color to almost a transparent color. Flask 1 was the most transparent. Flask B and 3 were similar. The control (flask A), had no color change, and flask 2 had a slight color change.

5.3.2 Yeast Batch Tests

Figure 5.8 illustrates the organic degradation of the control and cultures without ozone. The COD concentrations had slight decreases. The control was reduced from an organic content of 2330 mg/L to 1915 mg/L, a reduction of only 17.81 %. The *Rhodosporidium sp* reduced the organic content from 23330 mg/L to 1935 mg/L, a reduction of 16.95 %, while the *Candida sp* reduced the COD to 1780 mg/L, a slightly higher removal of 23.61 %.

Figure 5.9 illustrates the organic content of the control and cultures with ozone. The organic content of the control declined to 1965 mg COD/L, a 15.67 % reduction. The *Rhodosporidium sp* had a slightly higher removal as compared to the last batch, which was 1872.5 mg COD/L by day 7, a 19.66 % reduction. The *Candida sp* reduced the organic content to 1945 mg COD/L, a 16.52 % reduction.

Figure 5.10 shows the oleic acid degradation by the *Rhodosporidium sp* with and without pretreatment. On the first day for both the batch tests, the oleic acid concentration was reduced to almost the same concentration (~ 1.4×10^7 ppm), after day 2 the concentration of the pretreated batch increased (1.88×10^7 ppm), while the concentration of the batch without ozonation continued to decline reaching 1.27×10^7 ppm on day 3.

Figure 5.11 shows the oleic acid degradation by the *Candida sp* with and without ozone. For this organism, the batch with ozone had a higher removal on day 1 (from 1.68×10^7 ppm to 1.47×10^7 ppm) than the batch without ozone. However after day 1 the concentration of the oleic acid increased to 2.33×10^7 ppm on day 3, while the batch without ozone had an increase on day 1 (1.97×10^7 ppm) but thereafter decreased in concentration to 1.23×10^7 ppm on day 3 from an initial concentration of 1.68×10^7 ppm.

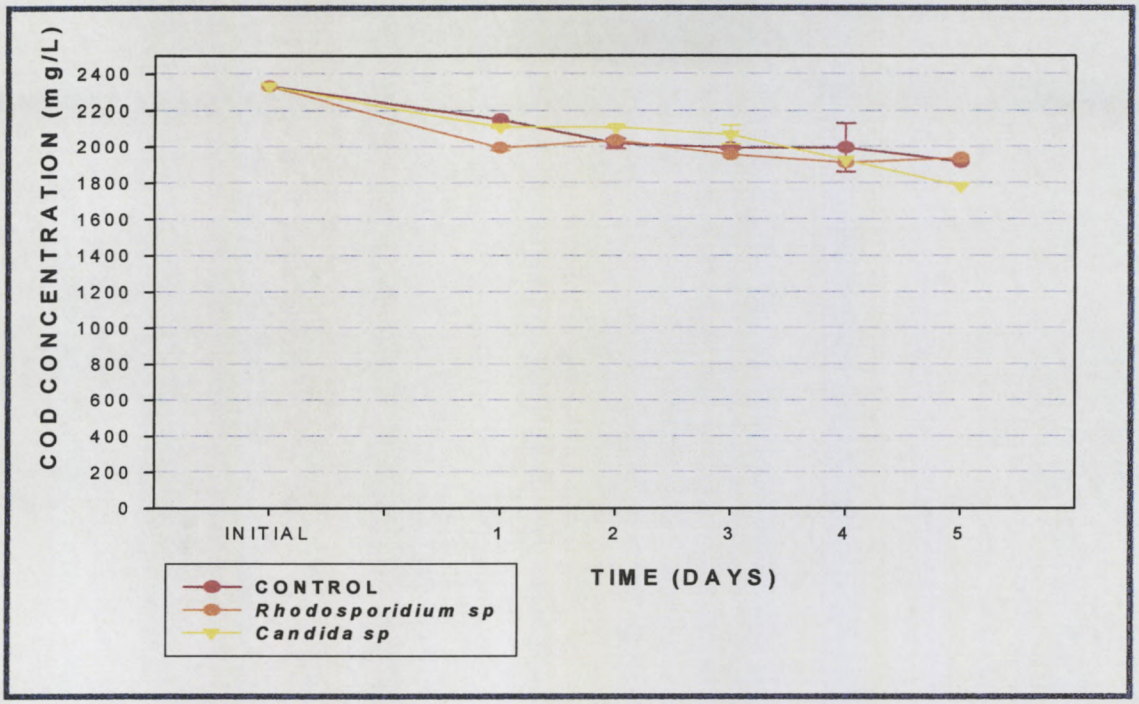

Figure 5.8: Mean COD profiles of control, *Rhodosporidium sp* and *Candida sp* without ozone

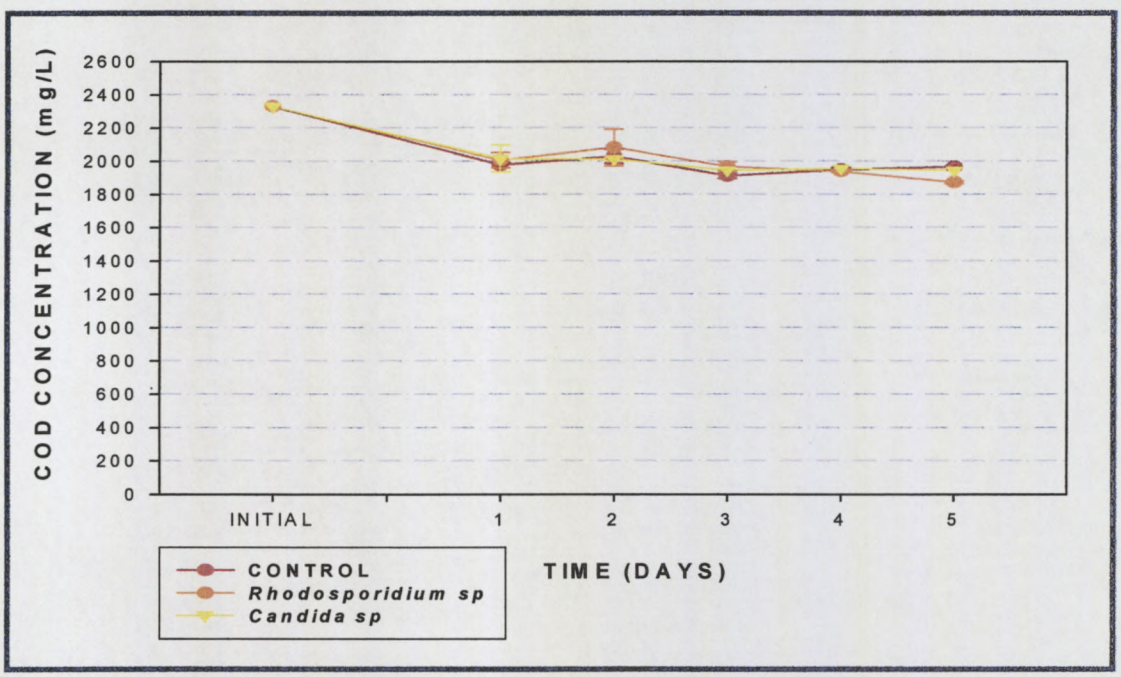

Figure 5.9: Mean COD profiles of ozonated batch tests with control, *Rhodosporidium sp* and *Candida sp*

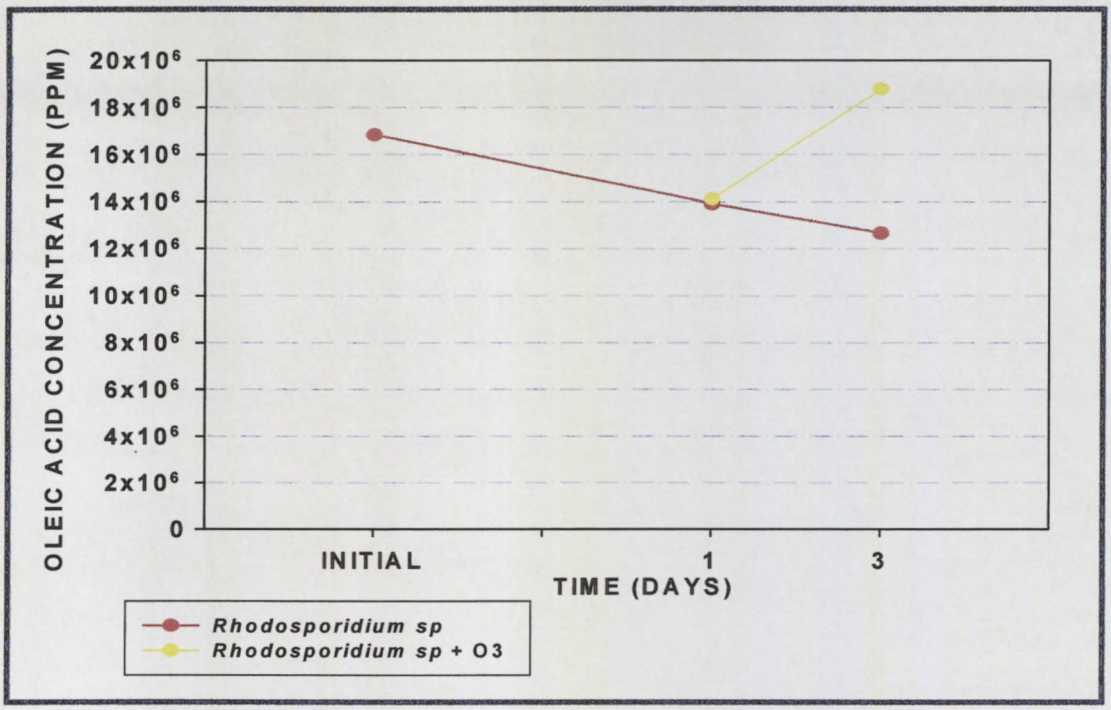

Figure 5.10: The degradation of oleic acid by *Rhodosporidium sp* with and without ozone

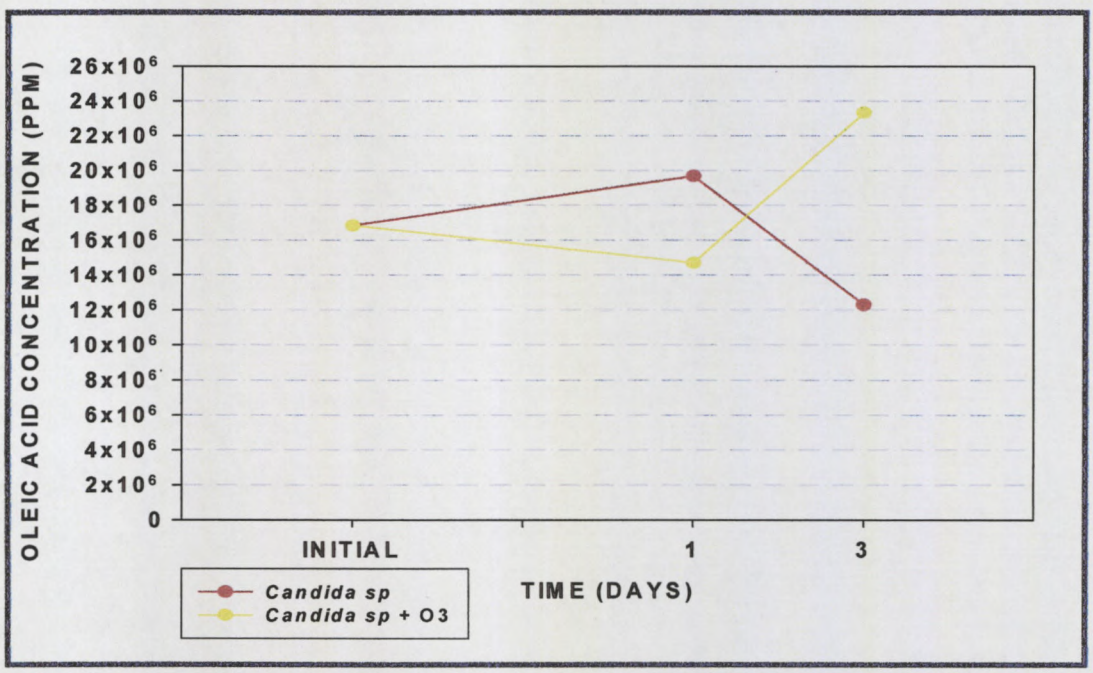

Figure 5.11: The degradation of oleic acid by *Candida sp* with and without ozone

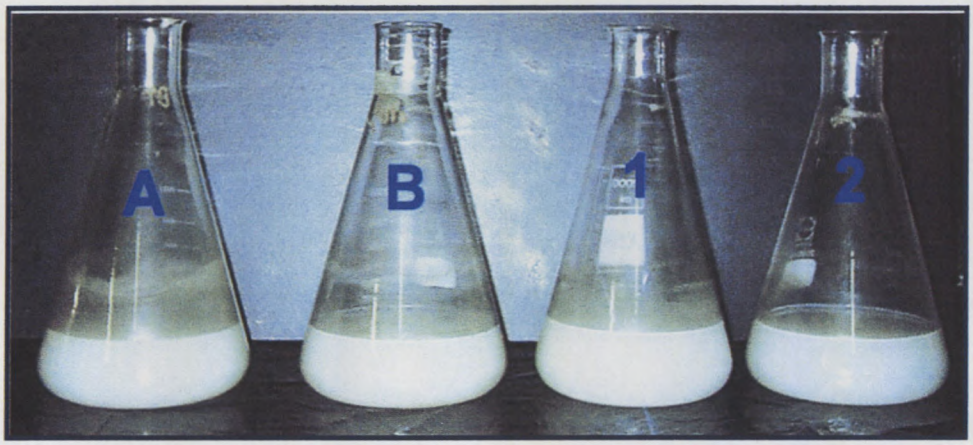

Figure 5.12: Batch tests showing A: control, B: control with ozone, 1:*Rhodosporidium sp* with ozone, 2: *Candida sp* with ozone

Figure 5.12 shows the batch test flasks for the control, the control without ozone and the two isolates with ozone. It can be seen that there was a change in the effluent color. This time instead of becoming a transparent yellow, it became more white in color after the 5 day period.

5.4 DISCUSSION

The carbonaceous material in an effluent can be divided in to two principle forms, i.e., the biodegradable and non-biodegradable. Each form can be further divided in to two principle fractions i.e., soluble and particulate. The readily (soluble) biodegradable material is rapidly utilised by microorganisms whilst the particulate fraction or slowly biodegradable COD is effectively absorbed but assimilated more slowly. The non-biodegradable particulate material cannot be hydrolysed by individual microorganisms (Lilley *et al.*, 1997, and Department of Natural Resources, 2001b).

Referring to figures 5.2 and 5.3 the fungal metabolism and figures 5.8 and 5.9 the yeast metabolism, the biodegradability is shown by the decrease in COD values.

For the fungal batch tests without ozone (fig. 5.2) and with ozone (fig. 5.3), the *Alternaria sp* degraded the highest COD concentration in both batch tests. However the degradation in the

ozonated batch was better than in the batch without ozone. As mentioned in the previous chapter the *Alternaria sp* has unique capabilities to breakdown FOG 's in the effluent efficiently under varying pH and temperature and that it has a high specificity for unsaturated fatty acids in an aqueous system i.e., their lipases reveal their highest activity at an oil-water interface. With this property this species can grow efficiently in the presence of long chain fatty acids in aqueous system. Also the lipase producing system of *Alternaria sp* could also be regarded as specific as a study done by Devnath (2001), observed that this organism grew poorly on fatty acid agar plates containing oleic acid as part of the medium, which was probably due to lack of an increased surface area of exposure to the fatty acid and lack of nutrient diffusion on the fatty acid agar plates.

Mucor sp 2, functioned more efficiently in the ozonated batch than in the batch without ozone. *Mucor sp* 2 also managed to degrade a higher amount of oleic acid in the ozonated batch as compared to the batch without ozone depicted in figure 5.6.

Ozonation is usually adopted for water disinfection but also has a high potential as pretreatment method. The characteristics of ozone is that it is rather selective towards double bonds. Theoretically; it would leave intact the proteins and the sugars of the effluent, which are biodegradable, and attack selectively the double bonds of unsaturated fatty acids and phenols (Andreozzi, *et al.*, 1998).

Mucor sp 2 could not utilise the unsaturated fatty acids in the effluent without ozone, but after ozonation, (which breaks double bonds in unsaturated fatty acids) made it easier for the *Mucor sp* 2 to assimilate the oleic acid. This also occurred for the other two organisms, but this only occurred after day 2. A probable reason for the *Alternaria sp* and *Mucor sp* 1 degrading the oleic acid late, was probably due to the organisms adjusting to the environment. Occasionally, prior to degradation of many organic compounds, a period whereby no destruction of the organic substrate or pollutant is observed but thereafter disappearance or rapid degradation of this pollutant or substrate occurs. This period of no destruction is referred to as an acclimation period (Alexander, 1999).

A number of fungi belonging to the *Mucor sp* have strong lipolytic activity when grown in the presence of triglyceride components, or oils, as an organic substrate (Akhtar *et al*, 1980).Also some reports show that extracellular lipase produced by *Mucor sp* is both constitutive and inducible, though the nature of the triglyceride inducing the lipase is specifically inhibited by its hydrolysis products (Nagaoka *et al*, 1969). The lipase produced by the *Mucor sp* depends largely upon the prevailing culture conditions such as pH and temperature of the growth environment. The optimum range of pH 6 to 8 is considered essential for lipase excretion. Significant lipolytic activity has been evident at pH 5 provided that the required substrate is available (Lazar & Schröder, 1992).

For the yeast batch tests (figs. 5.8 and 5.9), the organic degradation for the *Rhodosporidium sp* increased after ozonation. Hence ozonation made it easier for the *Rhodosporidium sp* to assimilate the constituents of the effluent. However for the *Candida sp*, the organic removal decreased after ozonation. A possible explanation could be that the ozone restricted the organism from assimilating the effluent.

An assumption made from works from Huss, (1908), and Harrisson, (1927) (cited in Rathledge, 1994), was that yeasts are able to reduce FOG levels in an edible oil effluent. They found that bacteria and yeasts were able to be cultivated on oils and fats. Also works by Pan, (1959),(cited by Rathledge 1994), showed that it was possible that vegetable oil could be utilized as a sole source of carbon and energy.

The results obtained shown in figures 5.10 and 5.11 show that the yeast cultures are capable of reducing the fatty acid concentration in the effluent. However the ozonation did not produce a significant change in oleic acid degradation for the *Rhodosporidium sp* (fig. 5.10). The concentration increased after day 1, this was probably due to the organisms dying and therefore releasing the internal lipid content of the cells causing an increase in oleic acid concentration. However the batch without pretreatment continued to degrade the oleic acid.

For the *Candida sp*, the initial oleic acid degradation was higher in the batch with ozone for day 1, but subsequently increased. However for the batch without ozone the concentration decreased after day 1. An explanation for this was the ozone could have inhibited the yeast cultures from degrading of the FOG content in the effluent.

Other studies have shown that the failure of microbes to use long chain fatty acids as a growth source could be attributed to their insolubility that makes it difficult to metabolise initially rather than because of its toxicity. However, growth by some microorganisms in the presence of long fatty acids has been observed when the pH of the growth medium is maintained between a pH of 5 to 8. It is considered that the composition of the growth medium, its pH, chain length of the fatty acid and inhibitory concentration of the fatty acids in the aqueous growth medium largely determines the microbial utilisation or breakdown of the fatty acid component (Rathledge, 1994).

The reason for the white color change of the effluent (Fig. 5.12), was due to the yeasts cultures acidifying the solution. Walker, (1998), found that yeasts acidify their growth medium through a combination of differential ion uptake and proton secretion during nutrient transport, hence the white color.

The following explanations could also explain poor FOG degradation for the fungal and yeast cultures:

- the available concentration or quantity of FOG present could attribute to poor microbial utilization of the FOG's. Rathledge, (1994), stated that if the FOG concentrations are too high, or even too low, it may inhibit the microorganisms.
- the viscosity and dispersal of oil and fats is another factor to be considered for successful microbial growth (Tan and Gill, 1985b; cited Rathledge, 1994). Rathledge, (1994) also stated that different agitation rates may be needed for different organisms. Wakelin and Forster, (1997), found that a high viscosity of FOG could result in poor FOG utilization. They found that high viscosity of castor oil resulted in it being less effectively dispersed in the culture medium.

- even though in this study the flasks were on a shaker, the flasks were not aerated. Oxygen is considered as an important parameter in microbial degradation potential. In this study oxygen was not added. This could explain why some organisms reduced FOG well and others did not. Lack of oxygen may have also affected yeast growth and respiration, as it is considered essential for microbial growth (Löser, *et al.*, 1998).
- the pH at which ozonation was carried could not have at the optimum. Research by Lalbahadur (2001), stated that a pH of 7 resulted in the ozonation procedure producing best results.

5.5 CONCLUSION

For the fungal batch tests, the *Alternaria sp* had substantial organic and lipid removal efficiencies as compared to *Mucor sp* 1 and 2.

Pretreatment with ozone reduced the organic content of the effluent but not significantly. Ozonation is not required for organisms such as the *Alternaria sp*, as this isolate displayed degradative capabilities without ozone. However ozone has the ability to aid certain organisms in degrading the organic and lipid content of edible oil effluent like *Mucor sp 2*. This organism functioned more efficiently in the ozonated batch test as compared to the batch without pretreatment.

For yeast batch tests, the pretreatment with ozone increased the organic removal efficiency for the *Rhodosporidium sp*, whereas the *Candida sp* organic removal efficiency decreased after pretreatment with ozone.

When determining the oleic acid degradation rates, it was found that ozone enhanced the removal efficiency in the *Candida sp*, while reducing the efficiency for the *Rhodosporidium sp.* However no conclusive results can be achieved from the oleic acid degradation as the degradation was only monitored for days 1 and 3 and not the entire duration of the experimental process.

It was noticed that the ozone enhanced the organic degradation capabilities of the Rhodosporidium sp while reducing the lipid degradation capabilities. The opposite occurred for the Candida sp, which illustrated poor organic degradation capabilities after ozonation but increased lipid degradation capabilities.

It can therefore be concluded that:

- ozone has the ability to aid organisms to degrade certain components of the effluent while restricting them from assimilating other components.
- ozone can be used to enhance biological treatment in certain organisms, under optimum conditions
- certain organisms (e.g., *Alternaria sp*) do not require pretreatment of the effluent in order to degrade the effluent, however in order for them to function efficiently the growth conditions need to be at the optimum.

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Considering the results achieved during this investigation the differing COD and FOG removal capabilities of the activated sludge, fungal and yeast isolates, suggested that the removal is likely to be affected by substrate specificity of the induced extra-cellular lipase, the physical and chemical characteristics of the substrate, the pH and the temperature of the culture medium.

Mkhize, (2002), stated that the edible oil effluent would require dilution with domestic wastewater prior to its subjection to activated sludge and that dilution of the edible oil effluent with domestic wastewater will ensure that the final characteristics are favorable for biological remediation techniques through balancing of the COD:N:P ratios. However in this study it was observed that with the procedure used the activated sludge has the ability to degrade the undiluted edible oil effluent also using the COD:N:P ratio of 100:5:1. The experimental procedures supplemented with nutrients maintained a more steady removal rate, whereas the experimental procedures without nutrients portrayed a decreasing efficiency as the latter end of the process was reached.

It was also deduced that with a higher temperature (31°C) the degradation efficiency was increased, but this increase was only efficient with the supplementation of nutrients, without nutrients at this temperature the degradation efficiency was reduced, i.e., an average removal of 91 % and 82 % was achieved with and without nutrients respectively. At the lower temperature (21°C), the degradation efficiency was similar with and without nutrients, i.e., an average removal of 87 % and 85 % was achieved with and without nutrients respectively.

When looking at the overall performance obtained from the pretreatment methods, the coagulation with DAF ultimately yielded more efficient results than ozonation.

Pretreatment with ferric chloride and DAF reduced the organic content, but is not required for certain like the Alternaria sp, as this organism has the ability to utilise the organic content without the aid of the pretreatment. Pretreatment with ozone reduced the organic content of the effluent but no significantly.

The *Alternaria sp* was the organism that removed the highest amount of organic and FOG concentrations. However further experimentation is required to determine whether this organism can withstand higher organic loads. For *Mucor sp* 2, which yielded more efficient results with ozone, further optimisation is required to increase the degradative capabilities of this organism. Also the lipase activity of both the *Mucor sp* are non-specific and those of the *Alternaria sp* is specific.

Comparison of the degradative potential for edible oil effluent showed the yeast displayed a lower potential for reducing COD and FOG loads as compared to the fungal isolates.

When comparing all three biological treatment methods, i.e., activated sludge, fungal cultures and yeast cultures, the activated sludge biological treatment process would be the treatment of choice. After tests conducted in this research project indicated that with the right conditions the activated sludge has the ability to degrade raw edible oil effluent and that the future requirements regarding the discharge quality of the edible oil wastewaters can be met. The contents of fat and easily degradable carbon compounds can be eliminated down to residual components.

It can therefore be concluded that the use of activated sludge to degrade long chain fatty acids in the sunflower oil effluent seems to be the best option as it performed more efficiently and effectively than the fungal and yeast isolates. Also the temperature, pH and pretreatment processes do affect microorganisms ability to utilize COD and FOG either simultaneously or individually.

6.2 RECOMMENDATIONS

- The recommended parameters of 21°C at pH 7, with nutrients added according to the C:N:P ratio of 100:5:1 offers the optimised controlled conditions for the removal of COD and FOG constituents in an edible oil effluent.

- Recommendations made that for the degradation of edible oil effluent using fungal isolates are a temperature of 31°C and pH of 5.

- A different pH or higher ozone demand may be required to enhance degradation by *Mucor sp* 2.

- The parameters for degradation of edible oil effluent using these yeast isolates are temperature of 31°C at pH 8. However it is recommended that other pH and further optimisation should be looked into for using these cultures.

- It is also recommended that the dissolved oxygen concentration should also be optimised.

- It was not conclusively found how pH, temperature and pretreatment parameters affected organism ability to utilize these substrates, in-depth studies need to be conducted in order to determine the exact relationship or effect these parameters has on COD and FOG utilization.

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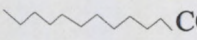
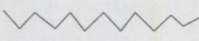
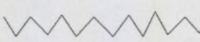
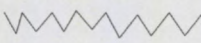
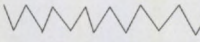
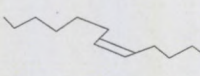
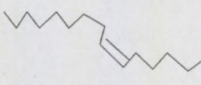
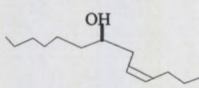
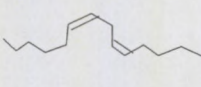
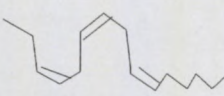
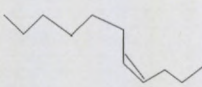
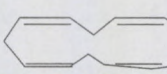
APPENDICES

APPENDIX 1

SOME COMMON NATURAL FATTY ACIDS (Palleros, 2000)

Number of carbons	Common name	Systematic name	Abbreviation	Structure	Melting point (°C)
			Saturated Fatty Acids		
12	lauric	dodecanoic	12:0		44
14	myristic	tetradecanoic	14:0		54
16	palmitic	hexadecanoic	16:0		63
18	stearic	octadecanoic	18:0		70
20	arachidic	eicosanoic	20:0		77
22	behenic	docosanoic	22:0		82
			Unsaturated Fatty Acids (all double bonds are cis)		
16	palmitoleic	9-hexadecenoic	16:1		-0.5
18	oleic	9-octadecenoic	18:1		4
18	ricinoleic	(R)-12-hydroxy-9-octadecenoic	18:1		6
18	linoleic	9,12-octadecadienoic	18:2		-12
18	linolenic	9,12,15-octadecatrienoic	18:3		-16
20	erucic	13-docosenoic	20:1		34
20	arachidonic	5,8,11,14-eicosatetraenoic	20:4		-50

APPENDIX 2

CHEMICAL OXYGEN DEMAND (COD)

Principle:

COD is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Due to its unique chemical properties, the dichromate ion ($Cr_2O_7^{2-}$) is the specified oxidant and is reduced to the chromic ion (Cr^{3+}). Both organic and inorganic components of a sample are subject to oxidation, but in most cases the organic component predominates and is of the greater interest. COD is a defined test; the extent of sample oxidation can be affected by digestion time, reagent strength, and sample COD concentration. COD often is used as a measurement of pollutants in wastewater and natural waters.

Apparatus:

- Thermoreactor (operating at 100 or 120°C)
- NOVA 60 Spectroquant
- Photometer tubes

For higher range (500 - 10 000 mg/L) (Method Number 024):

Sample	: 1mL
Solution A	: 2.2mL
Solution B	: 1.8mL

For middle range (100 - 1500 mg/L) (Method Number 023):

Sample	: 3mL
Solution A	: 0.3mL
Solution B	: 2.3mL

APPENDIX 2 continued...

For the lower range (10 - 150 mg/L) (Method Number 014):

Sample	: 3mL
Solution A	: 0.3mL
Solution B	: 2.85 mL

Preparation:

The procedure is the same for all three ranges:

Add the sample in to a photometer tube. Then add the Solution A and mix. Finally add Solution B and mix. Put tubes in thermoreactor for 2 hours at 100°C. Remove from thermoreactor and let tubes cool to room temperature before measuring COD concentration in NOVA 60 spectrophotometer.

APPENDIX 3

TOTAL SUSPENDED SOLIDS (TSS) DETERMINATION

Principle:

A well mixed sample is filtered through a weighed standard glass-fiber filter and the residue that is retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids (Standard Methods, 1998).

Apparatus:

- Glass Fibre Filters (pore size of 0.45μm)
- Desiccator
- Drying oven, for operation at 103 to 105°C
- Analytical balance, capable of weighing to 0.1mg
- Graduated measuring cylinder
- Filtration apparatus
- Membrane filter apparatus
- Aluminum weighing dish (if not available foil is used)
- Beaker

Procedure:

a) *Preparation of glass-fiber filter disk:* Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20mL portions of distilled water. Continue suction until all traces of water is removed, then turn off vacuum and discard washings. Remove filter from filtration apparatus and place on aluminum weighing dish. Dry in an oven at 103 to 105°C for 1 hour. Cool in dessicator to balance temperature and weigh. Store in dessicator until needed.

APPENDIX 3 continued...

b) *Sample analysis*: Put pre-treated and pre weighed filter in filtering apparatus. Turn vacuum pump on and add small volume of distilled water to seat filter. Mix sample in properly beaker and add 50mL of this to measuring cylinder. Add to filter. Wash filter with three successive 10mL volumes of distilled water. Continue suction until all washings are gone through. Carefully remove filter and place on aluminum weighing disk and dry in oven at 103 to 105°C for 1 hour. Cool in desiccator to balance temperature and weigh.

Calculation:

$$\text{mg total suspended solids/L} = \frac{(A - B) \times 1000}{\text{Sample volume, mL}}$$

where: A = weight of filter + dried residue, mg, and
B = weight of filter, mg.

APPENDIX 4

FATS, OILS AND GREASE (FOG) DETERMINATION

Principle:

In the determination of oil and grease, an absolute quantity of a specific substance is not measured. Instead, groups of substances with similar physical characteristics are determined quantitatively on the basis of their common solubility in an organic extracting solvent. "Oil and grease" is defined as any material recovered as a substance soluble in the solvent. It includes other material extracted by the solvent from an acidified sample and not volatilized during the test.

Apparatus:

- Separatory funnel
- Bulb or flat bottomed flask
- Funnel
- Filter paper
- Heating mantle
- Distillation unit
- Desiccator

Reagents

- Hydrochloric acid or sulfuric acid, 1:1: mix equal volumes of either acid and distilled water
- n-Hexane, boiling point 69°C
- Sodium sulfate, Na_2SO_4, anhydrous crystal

Procedure:

A sample volume of 30mL was used. The sample was acidified with HCl to pH 2 lower. The sample was then transferred to the separatory funnel. The container where the sample was kept was rinsed with 30mL of hexane and added to the separatory funnel. The funnel was shaken vigorously for 2

minutes. The layers were allowed to separatory. The bottom layer which was the sample was drained in to the original container. The top organic layer was drained through a funnel containing a filter paper and 10g Na_2SO_4, both have been solvent rinsed, into a clean, tared bulb flask. The extraction was done twice more with 30mL of solvent each time. The extracts were drained in the tared flask and the filter and Na_2SO_4 were rinsed with 10 to 20mL of solvent. Distillation from flask in a heating mantle was then carried out thereafter. A drip tip was connected to the end to of distillation apparatus to collect the solvent. When the solvent condensation came to an end the flask was removed and cooled in a desiccator. The flask was then weighed.

Calculation:

If the organic solvent is free from residue, the gain in weight of the tared distilling flask is due to oil and grease.

$$\text{mg oil and grease/L} = \frac{(A - B) \times 1000}{\text{mL sample}}$$

where: A = Total gain in weight of tared flask

B = Residue from solvent blank

APPENDIX 5

pH DETERMINATION

Principles:

Measurement of pH is a frequently used test in water chemistry. Almost every phase of water supply and wastewater treatment, e.g., acid-base neutralisation, water softening, precipitation, coagulation, disinfection, and corrosion control, is pH-dependant. pH is used in alkalinity and carbon dioxide measurements and many other acid-base equilibria. At a given temperature the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity.

Apparatus:

– Beckman 50 pH meter consisting of pH probe and temperature probe

Procedure:

a) *Instrument calibration:* The pH and Temperature probes were submerged in a pH 4 buffer solution, so as to calibrate the pH to a pH of 4. Both probes were then submerged in a pH 7 buffer solution, to calibrate the meter to a pH of 7. After each calibration, the pH and temperature probes were rinsed with distilled water.

b) *Measurement of pH:* the pH and temperature probes were place in the sample. The pH button on the meter pressed. When a fixed reading was obtained, this was regarded as the true pH of the sample. Both the probes were then rinsed using distilled.

APPENDIX 6

MIXED LIQUOR SUSPENDED SOLIDS (MLSS) DETERMINATION

Principle:

MLSS is defined as the total amount of organic and mineral suspended solids contained in the mixed liquor of the activated sludge reactor. This value offers the system operator a crude measure of the biomass contained within the process.

Apparatus:

- Centrifuge tubes (50mL)
- Centrifuge (capable of 3 000rpm)
- Crucibles
- Drying oven (operating at 103 to 105°C)
- Desiccator

Procedure:

A sample volume of 50 mL was added into a centrifuge tube. Tube was then centrifuged at 3 000 rpm for 6 min. The supernatant was discarded and the sludge pellet was quantitatively scooped into a pre-weighed crucible. The crucible was placed in the drying oven at 103 to 105°C and left overnight to dry. After 24h it was removed from oven and placed in desiccator. The cooled crucible was then re-weighed.

Calculation:

$$\text{mg MLSS/L} = \frac{(A - B) \times 1000}{\text{Sample volume, Ml}}$$

where: A = mass of crucible + sludge

B = mass of crucible

APPENDIX 7

VOLATILE SUSPENDED SOLIDS DETERMINATION

Principle:

The residue from the MLSS is ignited to a constant weight at 550°C. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

Apparatus

- Muffle furnace (operating at 550°C)
- Crucible with residue from MLSS determination.
- Desiccator

Procedure:

Once the MLSS determination is complete, the crucible is then place in muffled furnace at 550°C for 1 hour. The crucible is then place in a desiccator and cooled. The crucible is then re-weighed.

Calculation:

$$\text{mg VSS/L} = \frac{\text{A - B) x 1000}}{\text{Sample volume, mL}}$$

where: A = weight of residue + crucible before ignition, mg

B = weight of residue + crucible after ignition, mg.

APPENDIX 8

MICROSCOPIC EVALUATION OF FLOCS AND PROTOZOA

DATE: **ZONE:**

PROTOZOA - ROTIFERS - NEMATODES - AMOEBA

Ciliates		COUNTS	*Flagellates*		COUNTS
Carchesium			Bodo		
Epistylus			Hexamitus		
Opercularia			Monosiga		
Vorticella			Pleuromonas		
Aspidisca			Trepomonas		
Blepharisma					
Chilodonella			*Amoeba*		
Colpidium			*Thecamoebe*		
Euplotes			*Heliozoa*		
Lionutus			*Rotifers*		
Paramecium			*Nematodes*		
Spirostomum					
Trachelophylum					

MORPHOLOGY OF THE FLOC

FIRMNESS AND SHAPE OF FLOC	**STRUCTURE**		**SIZE**			**Various Features**
	Open	Compact	Small	Med	Large	
Firm, rounded Firm, irregular Weak, rounded Weak, irregular Agglomerates			S			Diversity Zooglea Free Cells Spirochetes Spirits Organic Fibres
Absent: -; Few: +; Moderate: ++; Many: +++ Remarks:						

APPENDIX 9

IDENTIFICATION OF FILAMENTOUS BACTERIA (adapted from Eikelboom & Buijsen, 1983).

NAME OF ZONE	DATE	FILAMENTOUS ORGANISMS								COUNTS
		1	2	3	4	5	6	7	8	
BRANCHING	real									
	absent									
	false									
MOTILITY										
MANY S GRANULES	in vivo									
	After S-test									
SEPTA	Clearly visible									
SHAPE OF FILAMENTS	straight									
	bent									
	coiled									
NEISSER STAIN	positive									
	negative									
GRAM STAIN	positive									
	negative									
DIAMETER FILAMENTS	<1.0 µm									
	1.0 - 2.0									
	>2.5 µm									
SHAPE OF CELLS	coccus									
	rods									
	square									
	rectangular									
SHEATH	present									
ATTACHED GROWTH										
- : ABSENT; +:FEW; ++: MODERATE; +++: LOTS/SUBSTANTIAL										

APPENDIX 10

SURVEY OF THE CHARACTERISTICS OF THE FILAMENTOUS MICROORGANISMS

(Adapted from Eikelboom & Buijsen, 1983)

	PHASE CONTRAST MICROSCOPE; 700–1000 x															BRIGHT FIELD			
	branching	motility	shape filaments: straight or slightly bent	shape filaments: coiled or twisted	length filaments <200 µm	attached growth of unicellular organisms	sheath present	crosswalls clearly visible	diameter cells: < 1.0 µm	diameter cells: 1.0–2.2 µm	diameter cells: > 2.5 µm	rectangular or square cells	cells rounded (ovoid, spherical, disc-shaped or rod-shaped)	sulfur deposited in the cells: in vivo	sulfur deposited in the cells: after applying the S-test	PHB9) granules present	Gram stain: positive	Gram stain: negative	Neisser stain: positive	Neisser stain: negative
Beggiatoa		+	+	+	±			+3)	+	+	+	+3)		+	+			+		+
"Cyanophyceae"		±	+				?	+			+	+					±	+		+
Flexibacter		+	+	+	+			±	+	+		+	+					+		+
Fungi	+		+					+		+	+	+					4)	4)		+
H.hydrossis			+		+	+	+		+			∞	∞					+		+
M.parvicella				+	+	+			+			∞	∞				+		+6)	
Nocardia	+			+	+				+			∞	∞				+			+
N.limicola I			+	+	+				+				+				+		+7)	
N.limicola II				+	+			+	±	+			+				+		+7)	
N.limicola III				+	+			+		+			+				+		+7)	
S.natans	+1)		+			+	+	+		+			+			±		+		+
Thiothrix	2)		+		+		±	+3)	+	+		+3)	+3)	±	+			+		+
Type 0041			+		±	+	+	+	±	+		+			+8)		+	±		+
Type 0092			+		+				+			∞	∞					+	+7)	
Type 021N	2)		+		+			+	±	+		±	+		±8)			+		+
Type 0581				+	+				+			∞	∞					+		+
Type 0803			+		±			+	+			+					+	+	±6)	+
Type 0914			+		+			+3)	+			+3)		+			+			+
Type 0961			+				?	+		+		+						+		+
Type 1701	±1)		+	+	+	+	+	+	+				+			±		+		+
Type 1851			+		±	+	+	±	+			∞	∞				+5)			+
Type 1863			+	+	+			+	+				+					+		+

± = sometimes; ? = unknown; ∞ = cell shape usually not visible by phase contrast microscopy;

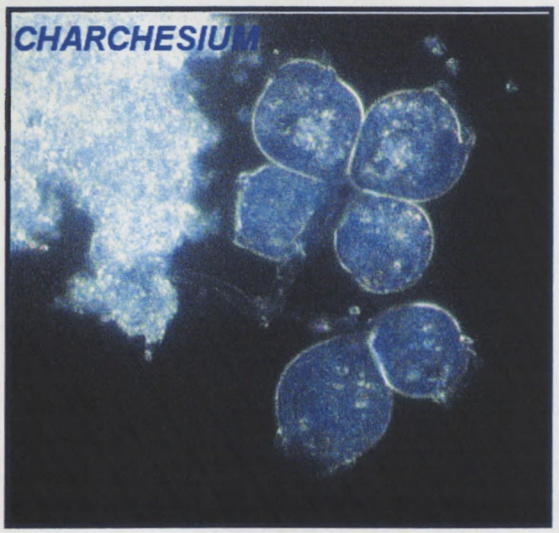
1) falsely branched; 2) sometimes formation of rosettes; 3) only visible after removal of the sulfur inclusions

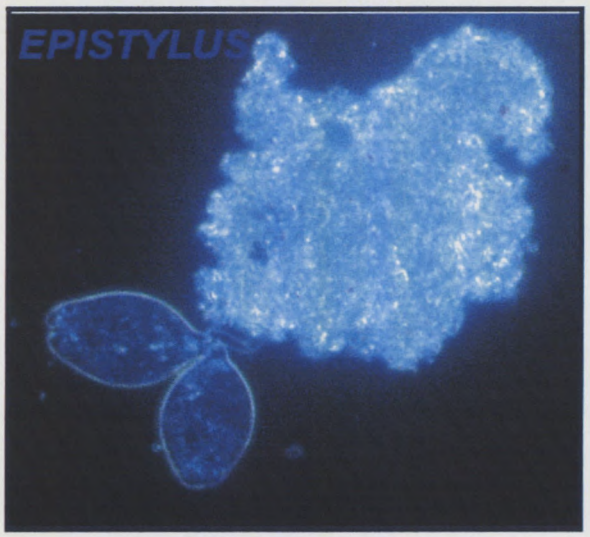
4) Fungi do not stain by the Gram stain; 5) faintly; 6) granules; 7) filaments grey-blue; 8) small granules;

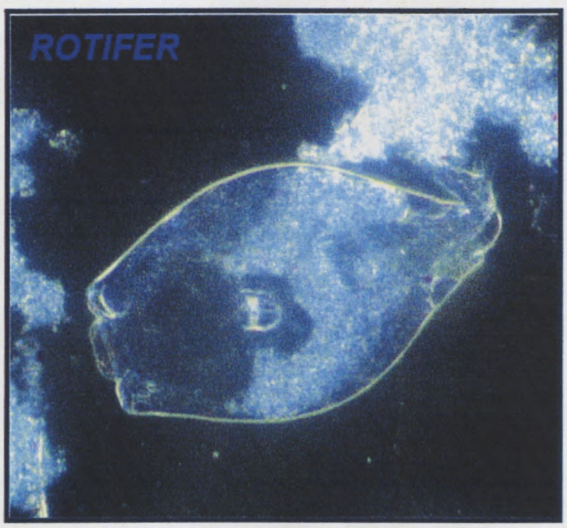
9) poly-β-hydroxy butyric acid

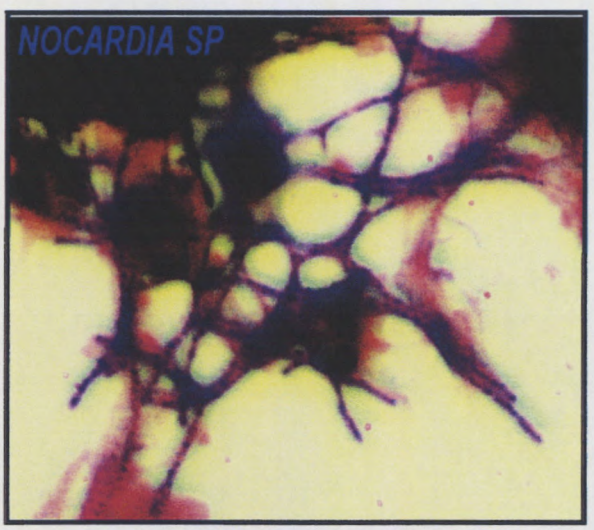
APPENDIX 11

IMAGES OF PROTOZOA AND FILAMENTOUS ORGANISMS FROM THE ACTIVATED SLUDGE PROCESS









Gram Stain

APPENDIX 12

PREPARATION OF GRAM STAIN, MODIFIED HUCKER METHOD

Solution 1: Prepare the following separately, then combine

A		B	
Crystal Violet	2 g	Ammonium oxalate	0.8 g
Ethanol, 95%	20 mL	Distilled water	80 mL

Solution 2:

Iodine	1 g
Potassium iodide	2 g
Distilled water	300mL

Decolorisation Solution

Ethanol, 95%

Solution 3:

Safranin O (2.5% w/v in 95% ethanol	10 mL
Distilled water	100 mL

Procedure:

1. Prepare thin smears on microscope slides and thoroughly air dry (do not heat fix)
2. Stain 1 min with Solution 1; rinse 1 sec with water
3. Stain 1 min with Solution 2; rinse well with water
4. Hold slide at an angle and decolorize with 95% ethanol added drop by drop to smear for exactly 25 sec. Do not over decolorize. Blot dry.
5. Stain with Solution 3 for 1 min; rinse well with water and blot dry.
6. Examine under oil immersion at 1000x magnification with direct illumination (not phase contrast): blue-violet is positive; red in negative.

APPENDIX 13

PREPARATION OF THE NEISSER STAIN:

Solution 1: Separately prepare and store the following solutions

A		B	
Methylene Blue	0.1 g	Crystal violet (10% w/v in 95% ethanol	3.3 mL
Ethanol, 95%	5 mL	Ethanol, 95%	6.7 mL
Acetic acid, glacial	5 mL	Distilled water	100 mL
Distilled water	100 mL		

Mix 2 parts by volume of A and 1 part by volume of B

Solution 2:

Bismark Brown, $C_{18}H_{18}N_{18}$ (1% w/v aqueous)	33.3 mL
Distilled water	66.7 mL

Procedure:

1. Prepare thin smears on microscope slides and thoroughly air dry (do not heat fix).
2. Stain 30 sec with Solution 1; rinse 1 sec with water.
3. Stain 1 min with Solution 2; rinse well with water; blot dry
4. Examine under oil immersion at 1000x magnification with direct illumination (not phase contrast): blue-violet is positive (either entire cell or intracellular granules); yellow-brown is negative.

APPENDIX 14

PREPARATION OF POLYHYDROXYBUTYRATE (PHB) STAIN

Solution 1:

Sudan Black B (IV), 0.3% w/v in 60%

Solution 2:

Safranin O, 0.5% w/v aqueous solution.

Procedure:

1. Prepare thin smears on a microscope slide and thoroughly air dry.
2. Stain 10 min with Solution 1; add more stain if the slide starts to dry out.
3. Rinse 1 sec with water
4. Stain 10 sec with Solution 2; rinse well with water; blot dry.
5. Examine under oil immersion at 1000x magnification with direct illumination (not phase contrast): PHB granules will appear as intracellular, blue-black granules, while the cytoplasm will be pink or clear.

APPENDIX 15

TOTAL NITROGEN DETERMINATION

Principle:

Organic and inorganic nitrogen compounds are transformed into nitrate according to Koroleff's method by treatment with an oxidizing agent in a thermoreactor. In concentrated sulfuric acid, this nitrate reacts with a benzoic acid derivative (Nitrospectral) to form a deep red nitro compound that is determined photometrically.

The TN was determined in order to established the C:N:P ratio, which was 100:5:1. This value was then used to supplement the effluent with Ammonium Chloride as a source of nitrogen.

Apparatus:

- Total Nitrogen Cell Test Kit (Number: 1.14537.0001)(Range:0.5 - 15.0 mg/L N)
- Thermoreactor (operating at a100 or 120°C)
- NOVA 60 Spectroquant
- Empty cell

Reagents in Kit

- 1 bottle of reagent N-1K: contains Potassium peroxodisulfide and potassium carbonate
- 1 bottle of reagent N-2K: contains Sodium Hydroxide
- 1 bottle of reagent N-3K

Procedure:

a) *Decomposition of sample:* 10 mL of sample was pipetted in to an empty cell. 1 level blue microspoon of reagent N-1K was added to the cell and mixed. 6 drops of reagent N-2K was added and mixed. The cell was then place in a thermoreactor at 120°C for 1 hour. The cell was then allowed to cool to room temperature.

b) *Preparation of measurement sample:* 1 level blue microspoon of reagent N-3K was added to the cooled cell and the cell closed tightly and shaken vigorously for 1 min. 1.5 mL of the

decomposed sample was added carefully down the side of a tilted reaction cell. The cell was once again closed tightly and shaken vigorously. The hot cell was then left to stand for 10min. After this duration measurement of the TN was done using a NOVA 60 Spectroquant.

APPENDIX 16

TOTAL PHOSPHATE DETERMINATION

Principle:

In a solution acidified with sulfuric acid, orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMG), which is then determined photometrically.

The TP was measured in order to established the C:N:P ratio, which was 100:5:1. This value was then used to supplement the effluent with Potassium Di-hydrogen Orthophosphate.

Apparatus:

- Total Phosphate Cell Test Kit (Number: 1.14729.0001) (Reagent 0.5 - 25.0 mg/L PO_4-P)
- Thermoreactor
- NOVA 60 Spectroquant

Reagent:

- 1 bottle of reagent P1-K contains: Potassium peroxodisulfate
- 1 bottle of reagent P-2K
- 1 bottle of reagent P-3K

Procedure:

a) *Digestion of sample:* 1 mL of sample was pipetted in to a reaction cell and 1 dose of reagent P-1K was added. The cell close tightly and mixed. The cell was then place in a thermoreactor at 120°C for 30 min. The cell was then allowed to cool to room temperature in a cell rack.

b) *Preparation of measurement sample:* 5 drops of reagent P2-K was added and mixed, followed by 1 dose of reagent P-3K. The cell was then closed tightly and mixed vigorously until the reagent was complete dissolved. The cell was then left to stand for 5 min. Thereafter the cell was wiped clean and measured in the NOVA 60 Spectroquant.

APPENDIX 17

DETERMINATION OF SLUDGE VOLUME (SV30) AND SLUDGE VOLUME INDEX (SVI)

SV30

Principle:

The settled sludge volume of a biological suspension is useful in routine monitoring of biological processes. For the activated sludge, a 30-min settled sludge volume has been used to determine the return sludge floe rate and when to waste sludge. The 30-min settled sludge volume is also used to determine sludge volume index.

Apparatus:

- Settling column: A 1L graduated cylinder
- A stopwatch

Procedure:

A 1L sample was placed in the settling column. The column was inverted to distribute the solids by covering the top and inverting the cylinder. At the end of 30 min the volume of the settled sludge is determined.

SVI

Principle:

The SVI is the volume in milliliters occupied by 1 g of a suspension after 30 min settling. SVI is used to monitor settling characteristics of activated sludge and other biological suspensions.

Procedure:

Determine the 30 min settled sludge volume (method mentioned above).

Calculations:

$$SVI = \frac{\text{settled sludge volume (mL/L)} \times 1000}{\text{Suspended solids (mg/L)}}$$

APPENDIX 18

EFFLUENT QUALITY FROM AN EDIBLE OIL PROCESSING PLANT (adapted from Steffen, *et al.*, 1989)

Average monthly effluent discharge (m^3)	**pH**	**COD (mg/L)**	**SOG (mg/L)**
Range	Range	Range	Range
2 180 - 8 200	1.8 - 10.5	1 100 - 8 990	80 - 1 360
Mean	Mean	Mean	Mean
5 130	5.9	4 580	630

APPENDIX 19

PREPARATION OF NUTRIENT AGAR PLATES

Composition	g/L
Meat Extract	1.0
Peptone	5.0
Yeast Extract	2.0
Sodium chloride	8.0
Agar	15.0

Preparation:

1. Suspend 31 g in 1 L distilled water
2. Bring to the boil
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. When almost cool, pour in to petri dishes and allow gel to form.

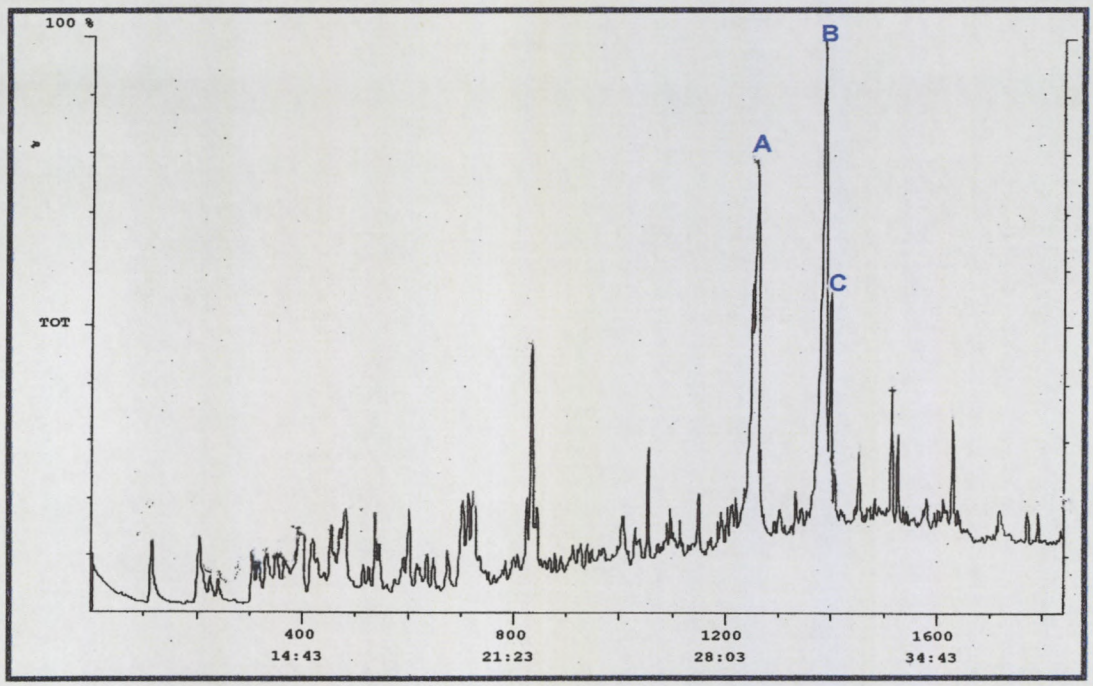
Contamination check:

1. One loopfull of the sample was streaked on the plate
2. The plate was then incubated at 31°C for 48 hours.

APPENDIX 20

GCMS GRAPH OF RAW EFFLUENT

A: Palmitic Acid, B: Oleic Acid, C: Stearic Acid



GC CONDITIONS

		FINAL TEMPERATURE	HOLDING TIME (MINS)
Column Type	**DB5-MS**		
Oven Initial Temperature	**50**	**50**	**5**
Ramp1	**20**	**160**	**0**
Ramp 2	**6**	**250**	**0**
Injector temperature	**250**		
Helium flow rate	**Constant velocity 40cm/sec**		

MS CONDITIONS

Mass Range	**50 - 450**
Transfer line temp	**275**
MS Acquire start time	**6**
Ion source temp	**200**

APPENDIX 21

PREPARATION OF FERRIC CHLORIDE ($FeCl_3$)COAGULANT (Pryor and Freese, 1998)

- 0.2 mL of 43 % $FeCl_3$ was pipetted into a 100 mL volumetric flask and was made up to the 100 mL mark with distilled water.
- When added to an 800 mL effluent sample, 1 mL of the $FeCl_3$ solution yielded 1 mg/L (1ppm) of $FeCl_3$.
- This solution was prepared fresh whenever required, since occurred causing a color change from yellow to cloudy orange.

APPENDIX 22

PREPARATION OF SABOURAUD DEXTROSE AGAR

1. 60 g of Biolab Sabouraud Dextrose Agar powder was weighed out using a top balance
2. This was then put in to a 1 L conical flask and 1 L of distilled water was added
3. The contents of the flask was then boiled until the agar dissolved
4. The contents was then poured in to Schott bottle and sterilised by autoclaving at 121°C for minutes.
5. When almost cooled the media was then poured in to the petri dishes and left to solidify.

APPENDIX 23

PREPARATION OF MALT EXTRACT AGAR

1. 17 g of the Malt Extract Agar was weighed out and put in to a conical flask.
2. 1 L of distilled water was added to dissolve the agar
3. The contents of the flask was then boiled until all the agar dissolved
4. The contents were then poured in to a 1 L Schott bottle and sterilised by autoclaving at 121°C for 15 minutes.
5. Subsequent to autoclaving the contents were cooled and poured in petri dishes and allowed to solidify.

APPENDIX 24

PREPARATION OF THE NUTRIENT SOLUTION (Griffin, 1994)

1. 1 L of distilled was added to a conical flask
2. The following constituents were weighed out on an analytical balance and then added to the distilled water:
   2 g NH_4CL; 1 g KH_2PO_4; 0.2 mg $FeCl_3$; 0.2 mg $MnCl_2$; 0.2 mg $ZnCl_2$; 0.005 mg biotin, 0.1 mg thiamine; and 0.5 g $MgSO_4.7H_2O$.

3. The medium was transferred to a schott bottle and sterilised by autoclaving at 121°C for 15 minutes.

Double strength solution: add two times the amount of nutrients in one liter

The solution was then autoclaved at 121°C for 15 minutes. The cooled solution was then used to dilute the effluent as required.

APPENDIX 25

PREPARATION OF METHYL ESTERS OF LONG-CHAIN FATTY ACIDS

Reagents:

- ❑ 0.5 M NaOH in Methanol Solution
- ❑ Boron triflouride - methanol (BF_3) complex
- ❑ Hexane
- ❑ Saturated Sodium Chloride Solution

Apparatus:

- ❑ 50 mL volumetric flask
- ❑ Glass beads
- ❑ Water bath set at 70°C
- ❑ 2 mL amber crimp vials
- ❑ Crimper

Preparation:

1. 0.5 mL was massed in to the volumetric flask with 1 to 2 glass beads
2. 6 cm^3 of 0.5 M NaOH in methanol solution was added
3. The solution was heated for 10 minutes and then cooled
4. 7 cm^3 of BF_3 was added and then heated for 2 minutes
5. 3 cm^3 of hexane was added and boiled for 1 minute
6. The solution was cooled and about 15 cm^3 of the saturated sodium chloride was added and the flask stoppered and shaken vigorously.
7. More saturated sodium chloride was added until the hexane came to the neck of the flask.
8. The top hexane was then pipetted in to the 2 mL crimp vials and crimped.

APPENDIX 25 continued ...

GC CONDITIONS

		FINAL TEMPERATURE	HOLDING TIME (MINS)
Column Type	**DB5-MS**		
Oven Initial Temperature	**50**	**50**	**5**
Ramp1	**20**	**160**	**0**
Ramp 2	**6**	**250**	**0**
Injector temperature	**250**		
Helium flow rate	**Constant velocity 40cm/sec**		

APPENDIX 26

IODOMETRIC METHOD I (Standard Methods, 1998)

Preparation of Reagents

- Potassium Iodide (KI) Solution
  - 20 g of KI crystals were added to a 5 L volumetric flask, which was, subsequently filled to the mark with distilled water
  - Potassium dichromate ($K_2Cr_2O_7$) solution
  - 4.8994 g of anhydrous potassium dichromate was dissolved in distilled water and made up to the 1 L mark.
  - The molarity of the solution was calculated using the following equations:

$$\text{No. of moles} = \frac{\text{mass(m)}}{\text{Molar mass (M)}} \quad \ldots (1)$$

$$\text{Molarity} = \frac{\text{no. moles}}{\text{Volume (v)}} \quad \ldots (2)$$

- Starch Indicator Solution
  - Cold water was added to 5 g of starch to produce a thin paste, which was ground in a mortar.
  - The paste was added to 1 L of boiling distilled water, stirred and allowed to settle overnight.
  - The clear supernatant was collected and used

APPENDIX 26 continued ...

- Sodium Thiosulphate
  - 25 g of sodium thiosulphate was added to 1 L of freshly boiled distilled water.

The Standardisation of Sodium Thiosulphate using the Dichromate Method

- 1 mL of concentrated sulphuric acid, 10 mL of $K_2Cr_2O_7$ and 1 mL KI were added to 80 mL of distilled water with constant stirring.
- the above mixture was allowed to stand for 6 minutes in the dark
- this was titrated against sodium thiosulphate, until the yellow color was discharged.
- 1 mL of starch indicator solution was added and titration continued until the blue color disappeared
- the volume of the titrant was recorded and inserted in to the following equation:

$$C_1 V_1 = C_2 V_2 \quad \ldots \quad (3)$$

Where: C_1 and C_2 = the concentrations of the sodium thiosulphate and the $K_2Cr_2O_7$ respectively.

V_1 and V_2 = the volumes of the sodium thiosulphate and the $K_2Cr_2O_7$ respectively.

Calculation:

- Calculation of the concentration of sodium thiosulphate:
  - Sodium thiosulphate : 25 g in 1 L
  - $K_2Cr_2O_7$: Mass of sample = 4.8994 g

    Molar mass = 294.1846 g/mol

    Volume of sample = 1 dm^3

    Using equation 1 : The number of moles of $K_2Cr_2O_7$ = 1.6654×10^{-2}

Using equation 2 : The molarity of $K_2Cr_2O_7$ = 0.01666 mol. dm^{-3}

The normality of the $K_2Cr_2O_7$ = 0.1 N.

- Concentration of Sodium thiosulphate

Using equation 3 : C = 0.1020 N.

- Calculation of standard curve:

a. The amount of $Na_2S_2O_3$ consumed per minute

TIME	$Na_2S_2O_3$ (mL)
1	0.2
2	0.4
3	0.4
4	0.6
5	0.6
6	1.0
7	1.0
8	1.2
9	1.4
10	1.6

b. The amount of ozone consumed per minute

- The results from the previous table were placed in the following equation to determine the amount of ozone in mg/L consumed in the corresponding minutes.

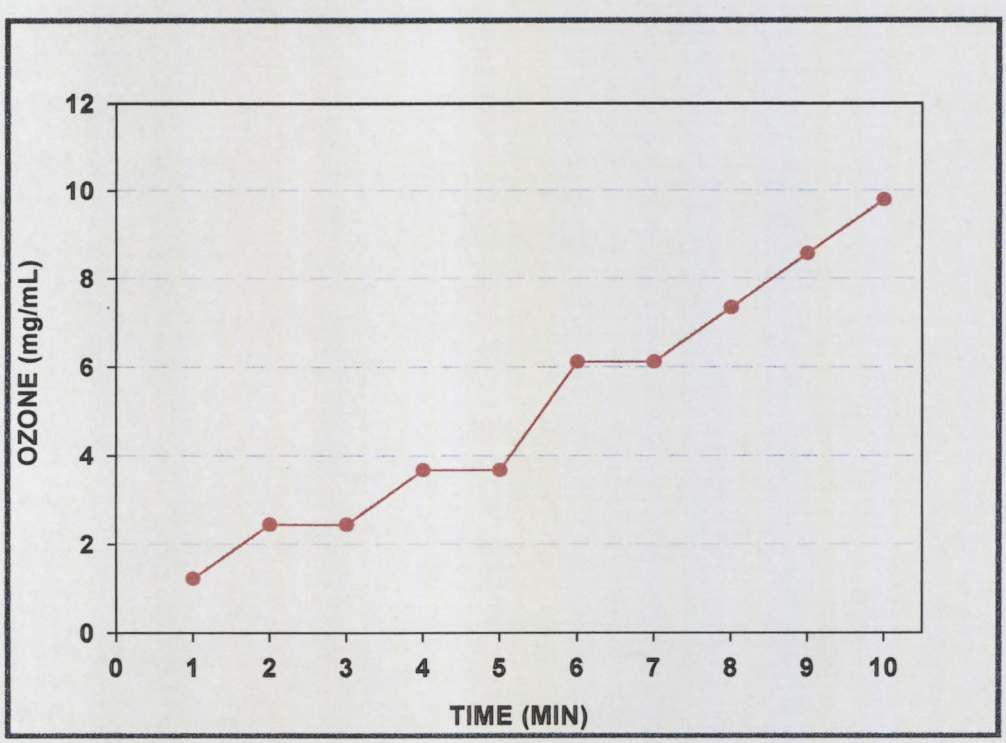
$$O_3 \text{ (consumed)} = \frac{\text{Vol}(Na_2S_2O_3) \times 24000 \times 0.1020}{\text{Vol (sample)}}$$

APPENDIX 26 continued ...

Amount of ozone consumed per minute

TIME	OZONE (mg/L)
1	1.224
2	2.448
3	2.448
4	3.672
5	3.672
6	6.12
7	6.12
8	7.344
9	8.568
10	9.792

The above points were used to plot the calibration curve.



Ozone calibration curve

APPENDIX 27

THE INDIGO COLORIMETRIC METHOD

- **Preparation of Reagents**
  - Indigo Stock Solution

    - 0.25 mL of concentrated phosphoric acid was added to 125 mL of distilled water in a 250 mL volumetric flask.
    - While being stirred, 192 mg of potassium indigo trisulphonate was added
    - Distilled water was added up to the mark
    - A 1:100 dilution exhibited an absorbance of 0.2 $\pm$ 0.010 at 600 nm using an Ultraspec 2000 spectrophotometer.

  - Indigo Reagent

    - 10 mL of indigo stock solution, 1 g of sodium dihydrogen phosphate and 0.7 mL of phosphoric acid were added to a 100 mL volumetric flask and diluted to the mark with distilled water.

2. The Spectrophotometric, Gravimetric Method

- 10 mL of indigo reagent was added to a 100 mL volumetric flask and diluted to the mark with distilled water. This was the blank.
- The tare weight of a beaker was obtained and 10 mL of indigo reagent was added.
- Sample was added to the beaker, until the blue solution turned faint.
- The beaker containing the solution was weighed.
- The blank and the sample were analysed in 1 cm cells using a UV/Vis spectrophotometer, Ultraspec 2000, at a wavelength of 600 nm.
- Residual ozone was calculated using the following equation:

$$mg\ O_3/L = \frac{(A_B \times 100) - (A_S \times V_T)}{f \times V_S \times b}$$

Where: A_B, A_S = Absorbance of the blank and sample respectively

V_S = Volume of the sample (mL)

= [(Final weight - tare weight) g x 1.0 mL/g] - 10 mL

V_T = Total volume of sample + indigo (mL)

= (Final weight - tare weight) g x 1.0 mL/g)

b = Path length

f = 0.42

- ❑ Total ozone consumed was calculated by the following equation:

Total O_3 consumed = Amount of O_3 added (from calibration curve) - Residual ozone

3. Use of the Ultrospec 2000

- ❑ The machine was allowed to self-calibrate
- ❑ Once calibrated, the required wavelength was chosen by pressing the "wave" button
- ❑ Distilled water was placed in a 1 cm cell and placed in the first cell of the machine. This is the reference and therefore, the " ref" button was pressed.
- ❑ Sample was placed in a quartz cell and the sample button was pressed and the absorbance measured and recorded.

APPENDIX 28

RESIDUAL OZONE CALCULATION

$$mg\ O_3/L = \frac{((A_B) \times 100) - (A_S \times V_T)}{F \times V_S \times b}$$

V_T = (final weight - tare) x 1.0 mg/L

= (890.4 g - 504.7) x 1.0 mg/L

= 386.33 mL

V_S = [(final weight - tare)g x 1.0 mg/L]

= 386.33mL - 10 mL

= 376.33 mL

b = path length = 1 cm

A_B = Abs Blank = 0.336

A_S = Abs Sample = 0.036

f = 0.42

$$mg\ O_3/L = \frac{((A_B) \times 100) - (A_S \times V_T)}{F \times V_S \times b}$$

$$= \frac{(0.336 \times 100) - (0.036 \times 386.33)}{(0.42)\ (376.33)\ (1)}$$

$$= \frac{19.59}{158.06}$$

= 0.1246 mg O_3/L