DEVELOPMENT OF BIOLOGICAL TREATMENT TECHNOLOGY FOR THE REMEDIATION OF EDIBLE OIL EFFLUENT

Final Report to the
Water Research Commission

by

S. Surujlal, G. Tivchev, H.C. Kasan and F. Bux

Centre for Water and Wastewater Technology
Durban Institute of Technology
P.O. Box 953
Durban
4000

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EXECUTIVE SUMMARY

1. BACKGROUND AND MOTIVATION

Eutrophication is a natural process that is greatly aggravated by the action of man in the natural environment. Deterioration of South Africa's natural water resources results directly or indirectly from the discharge of industrial effluent rich in nutrient nitrogen and phosphorus. The South African edible oil refineries generally discharge poor quality effluent, which causes threat to the water resources and wastewater treatment installations.

The edible oil industry has been identified to be amongst the 75 industrial groupings in South Africa. In all, there are about 16 edible oil-processing plants, run by 10 separate groups. These industries refine and process approximately 300 000 tons of crude vegetable oil per year, which increases annually by about 3%.

Edible oil effluent entering the sewer system consists mainly of fats, oils, greases (FOG), sulphate and phosphates resulting in both high inorganic and organic loadings at the respective wastewater works. Often effluents from edible oil industries entering wastewater systems have been pretreated to remove most FOG, however due to their natural triglycerides structure not all FOG is removed. In this regard it is noted that previous studies have shown that fatty material within waste streams from food industries are readily biodegradable and it, therefore, follows that these effluents are amenable to biological treatment.

2. STATEMENT OF OBJECTIVES

The research proposal submitted, and accepted, by the Water Research Commission detailed the objectives of the research which are as follows:

- To investigate the source of effluent production during the different stages of refining
- To chemically characterise the effluent

- To assess the efficiency of aerobic biological treatment for the removal of chemical oxygen demand (COD) and phosphate in the final effluent.
- To apply techniques for anaerobic digestion with retention of biomass for the treatment of vegetable oil effluent;
- To study the dynamics of anaerobic biodegradation of the lipid and non-lipid fractions of vegetable oil effluents;
- To develop a treatment protocol for on-site combined aerobic-anaerobic biological treatment of vegetable oil effluent.

Revised objectives:

- Due to time constraint, all research will be conducted at a laboratory scale and not pilot scale onsite.

From the, abovementioned, objectives it can be seen that the major aim of the project was to investigate the relationship between microbial activity and the three primary functions of a biological wastewater treatment facility viz., carbon (both aerobically and anaerobically), nitrogen and, phosphorous removal. Although, initially the main thrust of the research focused on biological phosphorous removal, the emphasis changed to mainly COD and FOG removal. This change was necessitated by alterations made on an operational level. The chief industrial partner in this research switched from a phosphoric acid based refining method to using caustic soda as an alternative method for oil refining. Therefore, the effluent phosphorous concentrations decreased dramatically and the need for biological phosphorous removal, prior to discharge, was made unnecessary, although, the need for COD and FOG removal remained a priority. For example, following this change, and decreases in phosphorous concentrations, resulted in all experiments requiring dosing with di-potassium hydrogen phosphate (K_2HPO_4) to enhance biological activity.

3. SUMMARY OF RESULTS AND MEETING OF OBJECTIVES

The main aim of this study was to assess the capacity of a laboratory scale effluent treatment process that will produce final effluent having a regulatory acceptable organic load and phosphate concentration prior to its discharge into the municipal sewer system. The study was conducted in various stages including: wastewater characterization,

treatability studies, and laboratory scale organic and nutrient removal treatment investigations.

With regards to the phosphorous removal objectives, following analysis for various effluent parameters, treatability studies were conducted using an aerobic-anaerobic sequencing batch reactor with a total hydraulic retention time of 24 hr. The results showed an average of 75% reduction of COD and more than 90% removal of FOG. Based on the results of effluent characterisation and treatability studies, a laboratory scale activated sludge effluent treatment process was designed and operated with two bioreactors (aerobic and anaerobic) in series. The system was operated for a period of 1 month resulting in 70% removal of COD and 4% phosphate reduction. After some structural and operational changes from the original design configuration, the system was the operated continuously for the duration of the study period. An optimum COD removal of 75% and 107 mg/L phosphate reduction was achieved during the last operational phase of the system. More than 95% reduction in FOG has been achieved in both semi-continuously and continuously operated systems.

In order to evaluate the effect sludge age has on biological phosphorus removal in oil effluent and to determine the optimum sludge age, a sequencing batch reactor was set up for the experimentation. The effluent was exposed to alternating anaerobic and aerobic conditions. The reactor had a hydraulic retention time of 12 hr. Sludge ages of 5, 10, 15 and 20 days were used. The COD, phosphorus levels, mixed liquor suspended solids, nitrates and ammonium levels were determined. The 10-day sludge age proved to be most effective in phosphorus removal and a removal efficiency of 84% was obtained. These experiments also indicated the complex interactions between design criteria and physiological requirements of the microorganisms. For example, initial anaerobic reactor volumes and hydraulic retention times showed a significant effect on subsequent microbial activity.

Additional experiments were made to optimise conditions for prefermentation in order to obtain the highest concentration of Volatile Fatty Acids (VFA's) to improve biological phosphorus removal from edible oil effluent. The conditions that were optimised were the hydraulic retention time, the effect of adjusted and natural pH and effect of mixing and

non-mixing. A single stage laboratory scale 10 L Primary Acid Fermenter Sequence Batch Reactor was set up for determination of hydraulic retention time. 4 x 1 L conical flasks were used as batch reactors for determination of total solids, pH and mixing effects. The laboratory scale results showed that an 8 day time period, 12 hr hydraulic retention time, a total solids concentration of 3 000 mg/L and adjusted pH to 7.0 with mixing showed optimum VFA production. When the optimised system was combined with a Biological Phosphorus Removal Sequence Batch Reactor, a 76% COD reduction and 78% phosphate reduction was achieved therefore it can be concluded that a prefermentation stage improves biological phosphorus removal from edible oil effluent.

The vegetable oil industry produces effluent containing quantities of fat, oil, sodium, phosphates as well as other pollutants. Physico-chemical treatment methods, such as Dissolved Air Floatation (DAF), gravity separation and the use of coagulants have been attempted providing a considerable reduction in organic loading; however, discharge standards are still not met. Thus, biological treatment methods are being sought after. Aerobic treatment has been attempted however, shock loads cause problems while running such a process. The objective of this phase of the study was to assess the efficiency of anaerobic digestion technology to degrade vegetable oil effluent as well as the efficiency of the Anaerobic Baffled Reactor. Anaerobic digestion involves the breakdown of organic matter by the action of microorganisms in the absence of oxygen, producing methane-rich biogas. The oil effluent was characterized, providing significant information on its chemical composition. It was found that the effluent had high sulphate content as well as a high COD. The high sulphate content of wastewaters has known to promote growth of Sulphate Reducing Bacteria, which utilize the same energy source as Methane Producing Bacteria and therefore compete for the same energy source. Sulphate and lipid reduction pretreatment experiments were carried out, using barium chloride and gravitational separation respectively. The results obtained, showed that the use of barium chloride to reduce sulphate content in oil effluents was successful, with significant sulphate reduction. The lipid reduction experiments however, did not show any significant lipid reduction. Batch tests were conducted in serum bottles to assess the extent of biodegradation of the oil effluent in its raw state as well as with reduced sulphate content. Methanogenic toxicity tests on the raw and pretreated oil effluents provided a range of toxicity results. These assays are relatively simple and inexpensive. Gas

production was monitored to determine the rate and extent of biodegradation. The efficiency of digestion was assessed by COD reduction. Results indicated potential inhibition of the methanogenic bacteria responsible for methane production by the presence of a toxic substance or substances (at elevated concentrations) in the oil effluents. Results showed raw effluent to be more susceptible to anaerobic degradation. A laboratory scale Anaerobic Baffled Reactor was assessed to treat oil effluents anaerobically and compared to Fed-batch digestion. Both reactors were fed a combination of oil effluents (COD 2000 mg/L) and artificial effluent. Results indicated that anaerobic fed-batch digestion is a promising method of treatment for oil effluents and that the anaerobic baffled reactor is not suitable for treatment of this type of effluent.

For the pretreatment of the effluent, all the results, viewed collectively, seem to indicate that the techniques of ozonation, peroxone and ultrasonics are ineffective methods for the pretreatment of an edible oil effluent.

It has been shown that ozonation performs optimally in the presence of unsaturated bonds (Kloos, 2000). Therefore, with these present in low concentrations, optimum ozonation proved unsuccessful. From all of the pretreatment results obtained it can be concluded that the alternative pretreatment methods were not as effective as the current pretreatment method. Thus, the method of chemical coagulation and flocculation with C40 appears to be the most effective pretreatment from the range of coagulants investigated for the edible oil effluent.

An activated sludge process was designed and the operation was based on a Modified Ludzak- Ettinger (MLE) configuration which was preceded with optimization of appropriate pretreatment technology. The commercial coagulant C40 showed comparatively superior performance and was selected as the pretreatment technology to prepare effluent for activated sludge treatment. The lab scale process was conducted in phases encompassing common operational parameters. Early stages of the lab scale process were unsuccessful since the wastewater was not pretreated. Subsequent to pretreatment, overall performance of the process was good recording maximum COD removal efficiencies of > 90% during steady state conditions. The process responded positively to

increase in the influent COD concentration (organic strength) with the maximum concentration of COD removed being > 1500 mg/L.

Apart from phosphorous removal total organic concentration removal became a critical focus point and in addition various cultures (yeast, molds and bacteria) were isolated from oil- contaminated soil to determine their relative oil degrading capacity.

Bacterial, yeast and fungal monocultures were used individually to determine their ability to grow on an agar medium containing commercial fatty acids (i.e. oleic and stearic acid respectively) as a carbon source and to determine their capabilities in degrading the fatty acid component in edible oil effluent samples which were monitored by COD and FOG analysis. Tests at discrete pH's of 5, 7, and 8 and at temperatures of 21^{o}C and 31^{o}C, respectively.

Results have shown that the isolates belonging to the *Mucor* spp. were capable of growing excellently on the agar plates, while the *Alternaria spp.* grew poorly on both oleic and stearic fatty acid agar plates. For the purpose in this study, FOG collectively represents the triglyceride or long chain fatty acid component present in the edible oil effluent. Using the raw effluent samples, the parameters of pH 5 and temperatures of 31^{o}C, showed that the best FOG removal rates of 90% by the *Alternaria spp.,* 89% by both *Mucor spp.* when compared to the other experimental parameters used.

For the bacteria and yeast, isolate F showed a 90% FOG removal at pH 5 at 21°C and *Rhodosporidium sp* showed a 91% FOG removal at 31°C respectively.

4. RECOMMENDATIONS FOR FUTURE RESEARCH

As a result of these studies various areas have been identified for future research. For example, the oil effluent was found to contain amounts of phytosterols, which could possibly be extracted, purified and sold as an animal feed supplement. In order to achieve this, it will be necessary to analyze edible oil effluents from all of the oil refineries over an extended time frame to determine the concentrations of the phytosterols in the different effluent streams, as well, as the extraction potential.

An additional area identified is the need for a comprehensive analysis of the effluent. This is necessary as operational and refinery procedure changes result in significant chemical characteristic changes in the effluents. This, in turn, effects the microbial associations required for further biological treatment of the effluents.

Settling problems as well as changes in microbial interspecies interactions have been noted in the pilot-scale activated sludge system. Further research will be required to determine the complex interactions between the pollutant and microbial associations and their interactions, which are required for effective activated sludge treatment of organic effluents.

5. COSTING ANALYSIS

The costs involved in biologically treating the raw edible oil effluent will include a substantial initial capital expenditure including the design and construction of the BNR process to the specifications required, as well as specialized equipment such as pumps, aerators and mixers. All of these start-up expenses are dependant on the wastewater characteristics of the effluent and will therefore differ according to the industry.

Other costs involved in biological treatment include:

a) Flocculation using C40, since this is a pre-requisite for biological treatment.
b) Chemical Consumables, such as H_2SO_4 to reduce the alkalinity of the effluent before flocculation, as well as NH_4Cl and KH_2PO_4 which are used to supplement the flocculated effluent to the ratio C:N:P = 100:10:1 in order for BNR to take place.
c) Running expenses, especially electricity as it is required for mechanical mixing and aeration of the mixed liquor.
d) Manpower, the BNR process will require at least one skilled process-controller to operate and maintain the process and 2 or 3 factory workers for maintenance, sampling and cleaning of the reactors in the BNR process.

Table 1: Costs and concentrations of chemical consumables used to flocculate the edible oil effluent.

Chemical	Price	Concentration required	Price/kilolitre
C40	R13.50/kg	8 g/kL	R108/kL
H_2SO_4	R29.20/L	1 L/kL	R29.20/kL
NH_4Cl	R66.00/kg	200 g/kL	R13.20/kL
KH_2PO_4	R210.00/kg	40 g/kL	R8.40/kL
Total			**R158.80/kL**

From Table 1 it can be seen that it would cost R158.80 to flocculate and supplement one kilolitre of effluent.

The following is the formula used by Durban Metro to calculate the relative effluent costs:

Cost (R/kL) = Volume of effluent (kL) x 0.334[Average COD (mg/L) – 350 mg/L]

The charge only starts at a COD over 350 mg/L; this is the average COD of domestic sewage. The idea is that the weaker effluents attract no charge other than the sewage connection costs.

Table 2: Trade effluent costs according to Durban Metro Wastewater Department for disposal of the various effluents.

Type of Effluent	Average COD (mg/L)	Cost (R/kL)
Raw Effluent	7500 mg/L	R23.88/kL
C40 Flocculated Effluent	1500 mg/L	R3.84/kL
Biologically Treated Effluent	500 mg/L	R0.50/kL

From Table 2 it can be seen that the biologically treated effluent, since having the lowest COD concentration will be the cheapest to discharge at only R0.50/kL. While the costs to discharge the raw and flocculated effluents are much greater and will therefore be R23.88/kL and R3.84/kL respectively.

6. TECHNOLOGY TRANSFER

(i) Capacity Building

The following students received relevant qualifications as a result of this project:

D. Tech (Biotechnology)	-	F. Bux
M. Tech (Biotechnology)	-	S Mkhize
	-	A Manganyi
		L A Frost
		S Surujlal
B. Tech (Biotechnology)	-	N Sithole
	-	S Surujlal
	-	P Mathibela
	-	Y Devnath
	-	K Reddy
Experiential Trainee	-	S Surujlal
	-	K Reddy
	-	S L Thomson
	-	T Lalbahadur

(ii) Industrial Liaison

Project findings were discussed with industrial partner on a regular basis. The research findings served as a basis for further investigation and up-scaling to pilot plant subject to a feasibility study.

(iii) Publications and conferences

Publications:

Mkhize, S. P., Atkinson, B.W. and Bux, F. (2000) Assessment of activated sludge process as a treatment alternative for remediation of edible oil effluent. *Water SA.* Vol 26(4) pp 555 – 558.

Mkhize, S. P. and Bux, F. (2001) Assessment of activated sludge to remediate edible oil effluent. *South African Journal of Science*. Vol 97. pp 380 – 382.

Reddy, K., Drysdale, G. D. and Bux, F. (2003) Evaluation of activated sludge treatment and settleability in remediation of edible oil effluent. *Water SA.* Vol 20(3) pp 245 – 250.

Presentations:

Mkhize, S. P., Bux, F. and Kasan, H.C. (1999) Assessment of a Biological Nutrient Removal Process for the Remediation of Edible Oil Effluent African International Environmental Protection Symposium (AIEPS '99), Fourth Southern Africa Anaerobic Digestion Symposium at Imperial Hotel, Pietermaritzburg, South Africa, on 4-8 July 1999.

Mkhize, S. P., Atkinson, B.W., Bux, F. and Kasan, H. C. (1999) Assessment of a biological nutrient removal process for the remediation of edible oil effluents IAWQ International Specialty Conference of the chemical industry group, waste minimization and end of pipe treatment in chemical and petrochemical industries. Merida, Yucatan, Mexico. November 4 - 18, 1999.

Mkhize, S. P., Atkinson, B. W. and Bux, F. (2000) Assessment of activated sludge process as a treatment alternative for remediation of edible oil effluent BIOY2K Combined Millennium Meeting, 2000-SASM, 23- 28 January 2000, Rhodes University, Cape Town oral presentations

Mkhize, S. P. and Bux, F. (2000) Assessment of biological treatment process for the remediation of edible oil effluent The Water Institute of Southern Africa, Biennial Conference and Exhibition, Sun City, 28 May - 1 June 2000, *Oral* Presentation

Manganyi, A., Surujlal, S. and Bux, F. (2001) Biological phosphorus removal from edible oil effluents. South African Society for Microbiology (KZN) 13th Annual Symposium, 19 October, Senate Chamber, University of Durban- Westville Poster presentation

Surujlal S. and Bux, F. (2002) Optimisation of Edible Oil Effluent Degradation by Microorganisms. International Symposium For Environmental Biotechnology (ISEB), 9 – 12 June 2002 Veracruz, Mexico. Oral Presentation

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The Steering Committee responsible for this project consisted of the following persons:

Mr. G Steenveld	Water Research Commission (Chairman)
Dr N Mjoli	Water Research Commission
Mrs. CM Smit	Water Research Commission (Committee Services
Ms S Chetty	Water Research Commission (Committee Secretary)
Dr PMM Jonas	Umgeni Water
Dr. H Kasan	Rand Water
Prof CA Buckley	University of Natal (Durban)
Ms J Bell	University of Natal (PRG)
Mr. K Beath	Department of Water Affairs & Forestry
Mr. P Viljoen	Department of Water Affairs & Forestry

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Mr. Mandelia	Sealake Oil Industries, Pietermaritzburg
Staff	Darvill Wastewater Works, Pietermaritzburg
Staff	Centre for Water and Wastewater Technology, Durban

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ABR	- Anaerobic Baffled Reactor
Al	- Aluminum
Al^{3+}	- Aluminium ion
Alum	- Aluminium sulphate
ATA	- Anaerobic Toxicity Assay
AE1	- 1st Aerobic zone
AE2	- 2nd Aerobic zone
AX	- Anoxic zone
BEPR	- Biological Excess Phosphorus Removal
BMP	- Biochemical Methane Potential
BNR	- Biological Nutrient Removal
BOD	- Biological Oxygen Demand
BOD_5	- 5-day Biological Oxygen Demand
Bx	- Organic loading rate
Ca	- Calcium
$CaCO_3$	- Calcium Carbonate
CH_4	- Methane
CO_2	- Carbon Dioxide
COD	- Chemical Oxygen Demand
DAF	- Dissolved Air Flotation
DO	- Dissolved Oxygen
DSVI	- Dissolved Sludge Volume Index
DWW	- Darvill Wastewater Works
EDTA	- Ethylene Diamine Tetra-acetic Acid
FAS	- Ferrous Ammonium Sulphate
Fe^{3+}	- Ferric ion
$FeCl_3$	- Ferric chloride
FFA's	- Free Fatty Acids
F/M ratio	- Food to Microorganism ratio
FOG	- Fats, Oils and Greases
F_{xa}	- Anaerobic Mass Fraction
H_2O	- Water
H_2O_2	- Hydrogen peroxide
H_2S	- Hydrogen Sulphide
HCl	- Hydrochloric Acid
HRT	- Hydraulic Retention Time
IR	- Infa red
kL	- Kilolitre
KHP	- Potassium Hydrogen Phthalate
LCFA	- Long Chain Fatty Acids
M^{2+}	- Divalent inorganic salt
M^{3+}	- Trivalent inorganic salt
Mg	- Magnesium
MLE	- Modified Ludzacl-Ettinger process
MLSS	- Mixed Liquor Suspended Solids
MLVSS	- Mixed Liquor Volatile Suspended Solids
MPB	- Methane Producing Bacteria

N	- Nitrogen
NH_4^+	- Ammonium nitrogen
NO_2	- Nitrites
NO_3	- Nitrates
NWA	- National Water Act
O_2	- Oxygen
O_3	- Ozone
OFN	- Oxygen Free Nitrogen
Os	- Sludge age
OUR	- Oxygen Utilization Rate
P	- Phosphorus
PAF	- Primary Aerobic Fermenter
PAO's	- Polyphosphate Accumulating Organisms
PHA	- Polyhydroxyacetate
PHB	- Polyhydroxybutyrate
PO_4 - P	- Orthophosphate
PO_4^{3-}	- Phosphate
ppm	- Parts per million
Q_i	- Influent flow rate
RBCOD	- Readily Biodegradable Chemical Oxygen Demand
Rpm	- Revolutions per minute
R_s	- Sludge age
SBCOD	- Soluble Biodegradable Chemical Oxygen Demand
SCFA	- Short Chain Fatty Acids
SDA	- Saboraud Dextrose Agar
SO_4^{2-}	- Sulphates
SRB	- Sulphate Reducing Bacteria
S-recycle	- Sludge recycle
SRP	- Soluble Reactive Phosphates
SWI	- Specific Water Intake
SVI	- Settling Velocity Index
TDS	- Total Dissolved Solids
TKN	- Total Kjeldahl Nitrogen
TN	- Total Nitrogen
TLC	- Thin Layer Chromatography
TP	- Total Phosphate
TS	- Total Solids
TSS	- Total Suspended Solids
UASB	- Upflow Anaerobic Sludge Blanket
VFA's	- Volatile Fatty Acids
VS	- Volatile Solids

CHAPTER 1
INTRODUCTION AND SCOPE OF RESEARCH

1.1 THE ORIGINS OF EFFLUENT/WASTEWATER

Due to difficulties encountered in obtaining effluent samples from the edible oil industries nationally, the current investigation was limited to selecting a company within the industry to serve as a case study. The process used to refine oil is common in the edible oil industry and therefore the wastewater generated could be regarded as similar in its chemical characteristics in the industry and a reflection of the broader industry in general. The only oil industry that collaborated was situated in Pietermaritzburg, in the KwaZulu-Natal midlands region. The name of the industry would not be revealed and would be referred to as Company X throughout the report. This company buys locally produced crude oil, and imports some from elsewhere to be refined on-site. Hence, the plant is mainly a refining factory; however, at the beginning of September 1999, milling was also introduced into the factory. Figure 1.1 illustrates the refinery process carried out at Company X. The principal products that are produced by Company X are refined sunflower oil, soaps and candles. The factory also produces and sells acid oil, which is a one of the by-products of soap production.

The factory is subdivided into four main plants, which are all located on the same premises. Three plants are mainly for production and the fourth plant is an effluent treatment plant. The three main production plants are: the refinery plant, which produces the refined oil; the acid oil plant, which produces soap stock and acid oil from fatty acids and sulfuric acid; and soap plant which produces soap from soap stock and candles.

All the three production plants are responsible for the production of different kinds of effluents at variable quantities and strength as shown in Figure 1.2. The volumes of effluents produced per plant vary on weekly basis depending on the refinery process employed as shown in Table 1.1. To understand the overall quality and quantity of effluent produced by the factory, it is better to consider individually the unit operations of each plant, its main product and effluents.

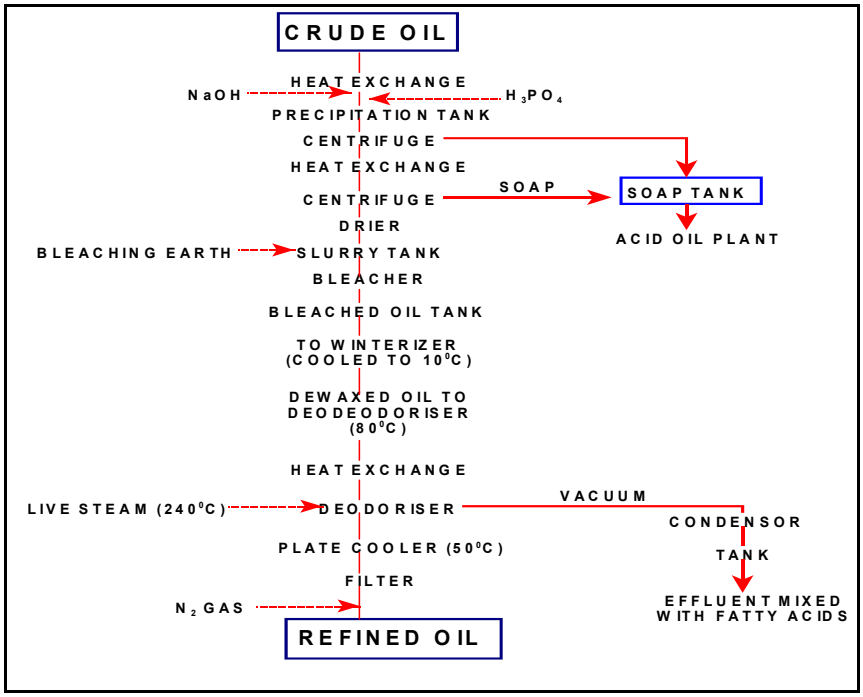


Figure 1.1: Schematic diagram of refinery process

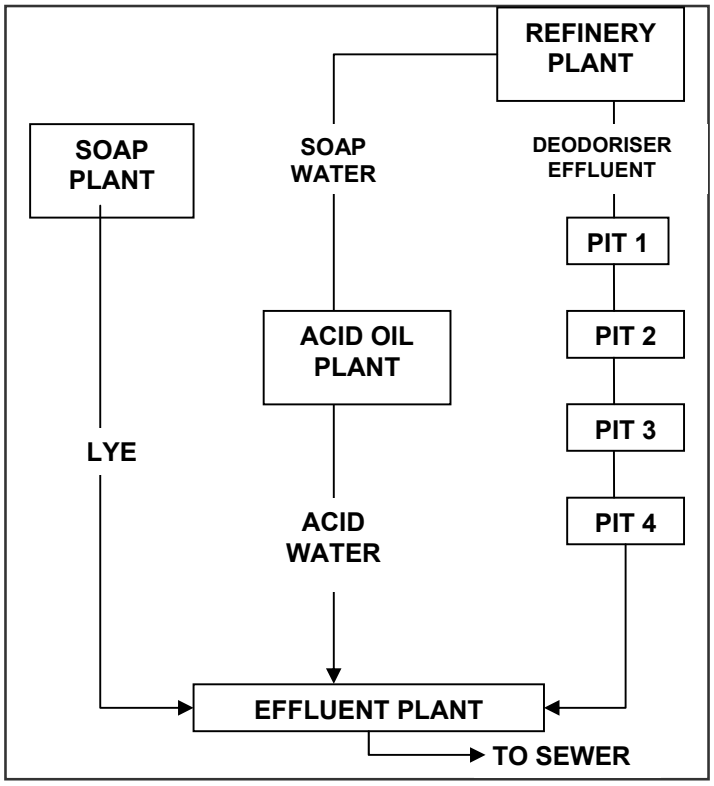


Figure 1.2: Schematic representation of the five plants at Company X and the effluents produced

Table 1.1: Typical effluent volumes produced by each plant per week

DAY (24 HOURS)	REFINERY EFFLUENT (tons/day)	LYE (tons)	ACID WATER (tons)
MONDAY	96	15	15
TUESDAY	96	10	15
WEDNESDAY	96	20	15
THURSDAY	96	7	15
FRIDAY	NIL	NIL	15

The processing of vegetable oil, both milling and refining, depends on water availability. During edible oil processing, the industry consumes approximately 2 million cubic meters of water annually. A typical oil plant discharges about 40% of the incoming water to the sewer system and the remaining 60% is either vaporised in many cooling circuits, or else leaves the site in one of the secondary products or by-products. Hence, the Specific Water Intake (SWI) for the edible oil industry is very high compared to other industries in South Africa. In a study that was conducted by Steffen, Robertson and Kirsten (WRC, 1989), they found that the SWI ranged between 2.1 and 3.1 m^3/ton for milling and between 3.2 and 4.6 m^3/ton for refining. Based on the results of their study, a target SWI of 2.0 m^3/ton for milling and 3.0 m^3/ton for refining was proposed. In addition to the proposed figure for each process, a further 5.0 m^3/ton SWI for a plant milling and refining all products on site was proposed. It was then concluded that improved SWI could be achieved only by improving water management by the edible oil industry. (WRC, 1989).

There were three different biological treatment methods used in this study for the treatment of edible oil effluent, i.e., Anaerobic Biological Treatment, Bench-scale Aerobic Biological Nutrient Removal and larger Laboratory-scale Aerobic Biological Nutrient Removal. Figure 1.3 illustrates the flow diagram of the different methods followed.

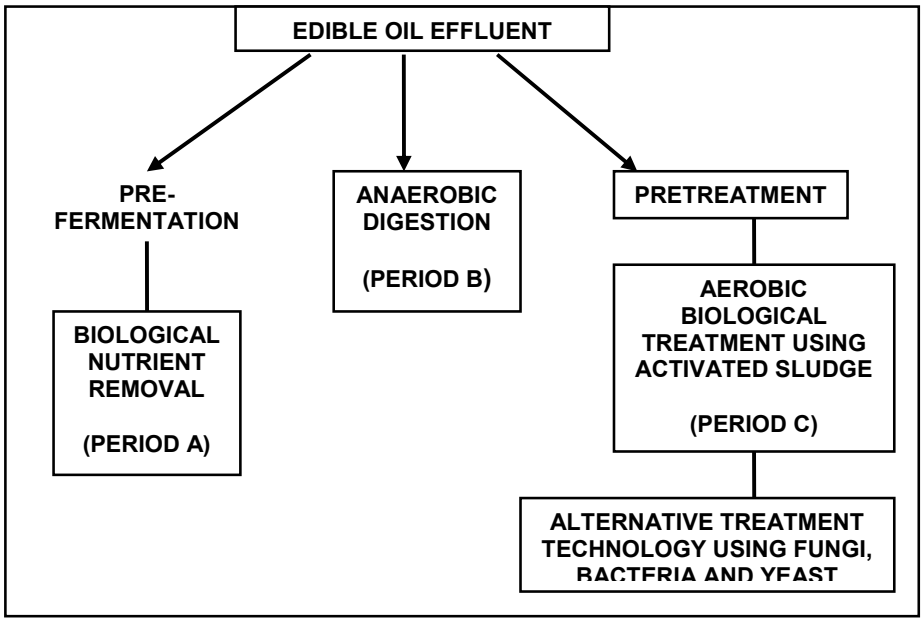


Figure 1.3: Flow diagram showing the research approach for treatment of edible oil effluent

1.2 WASTEWATER CHARACTERISATION OF THE EDIBLE OIL EFFLUENT

1.2.1 Introduction

Before a wastewater treatment plant can be designed for biological phosphorus removal, it is necessary to characterise the effluent quality and quantity. For this purpose, extensive monitoring of the effluent production is required, which includes the use of proper sampling techniques. Flow rates and water quality parameters often change sporadically, and these changes may affect the ability of a wastewater treatment plant to achieve consistent biological phosphorus removal.

Given the variable nature of the edible oil effluent, which results from the varying nature of edible oil refining process, and the necessity of attaining consistent phosphorus removal, it is necessary to collect samples that will represent “average” characteristics and approximate characteristics under more extreme conditions (WRC, 1989). According to Novotny (1998), a desirable sampling method is to collect 3 - 4 hour composite samples. This would provide data that may be considered representative of average effluent characteristics throughout the day. Usually a careful review of flow monitoring records and reports generated by the effluent plant over the past couple of years, if present, tend to be helpful in assessing the periodic and seasonal

characteristics of wastewater throughout the year (Novotny, 1998). Due to the variability of the effluent characteristics over the period of investigation, three tables have been presented to show the variable effluent characteristics over the respective periods, i.e., tables 1.2 to 1.4. Table 1.2 illustrates the chemical characteristics of 4 samples obtained per month, of the final effluent used for the bench scale biological phosphorus removal process.

Table 1.2: Wastewater characteristics of the final effluent (Period A)

Parameter (mg/L except pH)	June		July		August		September		October	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
pH	4.6 5.9	**5.6**	8.8 10.6	**6.5**	5.7 7.0	**6.5**	7.1 8.1	**7.7**	7.6 9.9	**8.6**
COD	7 590 7 380	**7 630**	7 550 8 710	**8 160**	1 025 1 270	**1 115**	7 240 7 590	**7 400**	11 700 11 810	**11 763**
PO_4^{3-} (-P)	500 590	**550**	910 1 140	**1 020**	1 640 1 680	**1 660**	4 320 4 510	**4 400**	2 110 2 180	**2 140**
TKN (-N)	6.08 7.96	**6.93**	3.21 6.26	**4.78**	6.54 7.19	**6.82**	6.98 8.67	**7.65**	4.36 5.81	**4.98**
NH_4^+ (-N)	0.98 1.51	**1.25**	0.41 0.76	**0.6**	1.39 2.62	**2.0**	1.09 1.21	**1.15**	2.09 3.6	**2.69**
SO_4^{2-}	4 980 5 910	**5 550**	5 280 5 830	**5 600**	3 410 3 530	**3 470**	5 690 5 980	**5 800**	1 170 1 400	**1 260**
Lipids (FOG)	249 266	**256**	103 121	**111**	325 352	**340**	581 631	**628**	297 319	**308**
TSS	239 281	**265**	379 388	**383**	98 134	**112**	256 274	**265**	309 340	**322**
Alkalinity	487 542	**520**	465 492	**480**	1 670 1 760	**1 720**	616 649	**630**	742 778	**766**

Table 1.3 illustrates the characteristics of the final effluent used for the anaerobic biological treatment process. Two batches of effluent were collected.

Table 1.3: Chemical Characteristics of Final Effluent used in Anaerobic treatment (Period B)

PARAMETER	SAMPLE A	SAMPLE B
pH	6.7	6.0
COD	6848 mg/L	7254 mg/L
Lipids (FOG)	2492 mg/L	456 mg/L
SO_4^{2-}	2590 mg/L	2140 mg/L
TSS	1830 mg/L	-
TDS	24820 mg/L	-
TS	-	11840 mg/L
VS	-	10670 mg/L

The Company X produced approximately 195 m^3 of effluent per day from the refinery process, during a 24 hr operational period. The effluent from the deodorisation process was flocculated and used for the laboratory scale aerobic biological treatment process. The characteristics of the raw wastewater, flocculated and corrected influent is shown in Table 1.4. Due to the large number of analysis conducted per parameter, the results are presented indicating the range i.e. highest and lowest values and the mean of all the analyses.

Table 1.4: Chemical characteristics of raw effluent after deodorisation and prepared effluent (Period C)

PARAMETERS	RAW EFFLUENT		POST-PRETREATMENT		PREPARED INFLUENT	
	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
Ph	3.0 - 4.9	3.8	9.4 - 8.5	9.2	6.8 - 7.3	7.1
COD (mg /L)	6190 - 8470	7250	1800 - 1950	1890	310 - 1928	N/A
FOG (mg /L)	4990 - 6840	5950	250 - 1350	440	156 - 395	N/A
TP (mg /L)		4.72		3.95		N/A
TKN (mg/L)		7.42		6.72		N/A

* Range - Parameters indicating the minimum and maximum values measured during the course of the entire investigation.

* Raw effluent □ effluent obtained from industry without any correction.

* Post - pretreatment □ effluent that is pre-treated with flocculent

* Prepared Influent □ effluent that is corrected after flocculation (pH adjusted /diluted/ supplemented)

1.3 THE NEED FOR PRETREATMENT

Pretreatment methods are used to treat wastewater in order to improve various conditions before subjecting to biological treatment. The effluent produced from the deodorisation process contained high levels of organics in the form of COD and FOG's. Preliminary findings showed that these concentrations caused shock loading in the aerobic biological treatment process and prevented it from functioning optimally, with regards to COD and FOG removal. Hence, from comparative analysis of various coagulants available, the commercial coagulant C40 was used to flocculate the raw effluent prior to aerobic biological treatment, to increase COD and FOG removal.

1.4 RESEARCH OBJECTIVES

The research project was therefore designed to:

- To investigate the source of effluent production during the different stages of refining
- To chemically characterise the effluent
- To assess the efficiency of aerobic biological treatment for the removal of COD and phosphate in the effluent
- To apply techniques for anaerobic digestion with retention of biomass for the treatment of vegetable oil effluent;
- To study the dynamics of anaerobic biodegradation of the lipid and non-lipid fractions of vegetable oil effluents;

1.5 REPORT STRUCTURE

The report is structured and will be presented as such:

Table 1.5 : Scope and structure of report

CHAPTER	SCOPE
1	General introduction and scope of research
2	Literature review
3	(A) Prefermentation (B) Assessment of bench scale biological nutrient removal processes
4	Anaerobic Digestion
5	Pretreatment methods to enhance secondary biological treatment of edible oil effluent
6	Laboratory scale aerobic biological treatment process
7	Alternate treatment technology using yeasts, bacteria and fungi for edible oil effluent degradation.
8	General conclusions and recommendations

CHAPTER 2
LITERATURE REVIEW

2.1 THE SOUTH AFRICAN EDIBLE OIL INDUSTRY

The edible oil industry has been identified to be amongst the 75 industrial groupings in South Africa. In all, there are about 16 edible oil-processing plants, run by 10 separate groups. These industries refine and process approximately 300 000 tons of crude vegetable oil per year, which increases annually by about 3%. The amount of oil that is produced locally depends very much on the climatic conditions. Good rains lead to large maize, groundnuts and sunflower crops, which results in good oil seed production. However, drought has negative impact in the industry, resulting in decline raw material production. To make up for the short fall in local oil production, the balance of oil is imported, in crude form, to be refined in the South African refineries (WRC, 1989).

Vegetable oil production can be divided into two distinct stages: crude oil production in an oil mill; and crude oil processing conducted in a refinery. Thus the vegetable oil industry can be divided into two main groups based on the main production process being used. The milling industry produces crude oil from raw materials such as seed, and the refining industry purchases and refines crude oil into final products. In South Africa, the two stages of processing are usually conducted on the same site, although marine oils and animal fats, where used, are purchased as such. (WRC, 1989).

The principal product of edible oil refinery is liquid oil, which may be sold as cooking or salad oil, or may be further processed to increase the market value of the final product, for example, margarine, peanut butter and mayonnaise. Vegetable oil may be obtained from large variety of monocotyledonous and dicotyledonous seeds. The most commonly grown oil bearing crops in South Africa are the sunflower, groundnuts, and maize although other seeds such as cotton and soya are also processed. (WRC, 1989)

2.1.1 The Refinery process and its effluents

The refinery is associated with the removal of phospholipids, colour bodies and other soluble and insoluble impurities from crude oil. The production of refined vegetable oil can be divided into two variable processes or stages viz., chemical refining and physical refining. Although the two stages are different from each other and produce different effluents, the products in both stages are similar in quality i.e. refined vegetable oil ready for commercialization.

2.1.1.1 Degumming

Degumming may be considered the first step in the refining process and is designed to remove the phosphatides that interfere with subsequent processing. Oils high in phosphorus such as soybean, corn and sunflower, may require degumming prior to the refining process. The primary reason for degumming is to either provide crude-degummed oil suitable for storage or long transit, to prepare oil for physical refining, or to produce lecithins. There are three main problems that are associated with the presence of gums in the crude oil, which are:

(a) Phospholipids have excellent emulsifying abilities. Thus when discharged as soap stock, they introduce problems for oil-water separation in the acidulation process, thus, resulting in increased product loses during chemical refining.
(b) Gums have the tendency to impart deep brown colouring to finished oils during the deodorising stages as a result of the high temperature that are employed.
(c) They tend to form complex compounds with certain trace metals that adversely affect product stability.

Water degumming is effective only for water-hydratable phosphatides i.e. those having a greater affinity for water phase rather than remaining in the oil phase. The addition of hot water and, subsequent, separation of the swollen insoluble gums, by centrifuge, easily removes them. For non-hydratable gums, pretreatment of the oil with phosphoric acid or citric acid is required to render them hydratable. The then hydratable gums are subsequently removed through addition of small amounts of water followed by centrifugation. Many variations on this two-stage process exist. (Horan, 1990).

The effluent produced during the degumming stage tends to contain quantities of phospholipids, inorganic phosphates (from phosphoric acid) and FOG.

2.1.1.2 Chemical Refining

Chemical refining, also known as caustic refining, generally refers to the process designed to neutralise free fatty acids present in the oil by introduction of an alkali, followed by centrifugal separation of the, heavy phase, insoluble material. Caustic neutralization is the traditional first step for edible oil processing if degumming is not included as a pretreatment step. Caustic refining is made up of five inter-related processes and each process produces its own unique effluent. The five processes are neutralization, bleaching, hydrogenation, winterizing and deodorizing. The advantage of caustic refining over the alternative method is its reduced sensitivity to the type of feedstock used.

A. *Neutralization:*

Crude oil contains a percentage of free fatty acids (FFA). The FFA's or carboxylic acids are a product of natural degradation of triglycerides. Dilute caustic soda solution of up to 4N strength is usually used for neutralisation. The oil-alkali solution is thoroughly agitated to ensure intimate contact, normally using an inline high-shear mixer. Both the oil and caustic soda should be cooled to less then 38°C. Careful control of the operating conditions is required at this stage as the strong caustic soda used tends to saponify the neutral triglyceride with the consequent loss of neutral oil (Hui, 1996a).

The neutralisation process also helps with the removal of metals, particularly magnesium (Mg) and calcium (Ca). Apart from saponification of free fatty acids in the crude oil, caustic addition tends to be more effective in hydration of gums than simple addition of hot water only. Thus, it is possible for crude oil containing low gum contents to be processed chemically without the need for an additional degumming pretreatment step (Hui, 1996a).

The immiscible soap or soapstock, produced during neutralisation, is separated from the neutralised oil using centrifuges or gravitational settling, depending on whether the operation is continuous, semi-continuous or a batch process. Phosphoric acid may be added in the wash water to reduce the residual soap in the refined oil, and to provide a

better split between the oil and aqueous phase. The soapstock is further treated in the acid oil plant (on-site) to produce acid oil (WRC, 1989; Hui, 1996a).

Chemical refining with caustic soda gives rise to the most potent effluent generated at an oil processing plant. The resultant effluent stream is known as soapy water and contains quantities of FFA's, free oils, gums or phospholipids, sodium ions and phosphates (Eroglu *et al*., 1990).

B. Bleaching

Bleaching is the adsorptive process that is associated with edible oil refining process. While the degumming operation is designed to remove phosphatides and caustic refining converts water soluble FFA's into soluble soaps, adsorptive bleaching provides the last practical opportunity to remove the remaining impurities, especially colour and phospholipids, to acceptable levels. The major colour pigments found in edible oil are chlorophyll (green) and caretenoids (orange) (Hui, 1996a).

C. Dewaxing

Dewaxing which is sometimes called winterising, refers to the removal of high melting point waxes extracted from certain oil seeds such as corn, sunflower and canola. The refined oil is cooled to approximately 5°C thus causing the high melting point esters and waxes to crystallize. These fat crystals are subsequently removed through filtering usually with the assistance of diatomaceous earth as a filter media (Hui, 1996a). The winterising process is only necessary for oil products that are going to be marketed as such without any further processing. It is usually not necessary to winterize the oil that is to be, further, hydrogenated. This stage of edible oil refining contributes little, if any, effluent to the final effluent stream both qualitatively and quantitatively (WRC, 1989).

D. Deodorising

This is typically the last step in the edible refining process. This step is included in almost all-refining operations regardless of other unit operations used. The deodorisation process is intended to remove the relatively volatile odoriferous compounds from the refined oil. This process involves steam distillation, under vacuum, which results in the removal of residual FFA's, aldehydes and ketones that are responsible for the unacceptable odours

and flavors in the final refined oil. Removal of pigments is through thermal decomposition. These decomposition products, from the pigments, are subsequently distilled off from the final oil product. After the deodorisation process has been completed, the refined oil is cooled in the lower tanks before being pumped for storage. Small quantities of citric acid may be added during the cooling stage as an anti-oxidant to prevent oxidation of the cooled oil (Hui, 1996a).

Deodorisation is the second greatest effluent generating stage following the neutralisation process. Most of the effluent that is produced during this stage consists of the distillate from the oil and contains volatile compounds responsible for the oil's characteristic odour, as well as, any remaining FFA's. A large quantity of water is also used during this stage for cooling purposes, which increases the SWI of the plant. Some of the stripping stream and the remaining FFA water vapors are mixed with the cooling water. After this has been recirculated over cooling towers, it is then discharged down the drain to join the main effluent stream (WRC, 1989).

2.1.1.3 Physical Refining

Physical refining refers to the process whereby the FFA's in the crude or degummed oils are removed by evaporation rather than by being neutralised and, subsequently, removed as soap stock similar to the alkali refining process. The physical refining technique has two main advantages compared with the conventional caustic refining route; reduction in oil losses and the elimination of soapstock and its associated treatment problems.

The physical refining process, however, requires that the feedstock or crude oil be rigorously pretreated to ensure it is free from phosphatides, impurities, trace metals and earth-removable pigments. If these impurities were allowed to remain in the oil, the high temperatures used during the processes would darken the oil and result in a poor quality product. The extent of pretreatment necessary depends on the particular oil type and its quality. Pretreatment of high fatty acid containing oils such as maize and sunflower, prior to physical refining, may comprise the addition of phosphoric acid or citric acid at temperatures of approximately 70°C followed by high-speed centrifugation to remove the hydrated gums. The centrifuged oil is then dried, bleached and winterized before being sent for physical refining. Both continuous and semi-continuous units may be used for

physical refining process. The effluent streams resulting from physical refining are similar in quality to those produced during the degumming and deodorizing stages of caustic refining.

2.1.2 The Acid Oil Plant and its Effluents

The main product of the acid oil plant is the acid oil, which is produced from a feedstock commonly known as soapstock. Soapstock is the byproduct of crude oil neutralisation with caustic soda during the chemical refining process. The resulting oil and water phase have very high concentrations of FOG's, Total Suspended Solids (TSS), Biological Oxygen Demand (BOD), COD loads, as well as, glycerin and FFA's. Due to these high concentrations, most processors include acidulation as part of the integrated facility.

Acidulation is one of the least desirable processes in the integrated facility. The process is rather difficult to perform effectively and it is generally the most cost ineffective process since it has no significant financial returns. The acidulation system is based on gravity separation that can be performed in either a continuous or a batch operation. The process involves collecting soapstock into an equalisation or holding tank. The mixture is heated and then treated with sulfuric acid at a controlled pH of about 2 to 2.2 units. After the reaction with the acid, the mixture enters a series of holding and settling tanks where the oil and the aqueous phases separate. Acid oil is skimmed off the top surface, and then dried. The acid oil may be sold, as is, or may be passed through an evaporative heat exchanger to remove excess water. The product is then sold as a feed supplement or may be used as a feedstock for soap and other industrial applications (Hui, 1996a).

The acid water effluent, produced during acidulation, may be neutralized with lime or other alkaline material prior to its discharge to the main effluent stream. This stream is heavily polluted with high concentrations of COD, BOD, TSS and sulfates. Most of the contaminants in this stream by far exceed the municipal discharge standards.

2.1.3 The Soap Plant and its Effluents

At the industry, the main product of the soap plant is bar soap. No powdered soap is produced or manufactured on site. The soap manufacturing process may be performed as either a batch or continuous operation. The acid oil, which contains fatty acids from the

acidulation phase, is neutralised using strong caustic hydroxide solution. The free fatty acids, which result from the acid oil, react with excess sodium hydroxide to form sodium salts which precipitates out of solution. After centrifugation and further treatment, the soap is then molded into desired shapes before being sold (Hui, 1996a).

The effluent stream produced in this plant is generally known as lye water and usually contains quantities of oils, FFA's, sulfates, and some sodium salts of FFA's. This stream is usually combined with acid oil water prior to its discharge to the final effluent stream.

2.1.4 Wash Down and Miscellaneous Effluents

Wash-down effluents are those effluents that emanate from regular cleaning of the edible oil factory. This cleaning is generally performed after a week's production or between changes of feedstock. Batch equipment may be cleaned after each batch has been processed. The cleaning of vessels is usually performed using live steam and hot water, to ensure maximum use of steam and the reduction in effluent volumes. Floor cleaning is conducted using hot water as necessary, which improves hygiene and reduces manual labor. The fats and oil bearing effluent stream resulting from cleaning is discharged to the main effluent stream via fat traps. The oil rich scum from the fat traps is recovered and recycled for reworking (Hui, 1996a).

2.2 IN-SITE EFFLUENT TREAMENT METHODS EMPLOYED AT COMPANY X IN PIETERMARITZBURG

The three main effluent streams that are generated from the four main plants (see Figure 2.1) are channeled to the on-site effluent treatment plant. This plant is dedicated for treatment or pretreatment of incoming effluent prior to its discharge to the municipal sewer system. The three main effluents are the refinery effluent from the refinery and packaging plants; the acidic effluent from the acid oil plant; and lye from the soap plant. The effluent plant comprises of two big holding tanks, pH correction tank, DAF unit and two settling tanks that are operated in series Fig. 2.1. Acidic effluents i.e. lye and acid water, are pumped and mixed together in the first holding tank. This effluent has a low pH of about 1 - 2 and is highly corrosive to concrete because of its sulfate content. On the other hand the refinery effluent has a high pH content ranging between 12 and 13. This effluent is

pumped to the second holding tank. There are two methods for effluent treatment that are currently being used at the plant. The two effluent treatment methods are pH correction and physical separation of oil and grease using DAF. After DAF treatment, lime and/or ferric chloride is added to the effluent, which is followed by settling in the two settling tanks in order to remove precipitated particles. The resultant effluent is discharged to the sewer system to be treated by Darvill Wastewater Works.

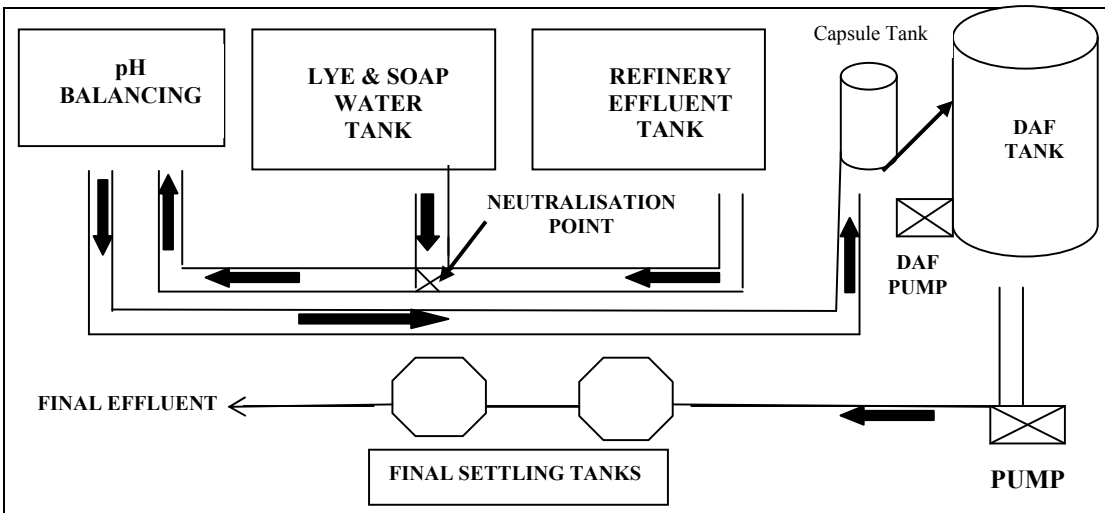


Figure 2.1: Schematic representation of the effluent treatment plant at Company X in Pietermaritzburg (Adapted from Surujlal, 1999).

2.2.1 pH Correction

The effluents from the two holding tanks are mixed together into a single effluent stream. Because of the pH differences between the two effluent streams, the two streams are mixed at different volume ratios by controlling the flow rates from the two tanks such that the desired final pH of between 5.5 and 8.5 is achieved in the final combined effluent stream. This effluent is finally directed to the acid equalisation tank for final pH adjustment. The effluent at this stage may be dosed with a polyelectrolyte such as lime or ferric chloride to coagulate fats and to precipitate phosphates. This effluent is now ready for DAF treatment.

2.2.2 Dissolved Air Flotation (DAF)

Dissolved air floatation process is a separation technique that employs the production of micro size (10-100 μm diameter) air bubbles to separate solids from liquids. A DAF system consists of three main unit processes, which are the pressurization system in the capsule, the floatation tank and the recycle system.

2.3 THE EFFLUENT PROBLEM AND LEGISLATION

Out of an estimated total of 16 edible oil factories in South Africa, only one factory was reported to be treating its effluent completely using biochemical methods. The rest of the edible oil plants generally use DAF for physical removal of FOG and pH correction (WRC, 1989). Even after application of these treatment methods, the remaining emulsified grease tends to clog the sewer pipes and pumps. The high organic (BOD and COD) and phosphorus loads create shock-loading problems for the receiving wastewater treatment installations (Eroglu *et al*., 1990).

At present the Department of Water Affairs and Forestry monitors water resources pollution from point sources by legislating that the effluents from industries must comply with the uniform discharge standards that are set at technologically attainable levels. These controls or regulations limit the rate of deterioration of the receiving water bodies. The National Water Act, 1998 (Act 36 of 1998) is the controlling legislation for the control, development, utilisation and protection of water resources in South Africa. The National Water Act, 1998 replaces the old Water Act, 1956 (Act 54 of 1956), which was motivated by the need for new legislation that would reflect democratic principles and equitable access to water resources by all symbolised by the slogan “Some for all, Forever” (Department of Water Affairs and Forestry, 1997). The Act provides for the compulsory purification of effluents by the user to specified standards and its subsequent disposal in a manner that will make it available for reuse.

The local authorities (the Pietermaritzburg-Msunduzi Transitional Local Council) and the municipal sewage treatment plants (Darvill Wastewater Works) have increased pressure on industries to increase the efficiency of in-house effluent handling and treatment

methods. Therefore industries are becoming more pro-active with regard to viable biological treatment methods which may be implemented on-site to supplement the existing physico-chemical effluent treatment methods.

2.4 THE EUTROPHICATION PROBLEM

Eutrophication is a natural aging process that occurs regularly in lakes over hundred of years and is usually limited to quiescent bodies of water such as lakes and impoundments (Lilley *et al* 1997). The natural eutrophication process results from continuous enrichment of impoundments with nutrients, notably phosphorus and nitrogen. This natural process is however greatly accelerated by human activities in the catchment areas of lakes and impoundments, through the increased input of nutrients (Bolitho, 1976).

Gross eutrophication is marked by massive increase in algal and/or mycrophytic growth in the catchment area. This condition is reached when the inorganic soluble nitrogen and phosphorus load to impoundments attains concentrations in excess of 0.3 mg/L nitrogen and 0.05 mg /L phosphorus, respectively (Lilley *et al.,* 1997). Algal species that are usually associated with eutrophication can be divided into four main groups: the blue green algae (Cyanobacteria), the green algae (Chlorophyta), the Diatoms, and the Flagellate. These organisms are used as effective indicators of eutrophication as they form the majority of species present under eutrophic conditions (Rudd, 1979).

Although algae form an essential part of the aquatic environment, their excessive growth is detrimental to the aquatic ecosystem. When algal blooms die in large numbers and decay a large pool of nutrients is released into the water body, which, results in accelerated growth of other organisms. Consequently, the oxygen content of the water body is depleted and the lower water (hypolimnion) becomes anaerobic due to thermal stratification. This eventually results in eutrophication significantly inhibiting other vertebrate animals in the water column.

Studies on causes and control of eutrophication (Chutter, 1990; Dillon and Molot, 1996) have shown that eutrophication can be effectively controlled if the nutrient load to the receiving waters is strictly regulated. Because of the ability of some blue-green algae to fix

atmospheric nitrogen gas to support primary production, it is therefore virtually impossible to control eutrophication by limiting nitrogen. Thus, in most cases phosphorus has been shown to be the limiting nutrient (Chutter, 1990; Dillon and Molot, 1996; Orhon and Artan, 1994).

In South Africa, eutrophication is enhanced by the long storage times of dams and reservoirs, with resultant accumulation of phosphate and nitrate, and the high summer temperatures, which promote algal growth (Bolitho, 1976). This eutrophication potential is still further enhanced by a rapid increasing population, irregular rainfall often leading to drought conditions; irrigation demands by the agricultural industry; industrial requirements; and loss of source supply due to degradation of rivers (Joska and Bolton, 1994).

In 1980, a special standard was promulgated in South Africa limiting the soluble ortho-phosphate concentration from point source discharges to less than 1 mg/L for certain sensitive catchment areas. A five years grace period was allowed before enforcement, to allow for the phosphorus removal technology to be developed and implemented. This stimulated considerable research in South Africa towards the understanding of the mechanisms that are involved in the biological phosphorus removal process and towards further development and refining the technology for practical implementation (Government Gazette, 1984).

2.4.1 Source of Nutrients (Phosphorus)

Nutrients that cause rapid eutrophication are introduced to the water environment by human activity from both diffused and point sources. In this study more attention was focused on the nutrient phosphorus.

2.4.1.1 Diffused Sources

Phosphorus is introduced to the environment in relatively small concentrations over large areas due to run-offs from rural and urban areas. The widespread use of agricultural fertilizers has a major contribution to this source of phosphorus pollution. In most instances, control and treatment to remove phosphorus from this source is not economically feasible, especially phosphorus originating from rural run-offs. For urban run-offs, natural and artificial reed beds are used as systems for phosphorus removal from

contaminated water. In these systems the soluble phosphorus in waste streams is converted to phosphorus trapped in the reeds that grows in the beds. The entrapped phosphorus in reeds is removed when the reeds are harvested. Some soluble phosphorus is not removed from the reed beds but remains trapped in the sediments lying at the bottom of the reed bed. This soluble phosphorus accumulates continually in the sediments (Lilley *et al.,* 1997)

2.4.1.2 Point Sources

This phosphorus pollution is mainly due to industrial and domestic effluent discharges. In South Africa 80% to 90% of nutrients in water originates from point sources. This type of phosphorus pollution is easy to control because pollutants from the residential and industrial areas are concentrated to a point by means of sewers and treatment methods are readily available (Wentzel, 1992). The edible oil industry in South Africa is both a major water consumer and polluter (WRC, 1989). In anticipation of water shortages in South Africa the then Water Act, (Act 54 of 1956), which was later replaced by the National Water Act, 1998 (Act 36 of 1998), made provision for the compulsory purification of effluents by industries (including municipal wastewater treatment works) to specified standards and its subsequent disposal in a manner that would make it available for reuse (Department of Water Affairs, 1986). To comply with the effluent discharge standard of 1 mg/L phosphorus, Darvill Wastewater Works is increasing pressure on the industries around the Pietermaritzburg area to implement cleaner production technologies and some form of an in-house effluent treatment system prior to discharge to the sewer system. As a result, the current study was initiated in conjunction with the edible oil industry to find a biological solution to reduce the phosphorus load in the effluent, prior to its discharge to Darvill Wastewater Treatment Works for final purification.

2.5 TREATABILITY OF EDIBLE OIL EFFLUENT

Food processing effluent including effluent from the edible oil processing industry is a complex mixture of floating, settleable, suspended and dissolved materials. Complete treatment of these effluents requires a combination of physical, chemical and biological treatment processes (Dalzel, 1994). The physical nature of fatty material is of great concern when considering any purification method. The fatty contaminants in the edible oil

effluent can be characterized in three ways viz.: by polarity, biodegradability and physical characteristics. Polar contaminants are usually derived from animal and vegetable sources such as food industry operations including edible oil processing industry. Where as, the non-polar contaminants are derived from petroleum and mineral sources and are generally non-readily biodegradable (Grant, 1980; Mcdermott, 1982; Sutton *et al.,* 1994)

It used to be a common practice to group effluents from the edible oil processing industry, which has polar and readily biodegradable fatty components, with effluents arising from the petrochemical industry, which is non-polar and non-readily biodegradable. As a result of this joint grouping of petrochemical effluents and the effluents from the food industry, the vegetable oil processing industry has been widely targeted as problematic (Grant, 1980).

2.5.1 Physical Treatment

The effluent from edible oil processing industry carries an appreciable amount of fatty material or FOG. Prior to any form of treatment, it is desirable to install an oil/water separation system as the first phase. This will reduce the pollution load being discharged and, also, facilitate the recovery of re-usable fat. The separation of the water and oily phases from the edible oil effluent can be archived using two simple methods, which are gravity settling and DAF process.

2.5.1.1 Gravity Settling or Fat Traps

The removal of fatty matter or FOG has for many years been achieved through fat traps. The gravity fat trap is usually installed as standard equipment on all process effluent streams and is the simplest form of physical treatment (Eroglu *et al.,* 1990). Fat traps are designed to produce a slow and gentle uniform flow through a tank, which allows density differences to bring the fatty material to the surface without disturbing any accumulated scum and sludge. Fat removal can then be done by using surface scraping mechanisms (Dalzel, 1994; Grant, 1980).

Fat traps are designed according to general settlement principle. According to Dalzel (1994) a typical fat trap has a length to width ratio of 2:1, a retention time of 10 - 40 minutes and the loading of 0.4$m^3/m^2/h$ to 3 $m^3/m^2/h$ at maximum flow rate. The problem

with fat traps is that they are expensive and occupy a large surface area, which makes them unsuitable for many industrial applications, especially for small industrial establishments (Dalzel, 1994; Grant, 1980). The disadvantage of using gravity fat traps is that they are unable to reduce the emulsified fatty material content of wastewater to under 500 mg/L, the maximum concentration permitted to be discharged to municipal sewer systems (Eroglu *et al.,* 1990).

2.5.1.2 Dissolve Air Floatation (DAF)

A common problem with fat containing effluents is precipitation and emulsification due to pH, temperature, pumping and detergents. Under these conditions, gravity separation with a simple fat trap rarely gives satisfactory results and an alternative that is often used is assisted floatation, in this case, DAF.

- Theory of Operation

Sedimentation is the separation of solids from liquids under gravity. Floatation is the separation of solids from liquids by means of buoyancy. DAF systems are, therefore, suitable for the removal of substances such as oils that do not settle by gravity due to their low settling velocity (Kiely, 1997).

There are three types of DAF systems: vacuum floatation, micro-floatation and pressure floatation. Pressure floatation is the most widely used (Letterman *et al*., 1999).

In pressure floatation, air is dissolved in water under a pressure of several atmospheres. The pressurised water then enters the floatation tank, containing the effluent or wastewater sample, by means of a pressure-release device. Different pressure-release devices may be used, such as: nozzles, needle valves or gate valves. In the pressure-release device, the pressure of the water is reduced to atmospheric pressure (Letterman *et al*., 1999). The sudden reduction in pressure results in the dissolved air leaving the water as fine air bubbles (Qasim, 1994). The bubble size is approximately 10 – 100 *u*m in diameter (Letterman *et al*, 1999).

High pressures produce small bubbles but at pressures higher than 500 kPa (5 Bar), there is no decrease in bubble size (Heinanen *et al*., 1992; De Rijk *et al*., 1994 as cited in

Edzwald, 1995). A pressure of between 400 and 600 kPa (4 and 6 Bar) is recommended for the production of small bubbles (Edzwald, 1995).

Following pressurization and release of the water, fine air bubbles enter the floatation tank containing the wastewater. The floatation tank is divided into two zones: the contact zone and the separation zone. In the contact zone, there is opportunity for the contact between the fine air bubbles and the solid particles. This leads to the formation of "bubble-solid agglomerates" which are less dense than water. In the separation zone, the bubble-solid agglomerates, due to their lower density, rise to the surface of the floatation tank allowing for the separation of the solid particles from the wastewater (Letterman *et al.*, 1999).

The agglomerates that collect on the surface of the floatation tank are called float and may be removed either by flooding or by mechanical scraping. With flooding, the water level in the floatation tank is raised in order that the float and water overflow into a float collection trough. Mechanical scraping involves the movement of a scraper, with rubber blades, over the surface of the floatation tank, which pushes the float into a collection channel. The clarified wastewater can then be removed from the bottom of the floatation tank (Letterman *et al.*, 1999).

- Enhancement of DAF using coagulation and Flocculation

A. Coagulation

The objective of coagulation is to promote the settling of solids that are suspended in the wastewater. Coagulation achieves this settling by utilising chemical coagulants that promote agglomeration of the solids (Kiely, 1997).

Coagulation may be described as a destabilisation process. This is because the chemical coagulant used alters the electrostatic charge of the particles so that they cease to repel each other, but rather, agglomerate or attach to one another. This increases their size and density resulting in an increase in their settling velocity. The particle suspension is no longer said to be stable since the particles agglomerate and destabilisation is said to have taken place (Kiely, 1997).

After having pretreated the wastewater by the addition of a coagulant, the wastewater still requires further treatment before being subjected to DAF. Flocculation is required, to facilitate the formation of large agglomerates or flocs (Letterman *et al.*, 1999).

B. Flocculation

Flocculation is the agitation, or mixing, of the wastewater either mechanically or by diffused aeration (Parker, 1975). The purpose of flocculation is to promote, by slow mixing of the wastewater, the interaction and agglomeration of particles destabilised by the addition of a coagulant, resulting in the formation of large flocs (McGhee, 1991).

Following the pretreatment of the wastewater - in the floatation tank – by means of coagulation and flocculation, fine air bubbles are introduced into the floatation tank. The bubbles make contact with the coagulation-agglomerates which have settled to the bottom of the tank. These results in bubble-solid agglomerates which rise to the surface of the tank due to their lower density compared with water.

Coagulation and flocculation may therefore be used to increase the amount of suspended particle removal by the DAF system.

Other types of pretreatment used in this study include:

2.5.2 Ozonation

2.5.2.1 Theory of Operation

Ozone reacts readily with substances comprised of unsaturated bonds, readily oxidising them. However, it does not as react readily with saturated substances, as there are no easy chemical pathways to follow (Kloos, 2000). Ozone reacts in three ways when introduced to organic materials.

A. Direct oxidation of ozone. This occurs at low pH and is slow and very selective.
B. Indirect oxidation of ozone whereby free radicals are formed. These are highly reactive and may lead to auto oxidation of organic matter. This occurs at high pH values and is fast and non-selective.

C. Ozonolysis where all three oxygen atoms are added at the site of a double or triple bond resulting in the production of ozonides. Decomposition of these ozonides at the position of the unsaturated bond forms aldehydes, ketones and acids (Hesby, 1998).

2.5.2.2 The Advantages and Disadvantages of Ozone shown in Table 2.1.

Table 2.1 The Advantages and Disadvantages of Ozone

ADVANTAGES	DISADVANTAGES
Generated on site.	Relatively costly.
Rapidly decomposes to oxygen leaving no traces	Produces biodegradable material that must be controlled
Reactions in potable water do not produce toxic compounds	Produces bromate in bromide rich waters

Sources: White, 1999, Singer and Reckhow, 1999 & Spellman, 1999.

2.5.3 Peroxone

When ozone is used as the solitary oxidant, the process is slow and very selective. However, the supplementation of hydrogen peroxide to the ozonation process results in the autocatalytic decomposition of ozone. This process is less selective, faster and more powerful than ozonation (Wentworth, 2001).

2.5.3.1 Theory of Operation

Peroxone is based on the use of ozone with hydrogen peroxide to produce hydroxyl radicals that oxidise most organic contaminants in solution (Arce Systems, 2000). Oxidation by peroxone is due to two reactions, namely:

1. Direct oxidation of compounds by ozone.
2. Oxidation of compounds by hydroxyl radicals produced by the decomposition of ozone (Wentworth, 2001).

Peroxone oxidises saturated organics to produce aldehydes, ketones, peroxides, bromate ions and biodegradable organics (Arce Systems, 2000).

The difference between the ozonation and peroxone procedure is that ozonation relies heavily on direct oxidation of aqueous ozone while peroxone relies on direct oxidation with the hydroxyl radical. In the peroxone process the ozone molecule is short lived. However, the increase in hydroxyl molecule concentration is greater than the decrease in ozone; therefore, peroxone oxidation is faster and more reactive than ozonation (Wentworth, 2001).

2.5.3.2 Advantages of Peroxone

Peroxone can be applied both in and ex-situ and removes taste, colour and pesticide residuals (Wentworth, 2001).

2.5.4 Ultrasonication

2.5.4.1 Theory of Operation

High-energy ultrasonics are stress waves which means that they create a stress or deformation of the medium through which they are passing (Encyclopaedia Britannica, 1983). The effects of these waves are irreversible and arise from a phenomenon referred to as cavitation (Blitz, 1963). When added to an aqueous medium, the sound produced acts as a source of vibration, causing the molecules to vibrate, which alternately compress and stretch the molecular structure (Dirkgroup, 2000). The compression and stretching causes pressure changes. Cavitation occurs when, in a liquid, the ultrasonic pressure exceeds the normal average pressure causing the pressure in the liquid to fall below zero. This results in ruptures in the liquid, facilitating the formation of small cavities, which, as the pressure increases, expand to form bubbles. As the pressure decreases, the bubble becomes unstable and collapses. At the end of the collapse, gas within the cavity will be extremely compressed. To relieve the pressure, shock waves will be released (Blitz, 1963). These waves have the ability to promote chemical reactions in certain liquids e.g. the depolymerisation of a high polymer substance (Encyclopaedia Britannica, 1983).

2.5.5 Chemical Treatment

Chemical treatment and/or pretreatment can, in many instances, improve the performance of physical and biological processes used in effluent treatment. The most commonly used chemical methods for effluent treatment are pH correction and coagulation to improve

settlement rates by increasing particle size density (Dalzel 1994; Lilley *et al.,* 1997; Eroglu *et al.,* 1990).

In South Africa, all but one edible oil manufacture uses chemical treatment as the sole effluent treatment method to reduce the pollution load prior to discharge to the municipal sewer system or receiving river body (WRC, 1989). Chemical treatment is used mainly for carbonaceous and phosphorus removal from wastewater. Lime is the most widely used chemical coagulant for both COD and phosphorus reduction (Lilley *et al.,* 1997). Chemical phosphorus removal through phosphate precipitation can also be achieved using iron (Fe^{3+}) and aluminum (Al^{3+}) salts such as ferrous sulphate; ferric sulphate; ferric chloride; aluminum chloride; and aluminum sulphate (Dalzel, 1994; Loots *et al.,* 1994; Lilley *et al.,* 1997; Grant, 1980).

At the edible oil industry, lime and ferric chlorides are added to the neutralized effluent up-stream of the DAF unit. Eroglu *et al.,* (1990) reported that ferric chloride was the most effective coagulant during physicochemical treatability studies, and that it resulted in a BOD_5 reduction of 36%.

2.5.5.1 Advantages of Chemical Treatment

Chemical phosphorus removal in wastewater is reliable, and with strict control, a consistently low effluent phosphorus concentration can be achieved (Lilley *et al.,* 1997). Chemically bound phosphorus is not easily dissociated in water, which prevents the release of the bound phosphorus back into the water body (Loots *et al.,* 1994). When alum is used as a coagulant, it is possible to recover both aluminum and fatty material from fat/alum flocks through acid splitting (Pope *et al.,* 1975 as cited from Grant, 1980).

2.5.5.2 Disadvantages of Chemical Treatment

The chemicals that are used during chemical treatment are corrosive in nature (strong oxidizing agents) and hence great care is required when handling them, which, in turn, necessitates the use of expensive equipment that is resistant to corrosion (Lilley *et al.,* 1997) Chemical treatment of effluent, is expensive due to the high price of chemicals. Pitman, (1991) estimated (10 years ago) that chemicals would cost the Johannesburg City council approximately 10 million rand per year if they were to continue using chemical

treatment alone. An additional problem associated with chemical usage, is the increased mineralization of water through the release of ions. Chemical coagulants usually contain chlorides or sulphates, which remain in solution, thus increasing the conductivity or the salinity of the receiving water body.

2.5.6 Biological Treatment

Biological treatment technology offers an efficient and cost-effective means for treating edible oil industrial wastewater. Biological treatment of edible oil effluent may be carried out either aerobically, anaerobically or using the combination of both (Hui, 1996b; Eroglu *et al.,* 1990, Grant, 1980, Seng, 1980).

2.5.6.1 Anaerobic Treatment Process

Anaerobic digestion has long been practiced as a stabilization process for waste sewage treatment sludges, but the process has not been widely adopted for effluent treatment, with only very high strength industrial effluent being seriously considered (Grant, 1980). The effluent from the vegetable oil refining industry is loaded with sulphates, fats and organic matter, which makes anaerobic treatment an attractive alternative prior to any aerobic treatment process. Studies by Eroglu *et al.,* (1990) have shown that lime addition and activated sludge treatment does not bring about appreciable decrease in the sulphate concentration from the acidic effluent.

In the anaerobic digestion of high strength industrial waste containing high level of sulphate, the two process of concern are sulphate reduction and methane production, the latter being inhibited by the former (Eroglu *et al.,* 1990). In their study, Eroglu *et al*., (1990) reported a 60% sulphate reduction in an anaerobic filter reactor with a concurrent reduction of 60% for COD. From this study the conclusion was that anaerobic treatment of acidic effluent from the edible oil refining industry might be used to replace chemical treatment as a pretreatment step.

2.5.6.2 The Activated Sludge Treatment Process

The activated sludge treatment is generally considered to have its origin in the aeration experiments which were carried out by Ardern and Lockett at Manchester in 1914 (Droste,

1997) Activated sludge is a suspended growth system which could be defined as a suspension of microorganisms, both dead and living, in wastewater.

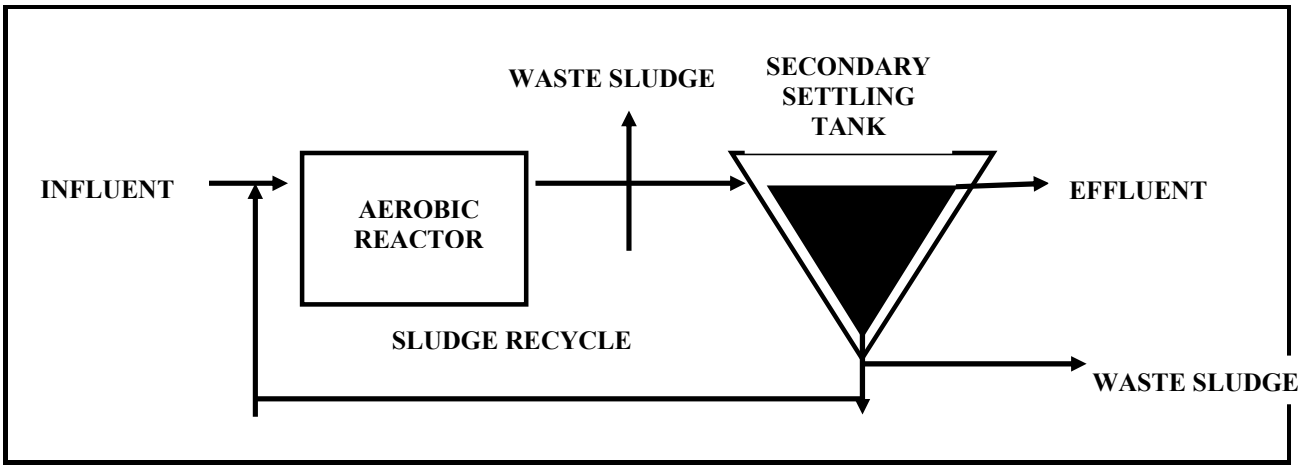


Figure 2.2: A diagrammatic representation of a conventional completely mixed activated sludge system with hydraulic control of sludge age and recycle.

Since its inception, the activated sludge process has become one of the main methods used world-wide for the purification of wastewaters that contain biodegradable organic compounds, for example, domestic sewage (Horan, 1990; Droste, 1997). The conventional activated sludge treatment process involves two distinct operations usually performed in two separate basins; aeration in the aeration chamber and settling in a secondary settling tank (Fig. 2.3)

The principal unit in the activated sludge process is the aeration tank or the biochemical reaction vessel. The content of the aeration tank comprises of an aerated mass of microorganism conglomerates, termed flocs, surrounded by the influent wastewater; this combined mixture of flocs and raw wastewater is termed the mixed liquor. The activated sludge flocs are aggregates of microorganisms, inorganic and organic colloidal material and larger particulate matter, all held together in a compact organic matrix. A large number of protozoa, free-swimming ciliates and flagellates are found both in the mixed liquor and in the flock matrix (Horan, 1990). The flocs are mixed with incoming sewage in the presence of oxygen, which is supplied through aeration. In the aeration basin, some of the substrate is completely oxidized into harmless end products of carbon dioxide, water, and other inorganic substances that are required to provide energy for the growth of microorganisms (Horan, 1990; Bitton, 1994; Droste, 1997).

The second phase in the activated sludge treatment process is the separation of the biomass and other suspended solids from the treated wastewater. This is accomplished in a secondary settling tank. Under the quiescent conditions of the secondary settling tank the activated sludge flocs settle rapidly to yield sludge with high concentrations of solids. A certain amount of the settled sludge is recycled back to the aeration tank while the remainder is removed from the system through wastage on a continuous or intermittent basis. Consistent wastage of sludge ensures that the concentration of biomass in the aeration chamber remains constant within the desired range (Droste, 1997; Qasim, 1994, Horan, 1990). The clarified effluent at this stage is relatively devoid of any suspended particles and may be discharged into a watercourse after tertiary treatment, which includes chlorination or irradiation.

Optimal operation of a conventional activated sludge process depends on the manipulation of the three basic design parameters, which are: organic loading rate (B_x) or the sludge age (R_s); maintaining the correct mixed liquor volatile suspended solids (MLVSS) value and dissolved oxygen (DO) concentration in the mixed liquor (Horan, 1990). B_x is frequently used as a key design parameter, since its use allows the determination of the required biomass without the need to make reference to the process kinetics coefficients. The choice of limiting values that may be assigned to the main design parameter to achieve a particular level of performance in the purification of a particular wastewater is most reliably made through pilot plant operation. The final quality of the treated effluent is always a key consideration in the design and operation of any activated sludge process although other operation factors or parameters also influence the choice of R_s and B_x. The more important factors are the degree of stabilization of the sludge biomass to be archived and the requirement to produce a sludge biomass with good settling properties (Horan, 1990, Qasim, 1994)

2.6 BIOLOGICAL NUTRIENT REMOVAL TREATMENT PROCESS

Biological nutrient removal (BNR) refers to the removal of primary nutrients (carbon, nitrogen and phosphorus) from wastewater that could, subsequently, cause eutrophication (Ekama and Wentzel, 1997; Lilley *et al.,* 1997). Nutrient removal is accomplished by manipulating the activated sludge process configuration to create environmental

conditions that are conducive to the optimal growth and activity of the microorganisms responsible for the removal of nutrients from wastewater (Wentzel and Ekama, 1997). BNR is mediated by a highly diverse mixed culture that develops in the modified activated sludge process. These mixed cultures work in sequence to remove different components at different stages of the process, that is, some lie dormant while others are actively metabolising (Wentzel and Ekama, 1997).

2.6.1 Carbonaceous Energy (COD) Removal

Carbon in wastewater streams occurs in organic and inorganic forms. Heterotrophic organisms use organic compounds for their metabolism while the inorganic compounds are metabolized by a group of organisms collectively termed autotrophs. Both forms of carbon are removed from wastewater through a series of oxidation and reduction (redox) reactions, oxidizing the carbon source to carbon dioxide and water. The carbon dioxide then escapes to the atmosphere, thus removing carbon from wastewater.

The energy content of wastewater can be expressed using common substrate parameters, which are 5-day biological oxygen demand (BOD_5) and/or COD (Orhon *et al.,* 1999; Lilley *et al.,* 1997). The COD test is the mostly used as a monitoring parameter at treatment works since it is quicker and gives a more accurate reflection of the energy content of the system than the BOD_5 test (Ekama *et al.*, 1984; Orhon *et al.,* 1999; Lilley *et al.,* 1997)

In a BNR process, the carbonaceous material or the COD content of the system is divided into three main forms viz. non-biodegradable; biodegradable; and heterotrophic active biomass (Wentzel *et al.,* 1995; Orhon *et al.,* 1999; Dold *et al.,* 1991). The non-biodegradable COD has two sub-fractions, the non-biodegradable particulate and the non-biodegradable soluble. The biodegradable COD, also, has two sub-fractions, the slowly biodegradable (SBCOD), and the readily biodegradable (RBCOD) fractions. Both the RBCOD and SBCOD fractions are based wholly on the dynamic response observed in an activated sludge system (Dold *et al.,* 1980, as sited from Wentzel *et al.,* 1995),

2.6.2 Biological Phosphorus Removal

Biological phosphorus removal is a well-documented phenomenon that is used to reduce phosphorus in wastewater. Soluble ortho-phosphate in wastewater is converted into stored phosphorus (trapped) in the biological sludge mass of the activated sludge system. The stored phosphorus is then removed from the system with the sludge that is wasted daily (Ekama *et al.*, 1984; Bdjanovic *et al.,* 1997). Excess biological phosphorus removal (BEPR) refers to the biological uptake and subsequent removal of phosphate by the activated sludge in excess of the amount that is removed by "normal" completely aerobic (or conventional) activated sludge systems. This process results from the cooperation of different groups of bacteria, primarily fermentative bacteria, phosphorus- accumulating bacteria (poly-P bacteria), other heterotrophs, and autotrophs (Danesh and Oleszkiewcz, 1997). Traditionally, bacteria of the genus *Acinetobacter* spp. were considered to be mostly responsible for the BEPR process (Fuhs and Chen, 1975; Buchan, 1981; and 1983). Other studies (Lötter and Murphy, 1985; Sidat *et al.,* 1999; Atkinson, 1999) have shown that, in addition to *Acinetobacter* spp., other bacterial species including Gram-positive, *Pseudomonas* spp., have the propensity to accumulate phosphorus in excess of their metabolic needs.

To stimulate the growth and dominance of the poly-P organisms in an activated sludge system to facilitate excess biological phosphorus removal, two conditions are required, which are:

(a) An anaerobic/aerobic sequence of reactors
(b) Presence of VFA's or short chain fatty acids (SCFA) (e.g. acetic acid) in the anaerobic reactor (Comeau *et al.,* 1986; Wentzel *et al.,* 1986; Bdjanovic *et al.,* 1997; Lilley *et al.,* 1997).

These organisms will be referred to here as poly-P organisms compared with non-poly-P organisms which cannot accumulate phosphorus beyond their metabolic requirements.

A number of mainstream processes have been developed for both nitrogen and phosphorus removal. These processes have evolved from the activated sludge process through the incorporation of the anaerobic sludge mass fraction (anaerobic and/or anoxic zones) at the upstream end of the aerobic zone. In South Africa, the 5 stage Bardenpho

process, 3 stage Phoredox process, the Johannesburg process, UCT process, and the Modified UCT processes are the wastewater treatment process that are commonly used for domestic wastewater treatment (Figures 2.3a, 2.3b and 2.4c) (Lilley *et al.,* 1997). The general mechanism of the BEPR process is discussed in detail in the following section.

The biological phosphorus removal processes are based on the circulation of the activated sludge biomass through a sequence of anaerobic and aerobic conditions or reactors (Kuba *et al.,* 1993). The exposure of the activated sludge to the alternating conditions, stresses the poly-P organisms such that their release and uptake of phosphorus is above the normal levels required for metabolism. The phosphorus present in wastewater is not only used for cell maintenance, synthesis, and energy transport, but is also stored for subsequent use by the poly-P organisms (Metcalf and Eddy, 1991).

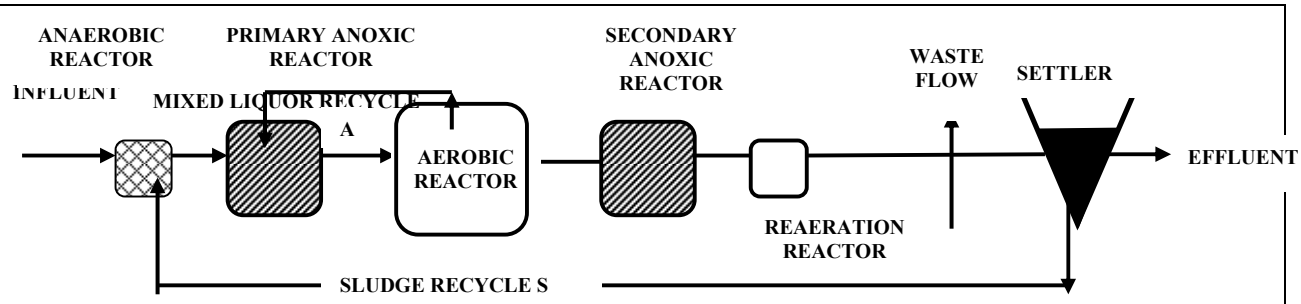


Figure 2.3a: The Phoredox process for biological nitrogen and phosphorus removal

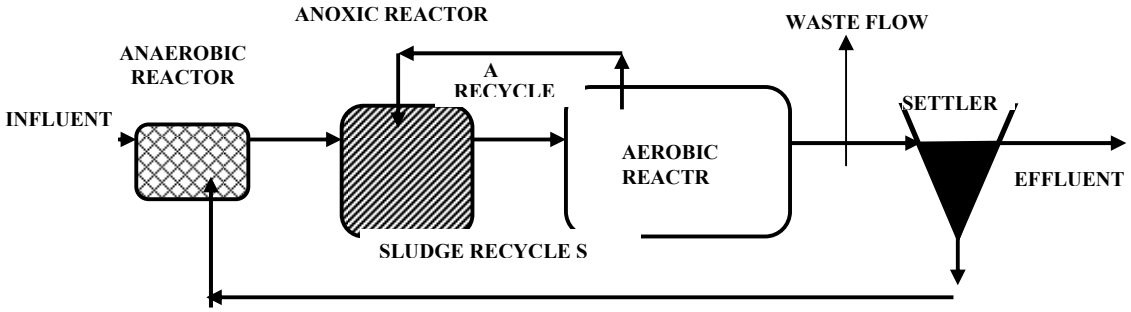


Figure 2.3b: The 3 stage Phoredox process for biological nitrogen and phosphorus removal

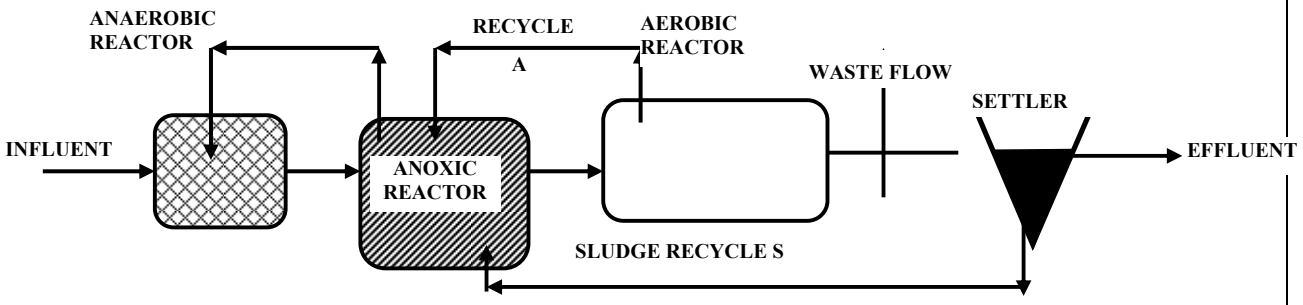


Figure 2.3c: The UCT process for biological nitrogen and phosphorus removal

2.6.3 Mechanisms of excess Biological Phosphorus removal

2.6.3.1 The Anaerobic Zone

The anaerobic zone (or reactor), in the non-conventional activated sludge system, is characterised by a lack of nitrates (NO_3), nitrites (NO_2) and dissolved oxygen (DO), and has two primary functions (Ekama and Marais, 1984):

(i) To stimulate the conversion of RBCOD into VFA's through fermentation by the non-poly-P organisms (heterotrophs).
(ii) Enables the poly-P organisms to take up and store VFA's as polyhydroxyalkanoate (PHA), a process termed sequestration.

The prerequisite for the BEPR process to take place is the presence of VFA's in the anaerobic reactor, up stream of the aerobic zone (Wentzel *et al.,* 1988). Upon entering the anaerobic reactor, the RBCOD fraction of the total influent COD is rapidly absorbed by the non-poly-P bacteria. Because of the absence of the terminal electron acceptor (O_2 and NO_3) in the anaerobic zone, these organisms are unable to metabolize the absorbed RBCOD to energy and for new cell synthesis. The RBCOD is then converted to VFA's through fermentative processes, which provide the non-poly-P organisms with very low energy yields.

The released VFA's are then taken up by the poly-P organisms and stored internally through the polymerization of VFA's into long chain carbon molecules called PHA, specifically, polyhydroxybutyrate (PHB). Once stored internally, this substrate is no longer available to other heterotrophic organisms, and is reserved by the poly-P organisms for their exclusive use in the subsequent aerobic reactor. The energy for substrate transfer and storage is derived from the hydrolysis of the energy rich polyphosphate (poly-P) bonds within the poly-P bacteria (stored-P). The hydrolysis of the poly-P molecules, in the anaerobic zone, leads to the release of phosphorus into the environment. This phenomenon is known as Phosphate-release (P-release). The uptake and storage of VFA's with the subsequent P-release by the poly-P bacteria is called sequestration. The phosphorus release associated with the transfer and storage of VFA's is referred to as

primary release, whereas, P-release that is not associated with VFA uptake is called the secondary release (Danesh and Oleszkiewicz, 1997). According to Wentzel *et al.,* (1990), 1 mole of P is released per mole of VFA sequestrated, that is, 0.5 mg P is released per mg VFA (as COD) sequestrated. Thus in the anaerobic reactor, ordinary heterotrophic bacteria convert the RBCOD to VFA's, which are then taken up by the poly-P bacteria and store as PHB (Wentzel *et al.,* 1990; Ekama and Wentzel, 1997, Banister and Pretorius, 1998).

2.6.3.2 The Aerobic Zone (Reactor)

In the subsequent aerobic reactor down stream of the anaerobic zone, the poly-P organisms are unable to compete with non- poly-P organisms for substrate (food source) such as glucose or other saccharides. The inability of the non-poly-P organisms to compete for food source in the aerobic zone/reactor is due to the presence of the terminal electron acceptor (O_2) (Lilley *et al.,* 1997). The poly-P organisms then use the stored PHB as a carbon and energy source for cell function and for the generation of new cells. The poly-P organisms also utilize the stored PHB as an energy source for taking up phosphate from the bulk of the solution. The uptake of phosphate helps to remanufacture the poly-P that was broken down in the anaerobic zone and store poly-P in the new cells that are generated. This gives rise to the phenomenon known as excess phosphate uptake, which occurs in the aerobic environment. The uptake of phosphate to manufacture cellular polyphosphate in the daughter cells results in phosphate being taken up in the aerobic reactor in excess of the phosphate that was released in the anaerobic reactor, thus resulting in the net phosphate removal from the liquid phase to the activated sludge biomass (Wentzel *et al.,* 1988; Mino *et al.,* 1998). Thus in the aerobic reactor, PHB is metabolised by the poly-P organisms and phosphate is accumulated above concentrations required for their metabolic needs (net phosphorus removal).

The uptake and storage of phosphate by the poly-P bacteria, at this stage, does not amount to phosphate removal from the system, but a mere displacement of phosphate from the wastewater to the biomass. Phosphorus is finally removed from the system through daily sludge wasting from the aerobic reactor or from the return sludge line. Wentzel *et al.,* (1990) reported that, at steady state, the biomass of poly-P wasted per day equals the mass of new poly-P organisms generated per day. Thus, the mass of poly-P

organisms in the biological system remains constant, which implies that, in the activated sludge process at steady state, there is neither a build up nor loss of poly-P organisms (Wentzel *et al.,* 1990).

There are two factors that must be borne in mind when designing and operating a BEPR system and are based on the abovementioned mechanisms viz:

1 RBCOD - the magnitude of phosphorus removal that can be achieved is directly related to the amount of RBCOD present in the influent.
2 Recycling of oxygen and nitrite to the anaerobic zone.

The introduction of oxygen and/or nitrates/nitrites to the anaerobic reactor through the sludge recycle (S-recycle) should be avoided since the non-poly-P organisms tend to rapidly metabolise the RBCOD in the presence of a suitable terminal electron acceptor such as oxygen. For example, for every 1 mg O_2 recycled to the anaerobic reactor, 3 mg COD as RBCOD will be consumed. The same applies for nitrogen, for every 1mg N as NO_3- recycled, 8.6 mg COD as RBCOD is consumed by the non-poly-P organisms for energy generation and new cell synthesis. This results in the RBCOD that is consumed by heterotrophs being unavailable for conversion to VFA's during the fermentation process. Hence the amount of VFA's generated and released to the solution will be reduced by the same amount of RBCOD that was consumed by the heterotrophic bacteria.

2.6.4 Historical Development of Prefermentation

2.6.4.1 Prefermentation Technology

The production of VFA's (mainly acetic and propionic acids) from domestic or industrial wastewater by subjecting the wastewater to anaerobic conditions for a period of time is known as fermentation. Wastewater fermentation has been occurring (unintentionally) ever since wastewater was transported in sewers with long residence times, or wherever wastewater was held under anaerobic conditions.

The biological processes involved in prefermentation are the same as those in anaerobic digestion, especially the first phase of the anaerobic degradation process, the acid-production phase.

During this phase in prefermenters VFA's are produced by bacteria and this phase consists of two processes, hydrolysis and acidogenesis. Hydrolysis is a process whereby particulate or high molecular weight soluble substrates are broken down to smaller molecules by the incorporation of water molecules. Hydrolysis is catalyzed by hydrolytic enzymes secreted by bacteria. Both the substrate and operational factors affect VFA product distribution and speciation. However, for prefermenters that are fed with raw wastewater or primary sludge, acetic acid is usually the most prevalent VFA produced, followed by propionic acid (Munch, 2000).

The second phase consists of two main processes; acetogenesis and methanogenesis.

Acetogenesis, or anaerobic oxidation of SCFA's, results mainly in the formation of acetate and hydrogen gas. Acetogenesis cannot be encouraged without also encouraging methanogenesis. This is unfortunate, as acetogenesis by itself would lead to "higher-quality" VFA's for the BNR process such as acetic acid.

Methanogenesis is the final step of anaerobic digestion, and results in the formation of methane from the decarboxylation of acetate and the reduction of carbon dioxide and hydrogen. Acetate is the prime precursor for methane production, which contributes to about 70% of the total methane produced (Munch, 2000).

2.6.4.2 Pre-fermenter Technology

Full-scale prefermenters that are fed with primary sludge are referred to as "side-stream prefermenters". Primary sludge is sometimes also called raw sludge and is the concentrated underflow of the primary clarifier.

2.6.4.3 Pre-fermenter Configurations

The prefermenter that is considered to be 'optimal' is one that consistently produces the desired amount of VFA's, is cheap to build and operate; and poses few operational

problems. To obtain these objectives, a number of different prefermenter configurations have been proposed, each of which represents a compromise between reliability of VFA production and associated costs. The prefermenter configurations can be classified according to the number of tanks needed for their realization. The most commonly used configurations are:

A. *Activated Primary Tanks (APT)*
B. *Single-stage Prefermenters*
C. *Two-stage Prefermenters*

2.7 CONCLUSIONS FROM THE LITERATURE REVIEW

Wastewater treatment methods were developed in response to the concern for public health and the adverse conditions caused by the discharge of these waters to the environment. Edible oil industries generate large volumes of effluent during the refinery process. Conventional effluent treatment technology has its limitation with regards to providing good quality final effluent. It is for this reason that various investigations using different types of biological treatment methods for degradation of edible oil effluent was warranted.

CHAPTER 3
(A) PRE-FERMENTATION

3.1 INTRODUCTION

Conventional processes for the removal of nitrogen and phosphorus from wastewater rarely attain the levels now required by environmental protection authorities unless supplemented with chemicals. Prefermentation technology enables conventional nutrient removal plants to achieve high nitrogen and phosphorus removal to levels well below international environmental standards without the costs involved in chemical addition for effluent filtration (Treatment of Wastewater, 2000). Fundamental data and full-scale plant evaluations indicate that fermentation of primary solids provide a VFA stream of sufficient strength to enhance biological phosphorus removal. Effluent total phosphorus concentrations in the range of 0.2 - 0.5 mg/L are possible with this technology compared to effluent total phosphorus concentrations in the range of 1 - 3 mg/L for systems without primary solids fermentation or chemical addition (Sedlak, 1991).

Prefermentation is a new unit of operation in Biological Nutrient Removal (BNR) wastewater treatment systems with the role of producing VFA's which can assist in the BNR process. Normally, the carbon source in the process is obtained from the raw wastewater feed (Munch, 2000). Therefore; prefermentation can increase phosphorus removal rates by facilitating the production of soluble organic products that are required as energy and carbon sources by the biomass responsible for nutrient removal. During the anaerobic degradation phase, in prefermenters, VFA's are produced by fermenting bacteria by two main processes; hydrolysis and acidogenesis. Therefore increasing the mass of substrate available to the phosphorus removing bacteria is achieved by conversion of primary sludge solids to VFA's which are subsequently made available to the anaerobic zone microorganisms of a biological phosphorus removal process. The amount of phosphorus that can be removed per unit of VFA or acetate generated (or added) in the anaerobic zone is a function of the cell yield and net amount of phosphorus stored in the wasted biological mass (Sedlak, 1991).

However, previous research done using prefermentation technology focused on the treatment of domestic or municipal wastewater. This technology has not yet been applied to treat edible oil wastewaters, so this project was aimed at determining the optimum time for VFA production, total solid concentration, adjusted and natural pH effect, and mixing/ non-mixing effect to increase the VFA production rate in order to enhance biological phosphorus removal from edible oil effluent.

3.2 AIMS AND OBJECTIVES

The aim of this study was to achieve optimised conditions for prefermentation in order to obtain the highest concentration of VFA's to improve biological phosphorus removal from edible oil effluent. The objectives were as follows:

1. To determine the optimum time for maximum VFA production;
2. To determine the optimum total solids concentration;
3. To evaluate the effect of adjusted and natural pH; and
4. To evaluate the effect of mixing as opposed to non-mixing.

3.3 MATERIALS AND METHODS

3.3.1 Optimisation of Total Solids (TS) Concentration

Experimental work was carried out in 4 x 1 L conical flasks as batch reactors on a shaker. The purpose of these batch experiments was to determine the TS concentration at which there was maximum VFA production. Primary sludge from Durban Northern Waterworks was diluted and inoculated in to three of the flasks at different solids concentrations i.e., 1 500, 3 000 and 5 000 mg/L respectively. The fourth flask was used as a control with only 800 mL of refinery effluent. Refinery effluent was then added to the experimental flasks to obtain a final working volume of 800 mL. The experiment was carried out over 15 days. Samples of 25 mL aliquots were taken daily and analysed for VFA production. pH and temperature were monitored periodically. For one test trial the refinery effluent was left at its natural pH of approximately 10 - 11 and another was conducted by adjusting the pH to approximately 7. (See Appendix 1 for VFA determination)

3.4 RESULTS AND DISCUSSION

Wastewater characteristics prior to and after treatment are summarized in Table 3.1.

Table 3.1: Wastewater Characteristics

PARAMETER	INFLUENT (MEAN VALUES)	PAF-EFFLUENT (MEAN VALUES)
pH	6.9	7.4
$COD_{soluble}$ (mg/L)	1 555	833
VFAs (mg/L)	1	15
ALKILINITY (mg $CaCO_3$/L)	79	51

The concentrations of VFA's produced over 10 days can be seen in Figure 3.1, where 8 - 9 days showed the greatest VFA production at a total solids content of 3000 mg/L.

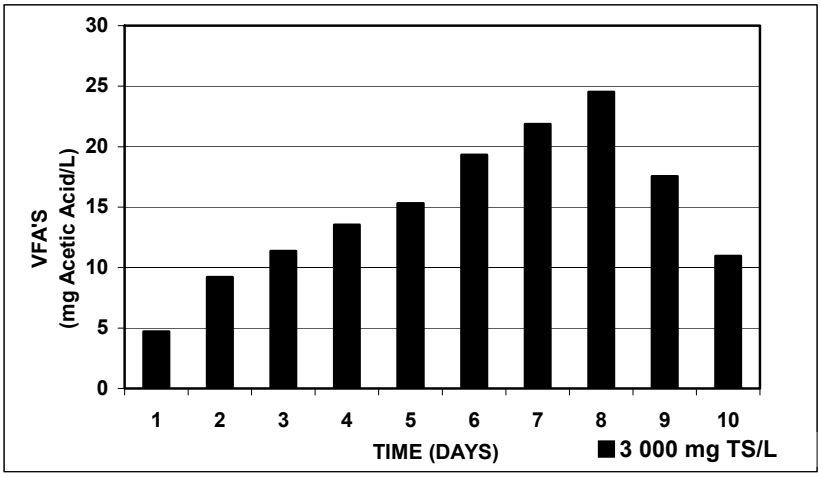


Figure 3.1: VFA concentration vs. time

In order to determine the optimum TS concentration, three experiments were conducted at TS concentrations of 1500, 3000 and 5000 mg/L over 15 days. The optimum TS concentration was 3 000 mg/L as this concentration yielded the highest produced concentrations of VFA's (Figure 3.2). Likewise, it can be seen from this figure that the highest concentrations of produced VFA's (24.51 mg/L) occurred on day 8 of the experiment.

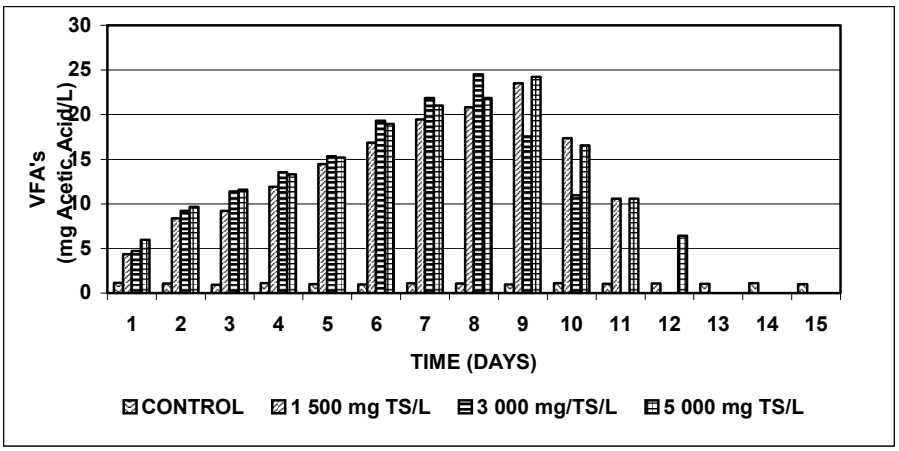


Figure 3.2: VFA production vs. time

The effects of the naturally developing pH and adjusted pH values on VFA production can be seen in Figures 3.3 and 3.4. From Figure 3.3 it can be seen that although VFA's are being produced the concentrations are very low, with the highest being 4.86 mg acetic acid/L. The adjusted pH (Figure 4.4) recorded higher concentrations of produced VFA's, with the highest being 24.51 mg acetic acid/L. These results seem to indicate that the microorganisms responsible for production of VFA's and soluble reactor phosphate (SRP) release prefer near neutral pH conditions.

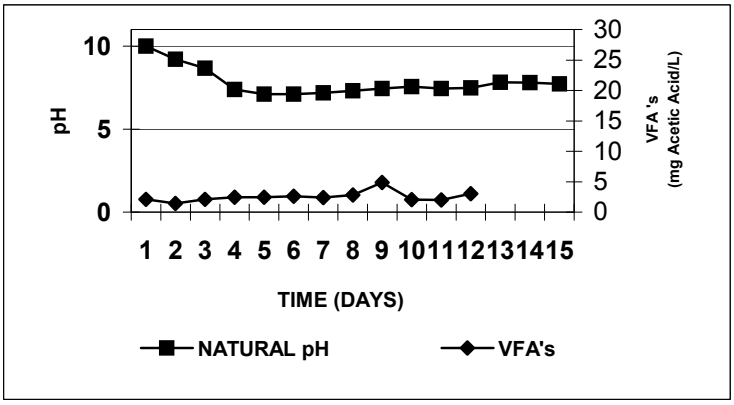


Figure 3.3: Effect of natural pH on VFA production vs. time

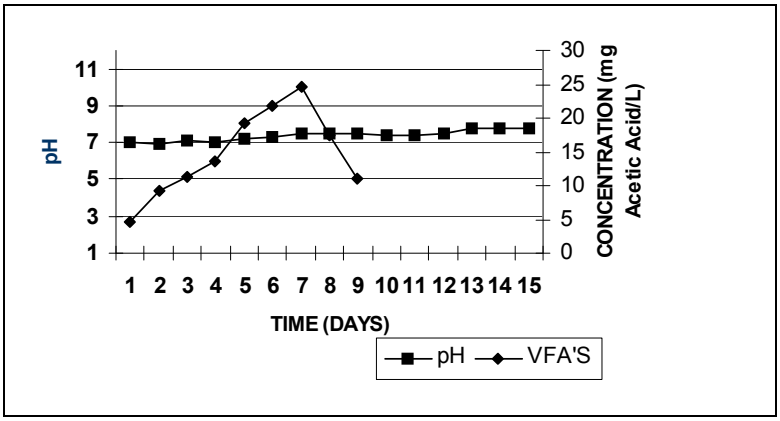


Figure 3.4: Effect of adjusted pH on VFA production vs. time

Although the temperature of the system was not optimised during this experiment, it was noticed that at lower temperatures the production of VFA's was greater than at higher temperatures. Circumstantial evidence of this was observed by the different rates of VFA production during different runs of the experiment. For example, experimental work conducted in the laboratory and at temperatures up to approximately 28^{o}C, resulted in exceedingly low VFA production rates, while experimental trials conducted in the cold room which has a temperature of approximately 17^{o}C the production of VFA's was much greater.

3.5 CONCLUSION

It can be concluded that the optimised conditions for prefermentation for treating edible oil effluent to enhance biological phosphorus removal are as follows:

- Time for maximum VFA production : 8 to 9 days
- Total Solids Concentration : 3 000 mg/L
- Adjusted pH : 7.0
- Mixing of the effluent during anaerobic phase

(B) ASSESMENT OF BENCH SCALE NUTRIENT REMOVAL PROCESS

3.6 AIMS AND OBJECTIVES

The aim of this study was to design and operate a laboratory scale activated sludge treatment process that would produce the final effluent having a regulatory acceptable COD and phosphate loads prior to its discharge to the municipal sewer system. The study was guided by the following objectives:

1. To conduct a preliminary effluent treatment investigation
2. To design and operate a bench scale activated sludge treatment process
3. To optimise the system operation for COD and phosphorous removal.

The investigation was partitioned into 3 aspects viz:

1. Wastewater Characterization
2. Laboratory Scale Bioreactor Design and Operation
3. Treatment Process Optimisation (Phase 4)

3.7 WASTEWATER CHARACTERIZATION

3.7.1 Materials and Methods

3.7.1.1 Characterisation of effluent

For the initial sampling survey composite wastewater samples were collected monthly over a period of five months (June, 1998 to October, 1998) from Company X in Pietermaritzburg. After sampling (25 L) the polluting parameters were determined directly and the effluent samples stored at 4^{o}C, until needed for additional tests and experiments. Colorimetric methods, using a spectrophotometer SQ 118 from Merck, was used for measuring COD (Appendix 3), phosphate (PO_4) (Appendix 4), total nitrogen (TN) (Appendix 5), ammonium nitrogen (NH_4) (Appendix 6), nitrates (NO_3) (Appendix 7) and sulphates (SO_4) (Appendix 8). All the samples were analysed in triplicate except for phosphate that was done in duplicates. The total suspended solids (TSS) (Appendix 9),

FOG's (Appendix 10), alkalinity as $CaCO_3$ (Appendix 2), were all analysed using standard methods (Standard Methods, 1989) and pH (Appendix 11).

3.7.2 Discussion

No previous effluent monitoring data was available at the start of this study, as the effluent plant at the Company X had carried out no on-site effluent monitoring. Wastewater characterisation studies of composite monthly effluent samples revealed that effluent characteristics fluctuated greatly and depended on the refining method employed at the plant at different times; i.e. whether chemical or physical refining was being used. In both cases the effluent would usually contain high organic (BOD & COD) and inorganic loads, as well as high quantities of free fats.

The organic load was mainly due to the presence of large quantities of FOG's in the final effluent, and also due to the chemical nature of the vegetable oil itself. The hydrolysis products of edible oils, long chain fatty acids; glycerol and glycerides; and proteins, all added to the organic load of the edible oil effluent (Eroglu *et al*., 1990). Ozturk *et al*., (1990) have estimated the organic load of the edible oil wastewater to vary between 0.85 to1.42 Kg BOD_5 /ton edible oil. They, also, showed that there was a direct relationship between BOD_5 and TSS, BOD_5 - COD, COD – FOG values. Its relationship between FOG and COD was also shown by Dart (1974) and later by Grant (1980) who both reported that 60% to 64% of BOD_5 was removed from edible oil wastewater after the removal of 88% to 91% of FOG during their physico-chemical studies.

The inorganic constituents of major concern in edible oil wastewaters are phosphates and sulphates. Phosphate in the effluent originates mainly from the use of phosphoric acid during the degumming stage and to a minor extent from the hydrolysis of phospholipids. Hui, (1996b) reported that crude oil, particularly soybean oil, contains significant quantities of organic phosphorus in the form of phosphatides, which are subsequently transferred from the oil phase to the water phase during the crude edible oil refining process. After acidification of the refinery wastewater, phosphatides get hydrolysed and the resulting phosphates are then released into the water phase.

Sulphates constitute a significant part of the inorganic constituents of edible oil wastewaters. Sulphate results from the use of concentrated sulphuric acid during acid splitting of the soap stock that, in turn, results in the FFA's being separated from the oil medium. Eroglu *et al*., (1990) and Hui, (1996b) reported, from separate studies, that the resulting effluent from acid splitting has an average pH of 1.7 and that it contains sulphate concentrations of 4000 mg/L. During this study, the sulphate concentration of the edible oil effluent was found to range between 1260 mg/L and 5800 mg/L SO_4^{2-} with a mean average of 4340 mg/L SO_4^{2-} during the five-month characterisation period.

Another complicating factor in the treatment of these effluents is the often-high concentration of salts, which results in high levels of conductivity being recorded. Salinization of wastewater results from the use of divalent (M^{2+}) and trivalent (M^{3+}) inorganic salts during physico-chemical treatment of wastewater to reduce waste loads of the final effluent. Salinity is indicated by the increase in conductivity of the wastewater. The edible oil industry reported that conductivity was one of their major effluent concerns and that the municipality was demanding that the conductivity levels in the final effluent be reduced. These conductivity concerns can often be solved through the optimisation of chemical effluent treatment methods after the effluent has been subjected to biological pre-treatment procedures, the optimisation of dosing techniques, careful pH and flocculation control

The nitrogen content of the effluents, both as total Kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4) were found to be below 10 mg/L. This seems to indicate that nitrogen could be limited for effective biological treatment. In an edible oil study by Ozturk *et al*., (1990) it was reported that the edible oil effluent BOD_5: N: P ratios were about 100: 4.4: 23, which indicated that nitrogen was present in low concentrations and could have been a growth limiting nutrient. During the present study, the N/COD ratio was found to be even lower and that this effluent, in its present form and due to its low nitrogen ratio, is not suitable for treatment using traditional three stage or five-stage BEPR processes. The effluent would, therefore, require nitrogen supplementation. Boyer (1996) reported that vegetable oil effluent could be effectively treated in a full-scale wastewater treatment installation if it was partially diluted with nitrogen rich domestic wastewater.

3.8 LABORATORY SCALE BIOREACTOR DESIGN AND OPERATION

3.8.1 Introduction

To achieve BNR of nitrogen and phosphorus, the process must incorporate an anaerobic zone, anoxic zone and aerobic zone in series (Ekama and Marais, 1984; Bdjanovic *et al.,* 1997; Starkenburg *et al.,* 1993, Wentzel *et al.,* 1990). For biological phosphorus removal only, as in this study, the anoxic zone is not necessary since it is specifically required for denitrification or nitrogen removal processes. The anaerobic zone, on the other hand, is of major importance for any biological phosphorus removal system. This zone is fundamental since it allows for the competitive selection of microorganisms (bacteria) that are responsible for the biological removal of phosphorus and enables the poly-P bacteria to proliferate in the system (Kuba *et al.,* 1993).

The objective of this phase of the study was to design and operate a biological treatment system, and to determine optimum conditions required for the treatment of edible oil effluents, by manipulating various process operation parameters. This part of the study was divided into 3 distinct stages.

3.8.2 Materials and Methods

3.8.2.1 Phase 1:

Bioreactor set-up and operation

Table 3.2: Summary of the process operating parameters during phase 1

PARAMETER	VALUE
$COD_{influent}$ (mg/L)	3850
PO_4-$P_{influent}$ (mg/L)	2155
$TKN_{influent}$ (mg/L)	<7.0
MLSS (mg/L)	4300
F/M (kgCOD/kgMLSS.d)	1.0
HRT (hr)	24
R_s (d)	30
Temp (°C)	Ambient
pH	6.10
$V_{Anaerobic\ reactor}$ (L)	0.34 l
$V_{Aerobic\ reactor}$ (L)	4.0 l
$V_{Secondary\ settling\ tank}$ (L)	1.8 l
Q_i (L/d)	4.34

A two-stage activated sludge process was selected for the edible oil effluent treatment. The two-stage laboratory-scale bioreactor chosen was modified from the 3-stage Phoredox process for biological phosphorus removal by omitting the anoxic zone and the A-recycle from the original design. This system consisted of an aerobic reactor (total volume = 5 L) connected to an anaerobic reactor (total volume = 1 L) which had two inlets, one on top through the lid for influent inflow, and one underneath for return sludge (S-recycle) flow. The mixed liquor from the anaerobic reactor was fed directly to the aerobic reactor through an outlet that could be either raised or lowered to vary the volume of the anaerobic reactor.

The overflow of the aerobic reactor flowed into the secondary settling tank (total volume = 2 L), which was constructed from a suitably shaped plastic bottle. The mixed liquor inlet for the aerobic reactor from the secondary settling tank was located at the bottom of the reactor, 0.5 L above the S-recycle sludge outlet point. A variable speed peristaltic pump pumped settled sludge from the secondary settling tank to the anaerobic reactor. Two peristaltic pumps maintained the flow through the system; one pumping the influent to the system and the other one pumping the settled sludge back to the anaerobic reactor. The internal flow rate was maintained by adjusting the S-recycle line peristaltic pump, whereas, the effluent overflow rate depended on the influent flow rate.

During this phase of the study, the reactor was operated on a semi-continuous basis for 30 days. Refer to table 3.2 for operating parameters. The reactor was inoculated with mixed liquor sludge from the aerobic zone of Darvill Wastewater Works, Pietermaritzburg. The mixed liquor suspended solids (MLSS) was maintained throughout the experiment by wasting 0.133 L of mixed liquor daily from the aerobic reactor. Activated sludge from Darvill Wastewater Works was chosen as the plant was designed for BNR.

As a result of the high strength of the edible oil final effluent, the influent was diluted to a desired COD concentration before being allowed into the system. Mixing or agitation of the mixed liquor was stopped during the influent pumping period to prevent dilution of the treated effluent and the untreated influent. The influent was prepared daily in a 5 L influent tank.

Aerobic conditions were maintained in the aerobic reactor by pumping in diffused air using an aquarium air pump. This sparging of air into the aerobic zone was sufficient to keep the mixed liquor well mixed and hence no additional mixing device was used. The anaerobic reactor mixed liquor was agitated using a magnetic stirrer at 100 rpm. The mixed liquor was rotated through the system for 24 hr by the peristaltic pump, connected to the S-recycle line. After 24 hr of treatment effluent samples were collected and analysed for COD (daily), soluble PO_4 (daily), and FOG (3 day interval).

3.8.2.2 Phase 2

Bioreactor Set-up and Operation

Table 3.3: Summary of the process operating parameters during phase 2

PARAMETER	VALUE
$COD_{influent}$ (mg/L)	5500
PO_4-$P_{influent}$ (mg/L)	430 - 450
$TKN_{influent}$ (mg/L)	<7
MLSS (mg/L)	3500 - 4000
F/M (kgCOD/kgMLSS.d)	1.5
HRT (hr)	24
R_s (d)	15
Temp (°C)	Ambient
pH	6.1
$V_{Anaerobic\ reactor}$ (L)	1.0 l
$V_{Aerobic\ reactor}$ (L)	4.0 l
$V_{Secondary\ settling\ tank}$ (L)	0.45
Q_i (L/d)	5.0

The results obtained from the first phase of this study showed that the design of the reactor was faulty, therefore, a second phase was initiated and the design parameters changed (Table 3.3). The new settling tank resulted in a 75% reduction of the settling volume, which resulted in an overall performance improvement. The mixed liquor inlet from the aerobic reactor into the secondary settling tank was moved to the S-recycle outlet. The new arrangement resulted in a more rapid settling rate of the sludge in the secondary settling tank.

The volume of the anaerobic reactor was also increased (Refer to Table 3.3). Mixing of the sludge was maintained using the magnetic stirrer at 200 rpm. The volume and operation of the aerobic reactor remained unchanged at 4.0 L. Aeration and agitation of the mixed

liquor was maintained by using diffused air into the system while the DO was monitored with a DO probe and maintained between 2 - 5 mg DO/L. The inoculum and substrate used was identical to that described in phase 1.

During phase 2 of the study the operation mode was changed from semi-continuous to a continuous mode. (Refer to table 4.3 for operating parameters)

3.8.2.3 Phase 3

Bioreactor set-up and operation

Table 3.4: Summary of the process operating parameters during phase 3

PARAMETER	VALUE
$COD_{influent}$ (mg/L)	2270
PO_4-$P_{influent}$ (mg/L)	190
$TKN_{influent}$ (mg/L)	<7
MLSS (mg/L)	4100 - 4200
F/M (kgCOD/kgMLSS.d)	0.5
HRT (hr)	24
R_s (d)	15
Temp (°C)	Ambient
pH	6.5 – 7.5
$V_{Anaerobic\ reactor}$ (L)	1.0 l
$V_{Aerobic\ reactor}$ (L)	4.0 l
$V_{Secondary\ settling\ tank}$ (L)	0.45
Q_i (L/d)	5.0

The reactor design and set-up remained un-altered and only the operating parameters were changed during this phase of the study (Table 3.4). Feed supplementation with sodium acetate was initiated in order to determine the effects of artificially increasing the VFA load on biological phosphorus removal in the edible oil effluent. Sodium acetate trihydrate ($NaCH_3C00.3H_20$) was added to give the influent at final concentration of 0.01M. After addition of Sodium acetate, and after being filtered through 0.45µm filter, the influent had a soluble COD concentration of 2000 mg/L.

- COD determination - Appendix 3a
- FOG determination - Appendix 10

3.8.3 Discussion

Phase 1

During Phase 1, the system had showed, compared with the preliminary investigation phase, an improved COD and phosphorus removal capability. No signs of overloading, according to the parameters analysed, were observed from the treatment process, despite the influent organic load being higher (0.2 - 0.3 kgCOD/kgMLSS.d) than the organic loads generally applied in domestic/industrial wastewater treatment processes (1.0 kgCOD/kgMLSS.d) (Ekama *et al*., 1984).

The system recorded an average COD removal of 70% with a low phosphorus removal efficiency of 4.4%. During the first 6 days of system operation, following the acclimatisation period of 15 days, an average phosphorus removal of approximately 330 mg/L, with the highest removal of 580 mg/L and the lowest of 43 mg/L, being recorded. The phosphorus removal rates fluctuated widely and after day 6, a sudden change in the phosphorus removal pattern was observed. Phosphorus release instead of the expected phosphorus uptake and removal was observed. This phenomenon started from day 7 to day 18 after which the process was stopped and this phase abandoned. During the 12 days of the observed phosphorus release, the rates of phosphorus release fluctuated widely with the highest phosphorus release of 270 mg/L and the lowest of 10 mg/L, being recorded. On average the process showed a phosphorus release of about 93 mg/L as compared to phosphorus removal of 331 mg/L.

Although, on the surface the percentage of phosphate may appear minimal (ca 4.4 percent), in real terms, the system was, during the first few days, actually removing approximately 238 mg/L. The reduction of phosphorus uptake and removal capabilities of the system was partly attributed to an insufficient anaerobic sludge mass fraction of 0.08 or 8% of the total system volume. It is, therefore, likely that synthesis of SCFA's or VFA's from the fermentation of the influent RBCOD was incomplete and the full acetate complement was not realised (Ekama *et al*., 1984).

The volume of the secondary settling tank could also have attributed to the reduced phosphorus removal ability of the system. The phosphorus release recorded from day 6 coincided with visually recorded blackening of the sludge in the anaerobic reactor and later

a black sulphur deposit on the sides of the secondary settling tank, due to the prevailing anaerobic conditions. The anaerobic conditions, in the secondary settling tank, resulted in the reduction of sulphate to sulphide ions which were confirmed by the strong smell of hydrogen sulphide (H_2S) eminating from the secondary settling tank. These anaerobic conditions in the secondary settling tank have been reported to trigger secondary phosphorus release in BNR systems. Although the anaerobic conditions were observed in the secondary settling tank, no attempt was made to verify that these conditions were directly responsible for the observed phosphorus release in the process after day 6.

Phase 2

Changing operation parameters such as, sludge age, anaerobic sludge mass fraction and the secondary settling tank volume, resulted in improved phosphorus removal. The average phosphorus removal increased to 8% of the total influent phosphorus, which is equivalent to 430 - 450 mg/L.

This improvement in phosphorus removal, during Phase 2, was thought to be due to the increased anaerobic sludge mass fraction and the reduced sludge age. Increasing the size of the anaerobic sludge mass fraction to 20% of the total reactor volume has been reported to increase the fermentation of RBCOD to VFA's in the anaerobic zone of the domestic wastewater treatment plants (Rustrain *et al*., 1999; Ekama *et al*., 1984). The decrease in sludge age is, also, known to have a beneficial effect on phosphorus removal efficiencies in BNR of domestic wastewater treatment systems (Wentzel *et al*., 1990). This is due to the increased removal frequency of the biomass, including the phosphate accumulating organisms (PAO's), from the system. This increased removal frequency of the PAO's, together with waste activated sludge, is thought to result in more cells, containing accumulated phosphorus, being removed from the system and, thus, stimulating the rapid reproduction of new biomass (PAO) that, also, has enhanced phosphate uptake capabilities.

However a gradual decline in the COD removal efficiencies was observed during Phase 2. This decline in COD removal started after day 16 when the COD removal efficiency suddenly dropped from an average of 69% - 68% COD removal to an average of 50%, which remained for the duration of the experimental phase. The observed decline in COD

removal efficiency coincided with similar decreases in phosphorus removal efficiencies of the treatment process. At, more or less, the same time when the COD removal efficiency dropped, the phosphorus release in the system was observed instead of the expected phosphorus uptake and removal. This decline in COD removal efficiencies was partly attributed to the increased operational F:M ratio (food to microorganism ratio) that was maintained at 1.5 kgCOD/kgMLSS.d. Increasing the F:M ratio of similar systems has been reported to have an overloading effect on the biomass, which, results in poor system substrate metabolism performance (Wentzel and Ekama, 1997; Casey *et al*., 1995).

The overall decline in the process removal efficiencies could also be attributed to the high sulphate concentrations of the original edible oil effluent. Although sulphates were not monitored in this study, their contribution to the process failure was evident from the observed sludge darkening in the anaerobic reactor and in the secondary settling tank. Increased production of H_2S gas was observed after day 16, which was accompanied by the increased oxygen demand of the activated sludge in the aeration reactor. This effect was similar to the process failure that was observed during phase 1 of the treatment process operation.

The relatively high sulphate concentrations in the influent may be responsible for the decrease in phosphorus removal efficiency of the system. As a result of the oxidation state of the sulphur in the sulphate ion (S^{6+} in SO_4^{2-}), other heterotrophic microorganisms such as sulphur reducing bacteria may have utilized the sulphate ions in the influent as a terminal electron acceptor. These sulphate reducing bacteria could have utilised a major part of the RBCOD, and thus out-compete the PAO for available substrates in a manner similar to that when oxygen and/or nitrates are present in the anaerobic reactor of the BNR system.

Phase 3

During this phase of the study the only process treatment parameter that was changed was the organic loading rate, which was reduced from 1.5 kg COD/kg MLSS/d to 0.5 kg COD/kg MLSS/d. The RBCOD of the diluted influent was increased by sodium acetate supplementation. The P/COD ratio remained constant between Phases 2 and 3, thus resulting in no change in P/COD ratio after effluent dilution with tap water.

The results, in Phase 3, showed improved phosphorus removal efficiencies, which were constant throughout the experimental phase. However, the improved phosphorus removal efficiency was concomitant with a gradual decline in the COD removal efficiency. The improvement in phosphorus removal efficiency may be, partly, attributed to the lower concentrations of sulphate ions as a result of the influent being diluted with water.

Further, the supplementation of the influent, with sodium acetate, also contributed to the increase in phosphorus removal efficiencies, as it resulted in the dominance of PAO's over non-PAO heterotrophs in the activated sludge biota. In essence, the increased VFA concentration in the anaerobic reactor is thought to have resulted in the increased dominance and proliferation of PAO's throughout the entire system, thus, out-competing the sulphur reducing bacteria for available substrates in the anaerobic reactor.

The sulphur reducing bacteria are characterised by slow growth rates (Metcalf and Eddy, 1991). This slow growth rate of the sulphate reducing bacteria is evident from the previous two experimental phases (Phases 1 & 2) while little or no evidence of the presence of the sulphate reducing bacteria was observed during the third phase of the treatment process study. The concomitant symptoms of sludge blackening, increased DO demand and the production of H_2S gas were not observed during this phase.

The gradual reduction in the recorded COD removal efficiency is thought to be due to the dominance of PAO's over non-PAO's of the entire activated sludge biota. Non-PAO's metabolise both RBCOD and SBCOD in the presence of a terminal electron acceptor (e.g. O_2, NO and SO_4), whereas, the PAO's take up and store RBCOD in the anaerobic zone (RBCOD from acetate supplementation of the influent). This uptake of readily biodegradable substrates in the anaerobic reactor by the PAO's is thought to have resulted in the poor removal of SBCOD in the subsequent aerobic reactor.

The results from the three experimental phases (Phases 1 - 3) appear to show that PAOs are unable to effectively and efficiently utilise the initial biodegradable COD factions in the influent wastewater. It was further, shown that high sulphate concentrations in the influent

have a negative impact on the wastewater treatment process efficiency in general and specifically for COD and phosphorus removal.

The removal of FOG, on the other hand, was consistent throughout the treatment experimental phases and was always above 95% FOG removal. The removal of fats from the influent wastewater by the treatment system may be due, in part, too adsorption of fats onto the activated sludge biomass, rather then through biochemical metabolism of fats into simpler organic molecules.

3.9 TREATMENT PROCESS OPTIMISATION (PHASE 4)

3.9.1 Introduction

As shown in the previous experiments there are a number of operational parameters that have a profound influence on the efficiency of BEPR. Therefore, manipulation of these design and process operations could result in performance optimisation of the BEPR process. In determining the effect of different operation parameters on the efficiency of BEPR, Wentzel *et al.* (1990) used a steady state model to predict phosphorus removal due to various parameters changes, such as sludge age (R_s), influent COD concentration, hydraulic retention time (HRT), the size of the anaerobic zone/reactor; number of anaerobic reactors; and influent characteristics (C: N: P ratio and RBCOD concentration).

3.9.2 Materials and Methods

3.9.2.1 Bioreactor Lay-Out and Operation

Table 3.5: Summary of the process operating parameters during phase 4

PARAMETER	VALUE
$COD_{influent}$ (mg/L)	2650
PO_4-$P_{influent}$ (mg/L)	600
$TKN_{influent}$ (mg/L)	< 7.0
MLSS (mg/L)	4100 – 4200
F/M (kgCOD/kgMLSS.d)	0.5
HRT (hr)	24
R_s (d)	15
Temp (°C)	Room
pH	6.5 – 7.5
V_r (L)	7
Q_i (L/d)	7

The general reactor configuration of a two-stage modified Phoredox process was maintained throughout the experimentation period. Only the anaerobic configuration was altered to allow for more increase in the anaerobic mass fraction (f_{xa}). The anaerobic sludge mass fraction was calculated as follows:

$$\text{Anaerobic sludge mass fraction } (f_{xa})\ (\%) = \frac{V_{anaerobic}}{V_{anaerobic} + V_{aerobic}} \times 100$$

Where: $V_{anaerobic}$ = Volume of sludge in the anaerobic reactor (l)

$V_{aerobic}$ = Volume of sludge in the aerobic reactor (l)

$V_{anaeroboic} + V_{aerobic} = V_{total}$ = Total volume of sludge in the aerobic and anaerobic reactor

The 1-litre conical flask shaped anaerobic reactor was removed and replaced by a 5-litre rectangular polyethylene anaerobic reactor. The influent and mixed liquor flow pattern remained unchanged, i.e. the influent flow entered through the top of the reactor whilst the S-recycle and outgoing mixed liquor flowed through the bottom inlet and outlet, respectively.

The reactor was seeded with 50% mixed liquor from the aerobic zone and 50% from the anaerobic zone of Darvill Wastewater Works. The anaerobic sludge mass fraction (f_{xa}) was increased from 1 litre (during phase 3) to 3 litres (total volume of the system increased from 5 litres to 7 litres), which represented the increase in the anaerobic sludge mass fraction of 23%, from 20% to 43% of the total sludge in the process (3 L V_{an}/7 L V_{total}).

A new batch of effluent was collected in 25 L containers from the edible oil industry prior to commencement of this fourth phase of the study. The effluent samples were stored in the cold room at between 0°C and 4°C after the necessary analyses was completed to prevent deterioration due microbial and chemical activity.

The influent was prepared in a large influent container (25 L) once every 4 days through dilution of the industrial effluent to give the desired final COD concentration. The influent supplementation was maintained at 1.75 g/l through the addition of sodium acetate trihydrate ($CH_3COONa.3H_2O$). An aquarium air pump pumping air through two diffusing

nozzles achieved aeration and agitation of mixed liquor in the aerobic reactor. A portable laboratory scale DO meter was used to measure the DO in the aerobic reactor. The DO concentration in the aerobic reactor was maintained between 2.0 – 5.0 mgO_2/L through on and off switching of the aquarium pump when necessary.

The anaerobic reactor contents were maintained in a well-mixed condition through the operation of the magnetic stirrer unit at lowest speed (ca. 50 rpm) so as to simulate a plug flow pattern. The treatment process was operated for 15 days without any analyses being done to allow for acclimatisation of the biomass to the influent substrate (i.e. achieve steady state). After the acclimatisation period was completed the system was then operated continuously for 82 days with sampling and analyses conducted daily during phase 3 of the study. Table 3.5 shows the summary of the operation parameters of the treatment process during phase 4 of the study.

3.9.3 Results

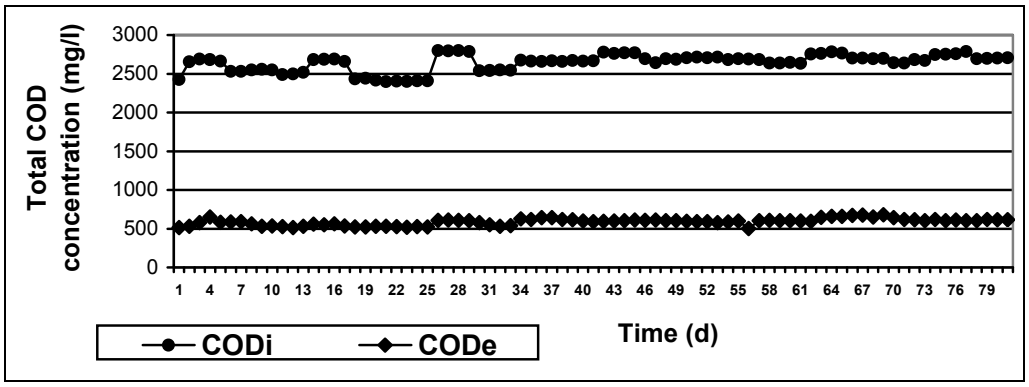


Figure 3.5: Total influent and effluent COD profiles for continuously operated biological treatment process at 0.5 F/M ratio (Phase 4)

CODi = COD concentration (mg/l) of filtered (0.45 µm) influent samples.

CODe = COD concentration (mg/l) of filtered (0.45 µm) effluent samples after biological treatment.

Influent COD strength varied between 2800 mg/L and 2400 mg/L. The COD removal efficiency remained stable despite the fluctuating influent COD strength. On average the COD removal efficiency remained 76% throughout the process operation (See Figure 3.5).

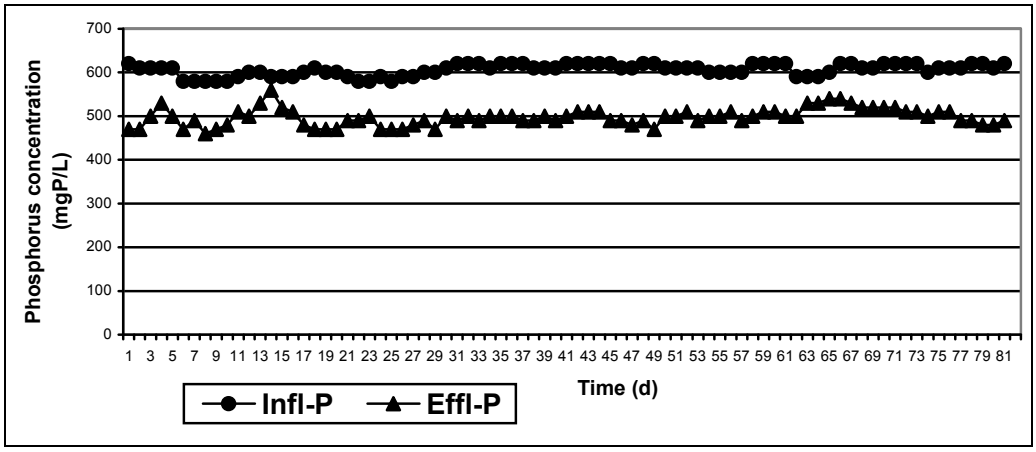


Figure 3.6: Soluble influent and effluent ortho-phosphate profiles for a continuously operated biological treatment process at 0.5 F/M ratio (Phase 4)

Infl-P = Phosphate concentration (mg/L) of filtered (0.45 µm) influent samples.

Effl-P = Phosphate concentration (mg/L) of filtered (0.45 µm) effluent samples after biological treatment.

On average between 76 mg/L and 116 mg/L phosphate was removed from the influent per day. No phosphorus release was observed throughout the treatment process operation at this phase (fig. 3.6).

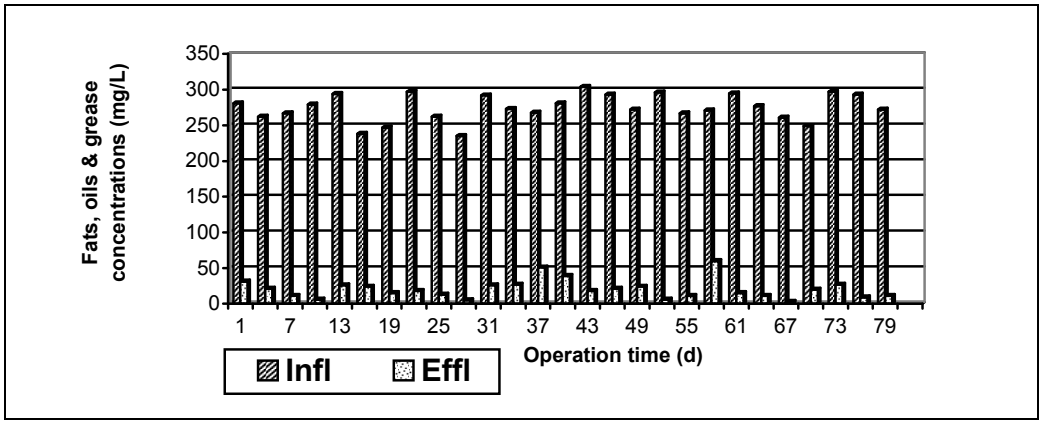


Figure 3.7: Influent and effluent FOG profiles during phase 4.

Infl = Concentration of FOG (mg/L) in the diluted influent samples.

Effl = Concentrations of FOG (mg/L) in the effluent after biological treatment.

On average FOG removal efficiency remained stable despite the fluctuating influent FOG concentrations throughout the process operation during this phase (Fig. 3.7). Effluent FOG concentrations were observed to be between 3 mg/L and 60 mg/L, from an average influent FOG of 275 mg/L.

3.9.4 Discussion

Increasing the anaerobic sludge mass fraction from 20% to 43% while maintaining a 0.5 kg COD/kg MLSS/d organic loading rate in the treatment process resulted in a general improvement in both COD and phosphorus removal. The system showed a consistent phosphorus removal efficiency of 17%, which is equivalent to a phosphorus uptake of 104 mg/L. This increased phosphorus removal efficiency, compared with the previous studies, was attributed to the greater anaerobic sludge mass fraction, which, in turn, resulted in an increased anaerobic contact time between the influent substrate and the anaerobic biomass. This increased contact time allowed for a higher amount of fermentation of RBCOD to VFA's, which were subsequently sequestrated by PAO's. This increased contact time in the anaerobic reactor also, allowed more time for anaerobic, non-fermentative bacteria to metabolise the SBCOD content of the influent substrate into RBCOD. Clearly, the increased production of RBCOD will make more substrate available to the anaerobic-fermentative bacteria for conversion into VFA's. Further, influent supplementation with 0.01 M sodium acetate could have ensured the dominance of PAO's in the anaerobic reactor over the non-PAO heterotrophic bacteria, as was postulated during phase 3 of this study.

The improved phosphorus removal was also mirrored by the improvement in the total soluble COD removal efficiency. The COD removal improved from 64% to an average of 76%. During this period of enhanced removal there was also, no inhibition observed in the biomass over time, as was the case during the preceding experimental phases (Phases 2 and 3).

Another factor responsible for the enhanced removal rates was the low concentration of sulphate during this phase of the study. The mixed liquor of the treatment system remained dark brown (chocolate brown) throughout this experiment, unlike previous experiments, where the liquor turned a black colour due to sulphate reduction.

The improved reduction of COD, together with the increased anaerobic sludge mass fraction, recorded during this phase, as opposed to the decreased COD removal efficiency during phase 3, is thought to be due to the increased activity of the anaerobic non-fermentative organisms and the fermentative micro-organisms in the increased anaerobic reactor volume. This increased activity, of the fermentative bacteria in the anaerobic reactor, could have increased the rate of metabolism of the SBCOD to RBCOD, which would otherwise have escaped from the anaerobic reactor and into the final effluent.

The effective COD removal recorded during this phase coincided with a reduced organic load rate of 0.5 kgCOD/kgMLSS/d. Similar results were observed for other treatment systems. For example, Eroglu *et al.,* (1990) and Ozturk *et al*., (1990) reported a consistent BOD removal of 85% from the activated sludge system treating vegetable oil effluent at an organic loading rate of 0.45 kgBOD/kgMLSS/d.

A shorter sludge age from 30 days to 15 days, on the other hand, had a minimal effect on the COD removal rates. As regards phosphorus removal a shortened sludge age from 30 days to 15 days resulted in significant increases in removal rates. This improvement in phosphorus removal efficiency with a decreasing in sludge age is consistent with the findings of Wentzel *et al*., (1990) who reported an increase in phosphorus removal efficiency with lower sludge ages, although this beneficial effect of a shorter sludge age became less significant below a sludge age of 3 days.

Despite the variations in COD and phosphorus removal the concomitant removal of FOG was always consistently better than 95%. One possible reason for these consistently high overall rates could be that the fatty material adsorbed to the biomass rather than simple metabolism of the FOG compounds.

The operating conditions in this forth phase appear to approach optimal and ideal biological conditions required for successful treatment of edible oil effluents as regards both COD and phosphorus reduction while, at the same time, reducing the FOG concentrations.

3.10 CONCLUSIONS

From the above experiments, and other experiments reported from literature, it is clear that edible oil effluents can be successfully treated using biological methods as a form of pre-treatment. Although, the total effluent COD (S_{ti}) values were considerably reduced during the present study, the effluent would require further polishing treatment to ensure that the regulatory discharge standards or municipal by-laws are successfully adhered to.

It is important to point out that effluent parameters are strongly dependent on the quality of crude oil and the refining method (physical or chemical/caustic refining) currently being employed and that these parameters change from batch to batch. Also, additional phosphorus removal from the final effluent, following biological treatment, using chemical precipitation methods, may be required before discharging to the municipal sewer system. Future recommendations include dilution of the edible oil effluent with domestic wastewater prior to its subjection to activated sludge treatment, as this will ensure that the final effluent characteristics are favourable for biological remediation techniques by adjusting the TKN/COD; P/COD; and RBCOD/S_{ti} ratios, as well as identifying possible limiting factors in the phosphorus removal process like, RBCOD and biomass concentrations.

CHAPTER 4
ANAEROBIC DIGESTION

4.1 INTRODUCTION

4.1.1 Anaerobic Digestion Process

Anaerobic digestion involves the breakdown of organic matter by the action of microorganisms in the absence of oxygen producing biogas, which contains methane and carbon dioxide as well as quantities of ammonia, nitrogen, hydrogen and hydrogen sulphide (Callander and Barford, 1983).

Although some fungi and protozoa can be found in anaerobic digesters, bacteria are the dominant microorganisms. The consortium of microorganisms involved consists of several trophic groups that possess different carbon catabolic functions (Stafford, *et al*., 1980). The four different groups include hydrolytic bacteria, hydrogen-producing acetogenic bacteria, homoacetogenic bacteria and methanogenic bacteria as shown in Figure 4.1 (Stafford, *et al*., 1980; Bulock and Kristiansen, 1987).

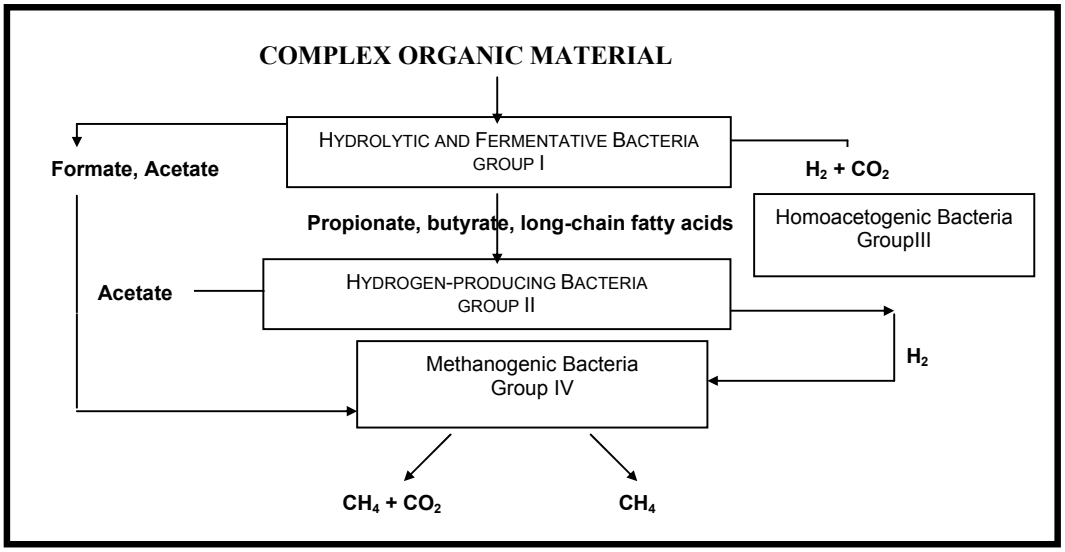


Figure 4.1: Diagram illustrating the four trophic groups involved in anaerobic digestion

4.1.1.1 Hydrolytic and Fermentative Bacteria

The group consists of both obligate and facultative anaerobes (e.g. *Bacteriodes, Bifidobacterium, Clostridium, Lactobacillus* and *Streptococcus*), which hydrolyse complex organic molecules (e.g. Proteins, cellulose, lignin and lipids) into monomers such as amino acids, glucose and fatty acids (Bitton, 1994).

4.1.1.2 Acetogenic Bacteria

Acetogenic bacteria, such as *Syntrobacter wolinii* and *Syntrophomonas wolfei*, consist of hydrogen-producing and homoacetogenic bacteria (Bitton, 1994). Hydrogen-producing acetogenic bacteria catabolize certain fatty acids (eg.propionic acid, butyric acid (Stafford, *et al*., 1980).

4.1.1.3 Methanogenic Bacteria

The Methanogenic bacteria consist of gram-positive and gram-negative bacteria (Bitton, 1994). From a taxonomic viewpoint methanogenic bacteria, belong to a separate kingdom (Archaebacteria). Methanogens differ from other bacteria in that methanogens lack muramic acid in their cell walls; they have a specific coenzyme, F_{420}, which acts as an electron carrier in metabolism and they possess ribosomal RNA sequences that differ from those of other prokaryotes (Bitton, 1994). Methanogens are grouped into 3 orders, namely, Methanobacteriales (e.g. *Methanogenobacterium, Methanobrevibacter, Methanothermus*), Methanomicrobiales (e.g. *Methanomicrobium, Methanogenium, Methanospirillum, Methanosarcina* and *Methanococcoides*), and Methanococcales (e.g. *Methanococcus*) (Bitton, 1994).

Methanogenic bacteria are often considered the key class of microorganisms in anaerobic digestion as well as the most fastidious of all the microorganisms responsible for anaerobic conversion of organics to methane. They are also known for their low synthesis rate (Speece, 1983). Methanogenic bacteria can be subdivided into two categories, namely, hydrogenotrophic and acetotrophic methanogens (Bitton, 1994). The hydrogenotrophic methanogens, hydrogen-utilizing chemolithotrophs, convert hydrogen and carbon dioxide to methane (Eq.1). The acetotrophic methanogens, also know as

acetoclastic or acetate splitting bacteria, convert acetate to methane (Eq.2) and are comprised of two main genera, namely, *Merthanosarcina* and *Methanothrix* (Bitton, 1994).

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ **Eq. 1**

$CH_3COOH \rightarrow CH_4 + CO_2$ **Eq.2**

An obligate, syntrophic relationship exists between the hydrogen-producing acetogenic bacteria and the hydrogenotrophic methanogens (Speece, 1983). The hydrogen partial pressure must be maintained at an extremely low level to ensure favorable thermodynamic conditions for the conversion of volatile acids and alcohols to acetate (Stafford, *et al*., 1980; Bitton, 1994; Bulock and Kristiansen, 1987; Speece, 1983).

4.1.1.4 Sulphate Reducing Bacteria (SRB)

Sulphate is present in many wastewaters as a result of the use of sulphuric acid in chemical processing. Sulphate is utilized by SRB as an electron acceptor, with hydrogen sulphide as the main product of the reaction. SRB may also utilize sulphite, which is an intermediate step in sulphate reduction. Methane producing bacteria (MPB) and SRB utilize the same energy sources, namely, acetate and hydrogen. Therefore the SRB compete with the MPB for the same energy sources (Sarner *et al*., 1988).

Methane Production

$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ **Eq.3**

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3$ **Eq. 4**

Sulphate Reduction

$4H_2 + S + H^+ \rightarrow HS^- + 4H_2O$ **Eq.5**

$CH_3COO^- + SO_4^- \rightarrow HS^- + 2HCO_3^-$ **Eq.6**

These two groups of bacteria have many similarities, namely, most are strictly anaerobic, chemoheterotrophic and have similar temperature and pH requirements. Therefore they are found co-existing in many anaerobic ecosystems (Zhou and Fang, 1998) although, SRB are less sensitive to pH and temperature variations (Sarner *et al*., 1988). Growth kinetics studies of SRB and MPB have shown that sulphate-reducing bacteria have a

higher affinity for acetate (k_s = 9.5 mg/L) than MPB (k_s = 32.8 mg/L), therefore SRB will outcompete MPB under low acetate concentrations (Schonheit *et al*., 1982). This competitive inhibition results in shunting of electrons from methane production to sulphate reduction (Schonheit *et al*., 1982). This competitive inhibition results in shunting of electrons from methane production to sulphate reduction (Schonheit *et al*., 1982; Speece, 1996).

4.1.2 The Anaerobic Baffled Reactor and Fed- Batch Digestion

The Anaerobic Baffled Reactor (ABR) was devised by Bachmann, *et al*., (1985), who initially named it the Modified Sludge Blanket Reactor. The ABR has been described as a series of Upflow Anaerobic Sludge Blanket (UASB) reactors, which do not require granulation (Bachmann *et al*., 1985). The ABR, as illustrated in Figure 4.2, contains alternately hanging and standing baffles, dividing it into a number of compartments, which segregate both biomass and gas phases (Nachaiyasit and Stuckey, 1995).

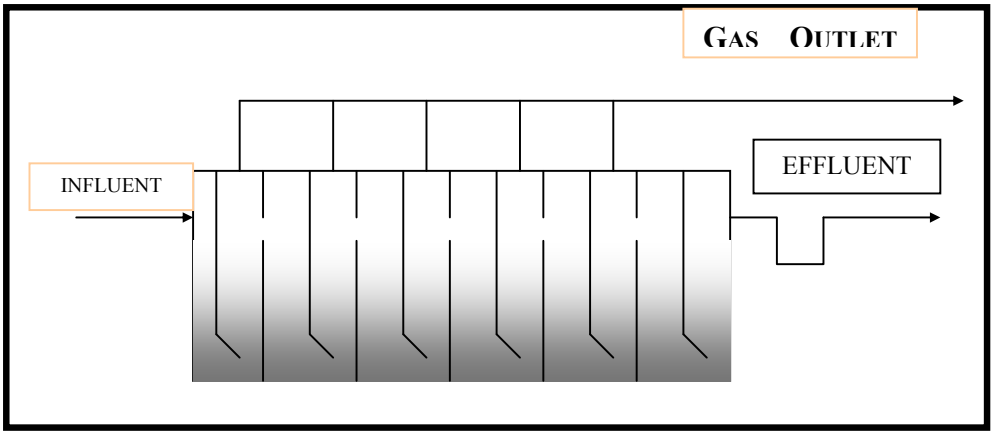


Figure 4.2: Schematic representation of the anaerobic baffled reactor

The slanted lower edges of the hanging baffles route flow of the liquid through the middle of the sludge bed, therefore reducing channeling. The liquid flow is alternately upward and downward between partitions. The biomass within the reactor tends to rise and settle with gas production and move horizontally down the reactor at a relatively slow rate (Nachaiyasit and Stuckey, 1995). The wastewater can therefore come into contact with a large active biomass as it passes through the ABR. The microorganisms in each compartment differ depending on the specific environmental conditions prevailing within

each compartment. Also, the remaining compounds or intermediates to be degraded influence the sludge characteristics (Nachaiyasit and Stuckey, 1997b).

The ABR is well suited to intermittent high organic or hydraulic loads. With biomass, which has been acclimatized to all possible components in the effluent, the ABR will withstand toxic shocks (Sacks, *et al*., 1998). These attributes are largely due to the compartmentalization of different microbial associations, which have been acclimatized to the range of constituents of wastewater (Sacks *et al*., 1998). The various microbial associations allow specialized bacteria to degrade the effluent stepwise, producing, degradation products, which may be toxic or inhibitory to a mixed culture (Sacks, *et al*., 1998). The compartmentalized structure of the ABR prevents much of the biomass being exposed to low pH during organic shock loads and maintains the biomass being exposed to low pH during organic shock loads and maintains the biomass within the reactor for long solids retention times (Nachaiyasit and Stuckey, 1997b). One of the most significant advantages of the ABR is its ability to separate acidogenesis and methanogenesis longitudinally, allowing the reactor to behave as a two-phase system without the associated control problems and high costs. The reactor design is simple, with no moving parts or mechanical mixing, making it relatively inexpensive to construct. High solids retention time is achieved without the need for biomass to be fixed to media particles or a solid-settling chamber. Increased volumes of wastewater can be treated since the hydraulic retention time and solids retention time are separate, relative to CSTR where hydraulic retention time is equal to solids retention time. The ABR has been found to be stable to hydraulic and shock loads and the reactor configuration provides protection of the biomass to toxic compounds in the effluent.

Digester start-up is one of the major problems associated with anaerobic treatment systems. Investigations have shown that with a long initial hydraulic retention time (80 hr), by gradually reducing it whilst keeping the substrate concentration constant, superior performance was observed in comparison to a reactor started up with a constant and a low hydraulic retention time coupled with a step-wise decrease in substrate concentration (Barber and Stuckey, 1998). Investigations involving operation of the ABR at low temperatures showed that biochemical reactions double in relative activity for every 10°C increase in temperature (Nachaiyasit and Stuckey, 1997a). However, no significant

reduction in overall COD removal efficiency was observed when the temperature was decreased from 35°C to 25°C. A further reduction to 15°C resulted in 20% decrease in COD removal (Nachaiyasit and Stuckey, 1997a). Changes in performance were gradual, which is an advantage, as this would provide improved protection to shocks, in comparison to other reactors.

4.2 AIMS AND OBJECTIVES

This research project investigates the potential usage of anaerobic digestion for treatment of vegetable oil effluent. The following objectives were followed:

1. To chemically characterize the effluent
2. To evaluate the use of gravity separators for lipid reduction
3. To evaluate the use of Barium Chloride for sulphate reduction
4. To investigate the anaerobic biodegradability of the edible oil effluent.
5. Using Biochemical Methane Potential (BMP) and Anaerobic Toxicity Assay (ATA) to measure the biodegradability of edible oil effluent in anaerobic treatment.

4.3 MATERIALS AND METHODS

4.3.1 Characterisation of the edible oil effluent

Final effluent was collected and stored at 4°C until required. Characterisation of the effluent was similar to the previous chapters where the organic load and FOG loads were determined.

4.3.2 Lipid Reduction by Gravitational Separation

A well mixed sample of edible oil effluent (100 mL) was placed into a 100 mL separating funnel and allowed to stand for 20 min. Thereafter, 50 mL was removed and referred to as the settled layer. The remaining 50 mL was removed and referred to as the top layer. Both samples were then analysed. The experiment was repeated with the following settling time periods (1 hr, 2 hr, 4 hr, 24 hr).

4.3.3 Sulphate Reduction using Barium Chloride ($BaCl_2$)

A $BaCl_2$ solution was prepared 5 times in excess of the sulphate content present in the wastewater sample. 2.125 g BaCl2 was weighed and then dissolved in 20 mL distilled water. The $BaCl_2$ solution was added slowly to the wastewater sample, with continuous mixing. The sample was allowed to stand for 1 hr, producing a white precipitate. The supernatant was decanted and analysed.

4.3.4 Biochemical Methane Potential and Toxicity Assays

The BMP and anaerobic toxicity assays were carried out according to the method described by Owen *et al*., (1979).

4.3.4.1 Anaerobic Sludge

The experiment was carried out with anaerobic sludge collected from a mesophilic digester at a wastewater treatment works. Properties of the anaerobic sludge used are outlined in Table 4.1.

Table 4.1 Properties of Anaerobic Sludge

PARAMETER	mg/L
pH	7.16
COD	14 520
Alkalinity	12 845
Lipids	16 400
Sulphates	60
VSS	4 385
TSS	8 690
TDS	800

The sludge was stored at 4°C until required. Sludge storage has been found to have no significant effect on the extent of degradation (Shelton and Tiedjie, 1984).

4.3.4.2 Preparation of Assay Bottles

The experiment was carried out in 125 mL bottles with butyl rubber septa and metal caps as well as 225 mL bottles with butyl rubber septa and screw caps. The bottles were gassed with OFN (oxygen free nitrogen), and then closed prior to the introduction of samples, defined medium and inoculum.

4.3.4.3 Defined Medium

The defined medium used for these experiments was adapted from the method outlined by Owen *et al*, (1979) (Appendix 12). The defined medium was revised to avoid precipitation. Sodium sulphide was used to provide a reducing environment (Owen *et al*., 1979). However, when added to the medium caused a thick black precipitate. Thus, sodium sulphide was substituted with cysteine, which also provides reducing environment. Ferric chloride was mixed with ethylene diamine tetra-acetic acid EDTA to facilitate dissolving. Sodium carbonate ($NaHCO_3$) was added to provide buffering which was important for pH control.

4.3.4.4 Procedure

Table 4.2 Sample Compositions for BMP and Toxicity assays

WASTEWATER % (V/V)	SLUDGE (mL)	DEFINED MEDIUM (mL)	WASTEWATER (mL)	DISTILLED WATER (mL)
Blank	300	0	0	700
Control	300	300	0	400
10	300	300	40	360
25	300	300	100	300
50	300	300	200	200
75	300	300	300	100
100	300	300	400	0

The components outlined in Table 4.2 were combined and mixed for each sample, and 100 mL was decanted into each 125 mL bottle. A working volume of 200 mL was used for the 225 mL bottles. The bottles were over gassed with OFN, then sealed with butyl rubber septa and capped with aluminum crimp seals. Gas volumes of the bottles were zeroed (ambient pressure) with a syringe and the bottles were ready for incubation at 35°C. The bottles were incubated in a shaking incubator to facilitate contact between the microorganisms and the substrate.

- BMP assay

Controls (duplicate) were prepared containing sludge and defined medium, without the addition of organic substrate. Blanks (duplicate) were prepared containing sludge and distilled water, without the addition of defined medium and organic substrate. The sludge and medium in the case of the controls were mixed together and the sludge and the distilled water for the blanks were mixed and a working volume of 100 mL for each sample

was decanted into each bottle, leaving a headspace of 25 mL. Duplicates of each sample were prepared incorporating a range of wastewater concentrations (Table 4.2). For the second BMP run as well as the run involving pretreated samples, 10%, 50% and 100% wastewater concentrations were used.

- Anaerobic Toxicity Assay (ATA)

Assay bottles were prepared as in the BMP assay, with defined medium, inoculum and wastewater samples. Two runs were carried out including the use of raw effluent as well as pretreated effluent. The wastewater concentrations investigated included 10%, 50% and 100% to provide a range from non-inhibitory to severely toxic. An additional 2 mL spike, containing acetate and propionate, was added to each bottle via syringe injection to give a final concentration of 75 mg acetate and 26.5 mg propionate in each sample. Two sets of controls were prepared (in duplicate). Control A contained only sludge, medium and 100% v/v wastewater. Control B contained sludge, medium as well as the acetate-propionate spike. A blank was also prepared containing only sludge and the spike.

4.3.4.5 Gas Measurement

Gas volume sampling and removal during incubation was performed with a graduated syringe (20 mL), fitted with a 22-gauge needle. Readings were taken at incubation temperature and the syringe was held vertical for measurement. During the second run using raw effluent as well as during pretreatment experiments, the syringe was filled to the 2 mL mark with 500 g/L KOH, which absorbs carbon dioxide present in the biogas. The gas is allowed to bubble through the KOH, thus gas volumes taken represented methane content of the biogas.

Gas production was measured daily for the first 5 days and periodically thereafter each measurement. Incubation was continued until gas production was complete.

4.3.5 Fed-Batch Digestion

4.3.5.1 Reactor

A reactor of 2.5 L with a working volume of 2 L was used. The reactor was equipped with a temperature control system mechanical mixer. The gas port was attached to a saline

displacement system to aid in monitoring gas production. The saline solution consisted of 20% (v/v) $NaCl_2$, which was acidified to pH 3 - 4. A glass syringe (50mL) was used to feed substrate to the digester. A peristaltic pump was used to feed samples with volumes larger than 50 mL.

4.3.5.2 Artificial Effluent

The artificial effluent used in this experiment is illustrated in Table 4.3.

Table 4.3: Composition of Artificial Effluent

COMPONENT	CONCENTRATION (mg/L)
MgCl2.6H2O	468
CaCl2.2H2O	117
FeSO4.7H2O	5.25
ZnSO4.7H2O	1.5
MnSO4	1.5
CuSO4.5H2O	0.3
CoCl2.6H2O	0.3
Na2MoO4.2H2O	0.15
H3BO3	0.3
KI	0.075
Yeast Extract	10

4.3.5.3 Digester Start-up

Prior to inoculation, the reactor was filled with water. The reactor was examined for gas leaks and the temperature control system and the mixer were switched on. This was carried out to ensure proper working order of the system. The digester was then inoculated with 2 L anaerobic sludge, which had a COD of 14 500 mg/L and pH 7.39. Digestion was carried out at 35°C, with continuous mixing.

4.3.5.4 Fed-Batch Digestion

Initially the digester was fed with 50 mL artificial effluent, which had a COD of approximately 400 mg/L. With regular pH and COD monitoring the feed volume was then increased to 100 mL/day except on Saturdays and Sundays. After 20 days, the maximum working volume was reached and the reactor contents were drained, leaving 950 mL of active sludge. Anaerobic conditions were maintained by pumping OFN in to the reactor after drainage. Effluent with a COD of 2000 mg/L was then fed to the digester and gas production, as well as pH and COD monitored.

4.3.6 Anaerobic digestion using the ABR

4.3.6.1 Anaerobic Baffled Reactor

The working volume of the ABR used in this experiment was 6 L. The reactor was operated at a temperature of 35°C, using a temperature controlled water bath. The reactor was equipped with an inlet for feeding and an outlet for effluent overflow and sampling. Gas ports enabled monitoring of gas production.

4.3.6.2 Digester Start-up

The ABR was inoculated with anaerobic sludge and allowed to stand for 15 days for sludge settlement and acclimatization of the microorganisms. A decline in pH was observed with no gas production. The drop in pH indicated that the methanogenic bacteria were not capable of utilizing volatile acids produced by Acetogenic bacteria, and as result, did not produce any biogas.

The second start-up involved of the reactor with 2 L anaerobic sludge, with a COD of 11 060 mg/L and pH 7.10. The remaining working volume of 4 L was composed of artificial effluent. The artificial effluent used is illustrated in Table 5.4. The reactor was allowed to stand for 7 days for sludge settlement. Feeding was initiated with artificial effluent, monitoring gas production and pH. The reactor was fed volume of 200 mL, composed of 90% edible oil effluent and 10% artificial effluent. Initially, a glass syringe was used to feed the reactor; thereafter a peristaltic pump was used.

4.3.7 Analyses

COD - The measurement for the first run of the BMP assay was carried out using the spectrophotometer as described in Appendix 3a. The measurement for the second run of the BMP assay as well as for the ATA, edible oil effluent characterization and pretreatment experiments were based on the open reflux, colorimetric method described in Appendix 1b.

Sulphates - Sulphates were measured colorimetrically using the Merck SQ118 Spectrophotometer as described in Appendix 8 to characterise the edible oil effluent as well as the BMP, ATA and pretreatment experiments.

TSS - Total suspended solids (TSS) and total dissolved solids (TDS) were measured gravimetrically according to Standard Methods as described in Appendix 9

FOG - The measurement of lipids was carried out gravimetrically according to Standard Methods as described in Appendix 10 for the BMP, ATA, Pretreatment experiments and characterisation of the edible oil effluent.

pH - pH was one of the parameters measured to characterise the edible oil effluent as well as the BMP, ATA and pretreatment experiments. pH was measured using a calibrated pH meter (Beckman); the process is described in Appendix 11.

MLSS & VSS - Total solids (TS) and volatile solids (VS) were measured gravimetrically according to Standard Methods as described in Appendix 13 for the second run of the BMP assay, ATA, pretreatment experiments and characterisation of the edible oil effluent.

4.4 RESULTS AND DISCUSSION

4.4.1 Chemical Characteristics of Edible Oil Effluent

Two batches of edible oil effluent were collected and analysed. The first was used for the first run of the BMP assay. The second batch was used for the second run of the BMP assay, ATA and pretreatment studies.

Both batches were final edible oil effluent, with the treatment process differing for each. A filtration system was implemented following the DAF unit, after sample A was collected and analysed. A significant change in lipid content of the effluent was seen, which suggests that the filtration system removed some of the lipids present in the effluent. No drastic changes were seen in the COD and sulphate content as well as the pH. The high sulphate concentration in both samples could be attributed to the use of sulphuric acid in the production process.

4.4.2 Lipid Reduction by Gravitational Separation

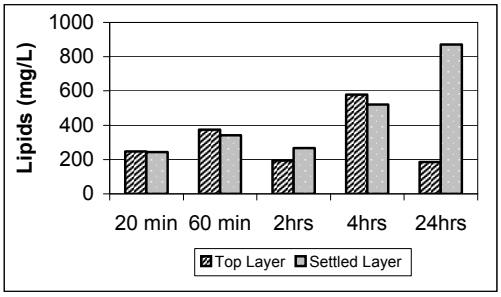


Figure 4.3: Lipids content (mg/L) of vegetable oil effluent after lipid reduction pre-treatment

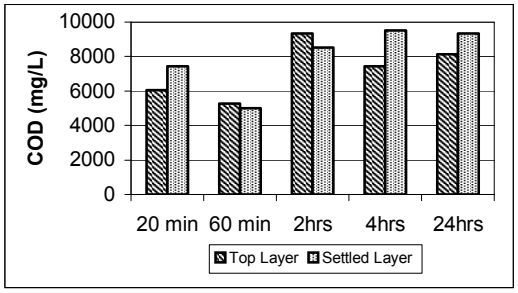


Figure 4.4: COD content (mg/L) of vegetable oil effluent after lipid reduction pre-treatment

Table 4.4: Results of lipid reduction by gravitational separation

SETTLING TIME	PARAMETER	TOP LAYER	SETTLED LAYER
20 min	pH	5.07	5.06
	TS	6030 mg/L	8320 mg/L
	VS	5553 mg/L	8042 mg/L
60 min	pH	5.08	5.07
	TS	11700 mg/L	11090 mg/L
	VS	10200 mg/L	9550 mg/L
2 hr	pH	5.07	5.06
	TS	13030 mg/L	13110 mg/L
	VS	2260 mg/L	2710 mg/L
4 hr	pH	5.09	5.07
	TS	11160	13070 mg/L
	VS	880 mg/L	2610 mg/L
24 hr	pH	5.06	5.07
	TS	14420 mg/L	14290 mg/L
	VS	3490 mg/L	3970 mg/L

The objective of this experiment was to observe the settling properties of lipids contained in the effluent. It was expected that the lipids would float to the surface of the solution if allowed to stand, with a decrease in lipid concentration in the settled layer of effluent. Figure 4.3 illustrates the lipid concentration of the top and settled layers for various time intervals. For the times intervals 20 min, 60 min, 2 hr and 4 hr there was no significant difference in lipid concentration for the top and settled layers. However, for the time interval of 24 hr a noticeable difference was seen, with the lipid concentration of the top layer at 184 mg/L and the settled layer at 870 mg/L. This suggests that the lipids settled in solution. It is possible that the fatty compounds adhered to suspended solids and settled out of solution, thus the higher lipid concentration in the settled layer. No significant difference was observed for pH (Table 4.4). The COD concentrations (Figure 4.4) for the

settled layers were slightly higher than the top layers since the TS and VS (Table 4.4) was higher in the settled layers.

4.4.3 Sulphate Reduction using Barium Chloride

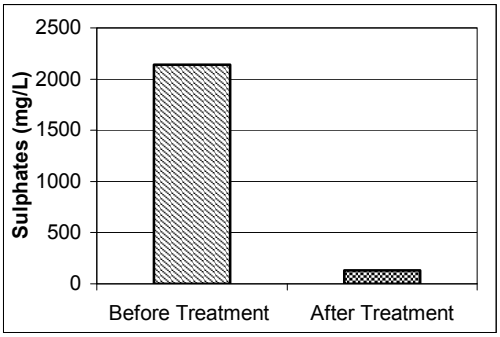


Figure 4.5: Sulphate content (mg/L) of vegetable oil effluent before and after sulphate reduction pre-treatment

Figure 4.5 illustrates the sulphate results obtained before and after treatment. A sharp decrease in sulphate content was observed as a result of the pretreatment process. No changes were observed for pH.

4.4.4 Biodegradability Assay

BMP is a measure of sample biodegradability. The objective of this experiment was to evaluate whether anaerobic microbial populations were able to utilize the edible oil effluent with resultant methane production. The BMP assay was run using raw as well as pretreated effluent. Measurement of gas production provided an indication of the metabolic activity and the degradability of the substrate. Experimental controls were run for each sample as well as a range of wastewater concentrations. The results of the two runs using raw effluent are illustrated in Table 4.6 to 4.16. Tables 4.17 to 4.21 illustrate the results of the BMP assay using pretreated effluent.

Table 4.5 and 4.11 show results of the experimental controls, for the first and second runs, respectively. Tables 4.6 to 4.9 and Tables 4.12 to 4.14 illustrate the results of the bioassay for the different substrate concentrations of the first and second runs respectively. Results of the experimental controls of the BMP assay using pretreated effluent are shown in Tables 4.16 and the results of the various wastewater concentrations

are shown in Tables 4.17 to 4.19. Average gas production samples (mean value of each sample) were corrected by subtracting the gas production from that of the control in order to show the gas production resulting from degradation of the substrate. The graphs of cumulative gas production are useful as they illustrate gas production rate (gradient of the slope) and time at which gas production stabilized.

4.4.4.1 Results of the Biodegradability Assay using Raw Edible Oil Effluent

A. ***Results of the BMP Assay (First Run)***

Table 4.5: Results of the controls in the BMP assay using edible oil effluent (first Run)

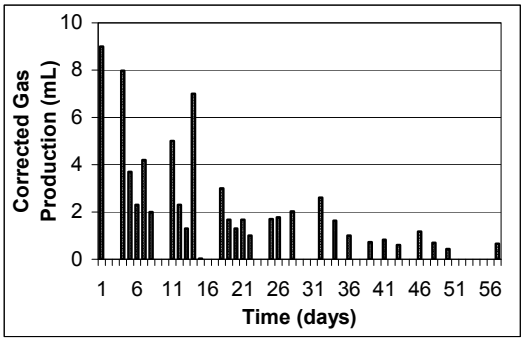


Figure 4.6: Average gas production of the controls in the BMP assay using raw edible oil effluent (First Run)

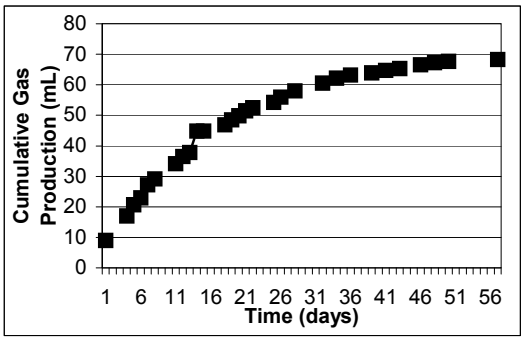


Figure 4.7: Cumulative gas production of the controls in the BMP assay using raw edible oil effluent (First Run)

Total gas production (35^0C):	68.33 mL	
%COD Reduction:	33.32%	
Mineralized COD:	-	
% Lipid Reduction:	92.8%	
Parameter	Before Incubation	After Incubation
pH:	7.27	7.42
Alkalinity	1844 mg/L	2128 mg/L
TSS:	8890 mg/L	5830 mg/L
TDS:	1790 mg/L	1315 mg/L

Table 4.6: Results of the BMP assay with 10% wastewater concentration using raw edible oil effluent (First Run)

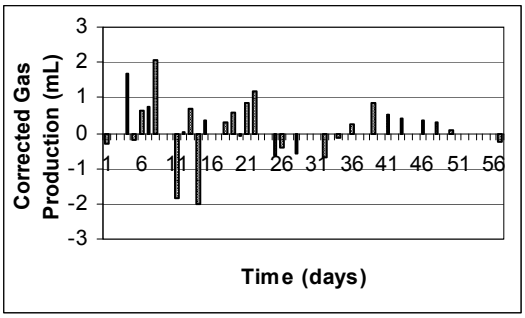


Figure 4.8: Corrected average gas production of 10% wastewater in the BMP assay using raw edible oil effluent (First Run**)**

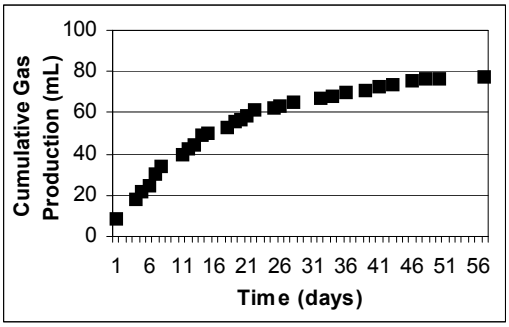


Figure 4.9: Cumulative gas production of 10% wastewater in the BMP assay using raw edible oil effluent (First Run)

Total gas production (35^{o}C):	77.46 mL	
%COD Reduction:	16.77%	
Mineralized COD:	4.14 mg	
Lipid Reduction:	25.34%	
Parameter	**Before Incubation**	**After Incubation**
pH:	7.34	7.53
Alkalinity:	1711 mg/L	2226 mg/L
TSS:	9360 mg/L	6070 mg/L
TDS:	2510 mg/L	1320 mg/L

Table 4.7: Results of the BMP assay with 25% wastewater concentration using raw edible oil effluent (First Run)

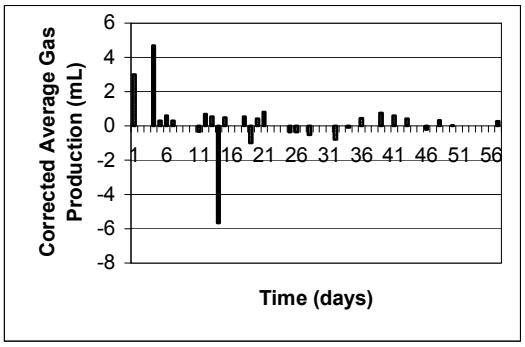


Figure 4.10: Corrected average gas production of 25% wastewater in the BMP assay using raw edible oil effluent (First Run)

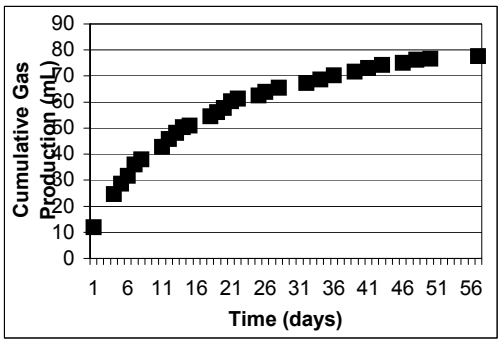


Figure 4.11: Cumulative gas production of 25% wastewater in the BMP assay using raw edible oil effluent (First Run)

Total gas production:	77.58 mL	
%COD Reduction:	15.19%	
Mineralized COD:	9.50 mg	
Lipid Reduction:	24.37%	
Parameter	**Before Incubation**	**After Incubation**
pH:	7.35	7.43
Alkalinity:	1716 mg/L	2472 mg/L
TSS:	9480 mg/L	6890 mg/L
TDS:	1790 mg/L	1650 mg/L

Table 4.8: Results of the BMP assay with 75% wastewater concentration using raw edible oil effluent (First Run)

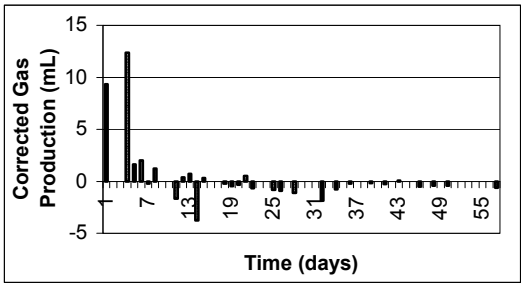


Figure 4.12: Corrected average gas production of 75% wastewater in the BMP assay using raw edible oil effluent (First Run)

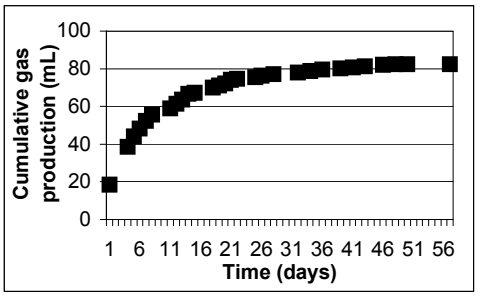


Figure 4.13: Cumulative gas production of 75% wastewater in the BMP assay using raw edible oil effluent (First Run)

Total gas production (35^0C):	82.32 mL
% COD Reduction:	8.01%
Mineralized COD:	15.16 mg
Lipid Reduction:	45.79%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.32	7.59
Alkalinity:	1863 mg/L	2766 mg/L
TSS:	991 mg/L	6365 mg/L
TDS:	3060 mg/L	2625 mg/L

Table 4.9: Results of the BMP assay with 100% wastewater concentration using raw edible oil effluent (First Run)

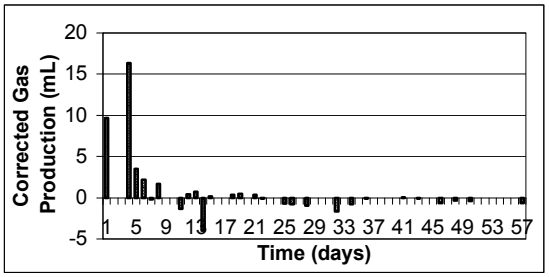


Figure 4.14: Corrected average gas production of 100% wastewater in the BMP assay using raw edible oil effluent (First Run)

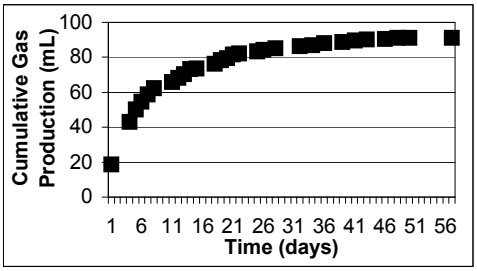


Figure 4.15: Cumulative gas production of 100% wastewater in the BMP assay using raw edible oil effluent (First Run)

Total gas production (35^0C):	90.95 mL
% COD Reduction:	4.93%
Mineralized COD:	14.55 mg
Lipid Reduction:	26.25%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.30	7.35
Alkalinity:	1746 mg/L	2962 mg/L
TSS:	9780 mg/L	7585 mg/L
TDS:	3630 mg/L	3180 mg/L

Table 4.10: Results of the biodegradability assay using raw edible oil effluent (First Run)

Wastewater Concentration	Wastewater COD (mg/bottle)	Mineralized COD (mg)	Gas Produced (mL/bottle)	Corrected Gas (mL/bottle)	Estimated CH_4 (mL)	% CH_4	% COD Reduction
Control	-	-	44.8	-	-	-	-
10	24.68	4.14	49.3	4.5	1.64	36.4	16.77
25	62.58	9.5	50.43	5.63	3.75	66.6	15.16
50	136.50	14.65	50.13	5.79	3.33	68.6	10.73
75	185.24	15.16	66.75	21.95	5.99	27.29	8.01
100	295.20	14.55	73.37	28.52	5.75	20.16	4.93

Looking at the graphs illustrating corrected gas production for the first and second runs using raw effluent, it can be seen that the highest gas production was recorded for samples containing 100% substrate solution. This suggests that the organics in the raw effluent were readily degraded by the microorganisms, with greater volumes of the effluent resulting in greater volumes of gas produced. Corrected gas production graphs (Figures 4.7, 4.9, 4.11, 4.13 and 4.15) for the first run using raw effluent illustrates gas volumes of between 0.5 mL and 12.5 mL for the first 9 days for the various wastewater samples. Thereafter gas production began decreasing, which suggested that the degradable organic materials were also decreasing.

The cumulative gas production graphs illustrate the total gas produced (i.e. not corrected using the control). Figures 4.7, 4.9, 4.11, 4.13 and 4.15 represent the cumulative gas produced for the various wastewater samples of the bioassay using raw effluent. After 30 d, the graphs indicate almost zero gas production suggesting that all degradable compounds present in the substrate had been utilised.

Table 4.5 to 4.9 illustrating the results of the BMP assay using raw effluent for the first run, includes results for pH, alkalinity, TSS and TDS before and after incubation as well as COD reduction and lipid reduction. Alkalinity indicates the buffering capacity of a digester or the ability of the digester to neutralize the effect of volatile acid formation. The results show an increase in alkalinity for all samples. The pH readings before and after incubation of both runs of the bioassay using raw effluent as well as the run using pre-treated effluent remained within optimal working range. The optimal pH range for methanogenic bacteria is between 6.5 and 8.2 (Ross *et al*., 1992). This suggests that the methanogenic bacteria were able to utilize the volatile acids produced by acetogenic bacteria at an optimum rate.

An accumulation of volatile acids exceeding the buffering capacity of the digester will lead to a drop in pH (McCarthy and McKinney, 1961; Switzenbaum *et al*., 1990).

The COD of a sample is an indication of the amount of the organic matter present. Degraded organic matter is converted to methane in the biogas, thus recalcitrant organic matter contributes to the final solution. From the results obtained, it was found that the %COD reduction for the various substrate concentrations of the first BMP run decreased with increasing wastewater concentration although all samples showed some degree of COD reduction. The average COD reduction for the first run of the 10% wastewater sample was 16.7% and 4.9% for the 100% wastewater sample. This suggests that the methanogenic bacteria responsible for methane production were inhibited by the presence of a toxic substance or substances (at elevated concentrations) present in the effluent.

B. *Results of the BMP Assay (Second Run)*

Table 4.11: Results of the controls in the BMP assay using raw edible oil effluent (second Run)

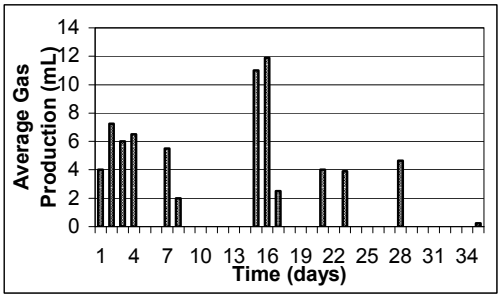


Figure 4.16: Average gas production for controls in the BMP assay using raw edible oil effluent (Second Run**)**

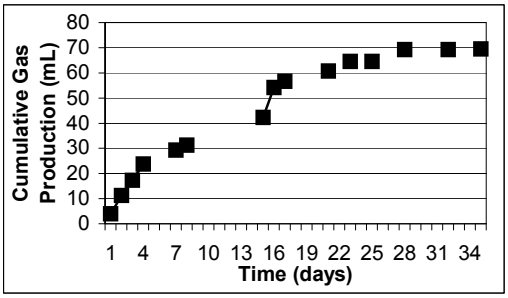


Figure 4.17: Cumulative gas production for controls in the BMP assay using raw edible oil effluent (Second Run)

Total gas production (35^0C):	69.45 mL
% COD Reduction:	-
Mineralized COD:	-
Lipid Reduction:	11.03%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.71	7.15
TS:	11940 mg/L	12260 mg/L
VS:	7670 mg/L	6380 mg/L

Table 4.12: Results of the BMP assay with 10% wastewater concentration using raw edible oil effluent (Second Run)

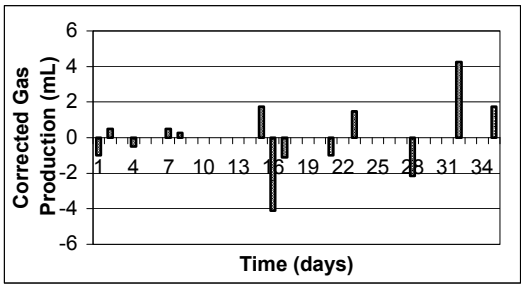


Figure 4.18: Corrected average gas production of 10% wastewater in the BMP assay using raw edible oil effluent (Second Run)

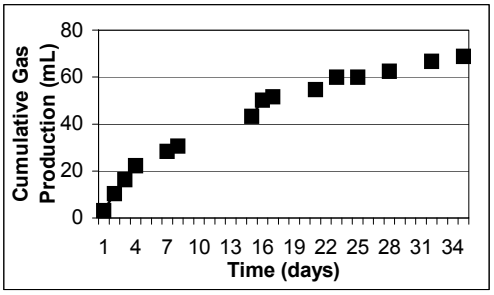


Figure 4.19: Cumulative gas production for 10% wastewater in the BMP assay using raw edible oil effluent (Second Run)

Total gas production (35°C):	68.7mL
% COD Reduction:	13.46%
Mineralized COD:	11.1mg
Lipid Reduction:	3.87%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.83	7.11
TS:	12910 mg/L	10920 mg/L
VS:	7370 mg/L	6350 mg/L

Table 4.13: Results of the BMP assay with 50% wastewater concentration using raw edible oil effluent (Second Run)

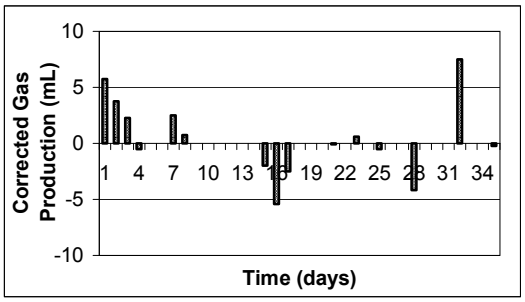


Figure 4.20: Corrected average gas production for 50% wastewater in the BMP assay using raw edible oil effluent (Second Run**)**

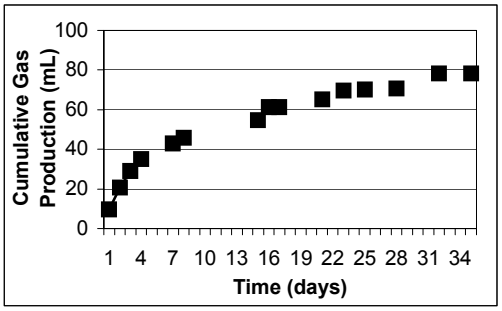


Figure 4.21: Cumulative gas production for 505 wastewater in the BMP assay using raw edible oil effluent (Second Run)

Total gas production (35^0C):	78.15 mL
%COD Reduction:	17.67%
Mineralized COD:	72.1mg
Lipid Reduction:	11.59%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.76	7.14
TS:	13020 mg/L	9200 mg/L
VS:	8090 mg/L	4970 mg/L

Table 4.14: Results of the BMP assay with 100% wastewater concentration using raw edible oil effluent (Second Run)

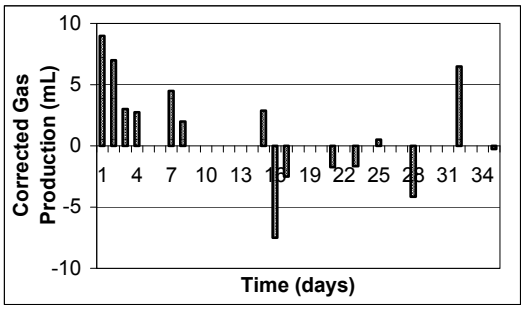


Figure 4.22: Corrected average gas production of 100% wastewater in the BMP assay using raw edible oil effluent (Second Run)

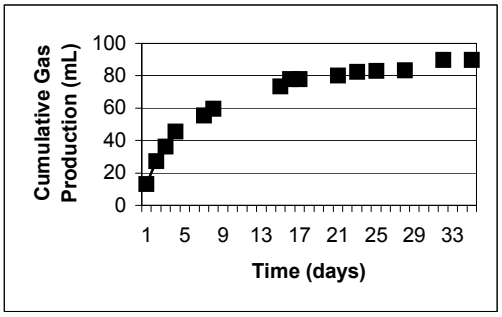


Figure 4.23: Cumulative gas production of 100% wastewater in the BMP assay using raw edible oil effluent (Second Run)

Total gas production (35^0C):	89.9 mL	
%COD Reduction:	24.14%	
Mineralized COD:	224 mg	
Lipid Reduction:	11.0%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.67	7.13
TS:	13650 mg/L	11860 mg/L
VS:	8290 mg/L	5380 mg/L

Table 4.15: Results of the biodegradability assay using raw edible oil effluent (Second Run)

%WASTEWATER CONCENTRATION	WASTEWATER COD (MG/BOTTLE)	MINERALIZED COD (MG)	GAS PRODUCED (ML/BOTTLE)	% COD REDUCTION
Control	-	-	69.45 mL	-
10	83.2 mg/L	11.1 mg/L	68.7 mg/L	13.46
50	408 mg/L	72.1 mg/L	78.15 mg/L	17.67
100	928 mg/L	224 mg/L	89.9 mg/L	24.14

4.4.4.2 Results of the Biodegradability Assay using Pre-treated edible oil effluent

Table 4.16: Results of controls in the BMP assay using pre-treated edible oil effluent

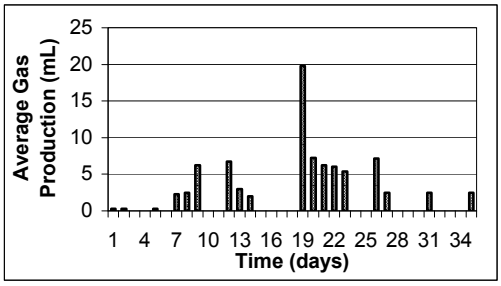


Figure 4.24: Average gas production of the controls in the BMP assay using pre-treated edible oil effluent

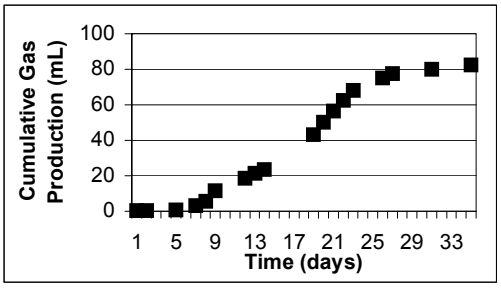


Figure 4.25: Cumulative gas production of the controls in the BMP assay using pre-treated edible oil effluent

Total gas production (35^0C):	82.8 mL	
%COD Reduction:	-	
Mineralized COD:	-	
%Lipid Reduction:	10.2%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.61	7.69
Sulphates:	510 mg/L	417 mg/L
TS:	13280 mg/L	11350 mg/L
VS:	8150 mg/L	6580 mg/L

Table 4.17: Results of the BMP assay with 10% wastewater concentration using pre-treated edible oil effluent

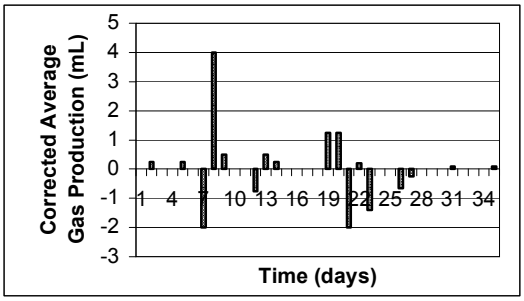


Figure 4.26: Corrected average gas production of 10% wastewater concentration in the BMP assay using pre-treated edible oil effluent

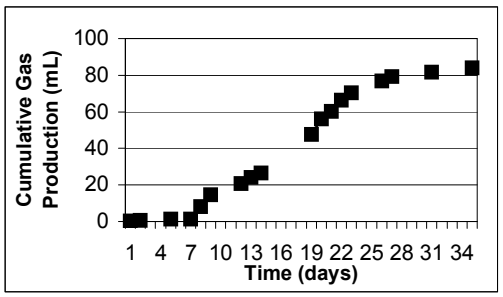


Figure 4.27: Cumulative gas production of 10% wastewater concentration in the BMP assay using pre-treated edible oil effluent

Total gas production (35^0C):	84 mL	
%COD Reduction:	12.5%	
Mineralized COD:	10.2 mg	
Lipid Reduction:	26.5%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.66	7.56
Sulphates:	430 mg/L	170 mg/L
TS:	13650 mg/L	10750 mg/L
VS:	8650 mg/L	5980 mg/L

Table 4.18: Results of the BMP assay with 50% wastewater concentration using pre-treated edible oil effluent

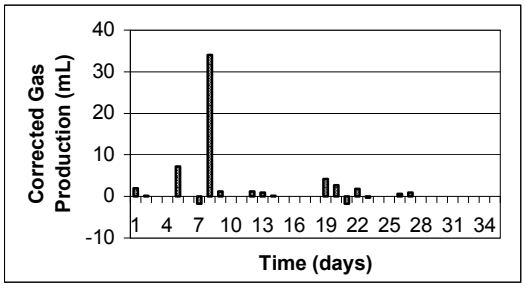


Figure 4.28: Corrected average gas production of 50% wastewater concentration in the BMP assay using pre-treated edible oil effluent

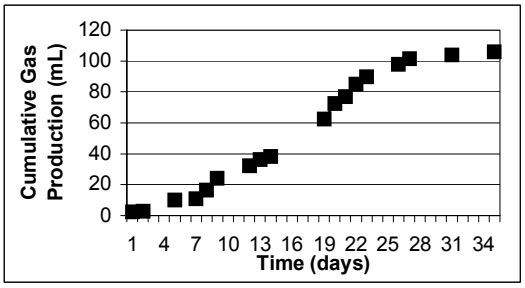


Figure 4.29: Cumulative gas production of 50% wastewater concentration in the BMP assay using pre-treated edible oil effluent

Total gas production (35^0C):	106.1 mL	
%COD Reduction:	14.9%	
Mineralized COD:	66.8 mg	
Lipid Reduction:	37.4%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.73	7.59
Sulphates:	353 mg/L	652 mg/L
TS:	12890 mg/L	10260 mg/L
VS:	7170 mg/L	5850 mg/L

Table 4.19: Results of the BMP assay with 100% wastewater concentration using pre-treated edible oil effluent

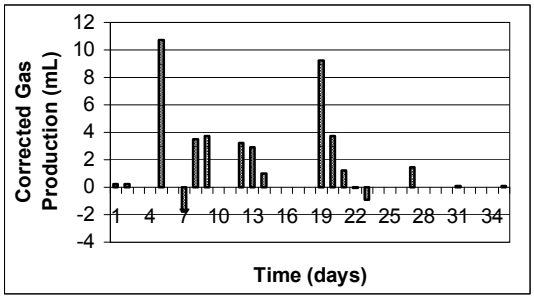


Figure 4.30: Corrected average gas production of 100% wastewater concentration in the BMP assay using pre-treated edible oil effluent

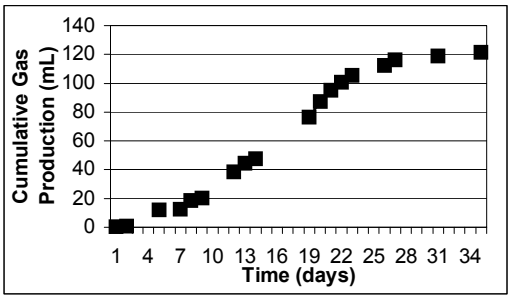


Figure 4.31: Cumulative gas production of 100% wastewater concentration in the BMP assay using pre-treated edible oil

Total gas production (35^0C):	121.25 mL	
% COD Reduction:	5.2%	
Mineralized COD:	47 mg	
% Lipid Reduction:	49.4%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.71	8.28
Sulphates:	333 mg/L	588 mg/L
TS:	14250 mg/L	11140 mg/L
VS:	7870 mg/L	5890 mg/L

Table 4.20: Results of the Biodegradability Assay using Pre-treated edible oil effluent

% WASTEWATER CONCENTRATION	WASTEWATER COD (MG/BOTTLE)	MINERALIZED COD (MG)	GAS PRODUCED (ML/BOTTLE)	%COD REDUCTION
Control	-	-	82.8	5.2
10	81.6	10.2	84	512.5
50	448	66.8	106.1	14.9
100	904	47	121.25	5.2

Figures 4.24, 4.26, 4.28 and 4.30 (in Tables 4.16 to 4.19) illustrate corrected gas production for pre-treated effluent samples. The graphs indicate the highest gas production for samples containing 100% wastewater concentration, which shows (as in the case with raw effluent) that the organics present in the pre-treated effluent were degraded by the microorganisms

Figures 4.23, 4.25, 4.27 and 4.29 (in Tables 4.14 to 4.18) illustrate cumulative gas production for the BMP assay using pre-treated effluent. It ca be seen that for the first 7 days gas production was low in comparison to the runs using raw effluent. Thereafter a sharp increase was seen between days 9 and 27, after which gas production seemed to stabilize. The low gas production in the first few days suggests an acclimatization period for the microorganisms thereafter the sharp increase in gas production suggests that the degradable organic material present in the edible oil effluent was utilized by the microorganisms.

The results of both runs of the bioassay using raw as well as pre-treated effluent indicate lipid reduction, which suggests that the microorganisms were capable of degrading lipids. The first run using raw effluent showed a general decrease in lipid reduction with increasing substrate concentration, whereas an increase in lipid reduction was seen in the second run with increasing wastewater concentration. Also, the percentages of lipids reduced in the first run are higher than the second run. The effluent sample used for the first run had a lipid content of 2492 mg/L, whereas the sample used for the second run had a lipid content of 456 mg/L. The higher lipid content of the effluent used in the first run could explain the decrease in reduction with increasing wastewater concentration, as methanogenic bacteria are inhibited by lipids (Hamdi, 1991). The results of the bioassay using pre-treated effluent indicate an increase in lipid reduction with an increase in wastewater concentration. This suggests that the microorganisms were capable of utilizing lipids with increasing lipid concentration in the pre-treated effluent.

BMP is referred either to sample volume (m^3CH_4/m^3 sample), sample mass (m^3CH_4/kg sample), or sample organic content (m^3CH_4/kg COD). The latter permits direct transfer of results into percent organic matter converted to methane (CH_4) by the theoretical 0.350m^3 CH_4 produced per kilogram COD catabolized (at STP) (McCarthy, 1964). Tables 4.10,

4.15 and 4.20 show results of the BMP assay for the first and second runs using raw effluent as well as pre-treated effluent. Looking at the estimated CH_4 and mineralized COD columns, the amount of CH_4 gas produced as a result of the mineralization of 1mg COD can be determined. It was found that the amount of gas produced per mg COD mineralized, did not correlate with the findings of McCarthy, (1964) for the first run using raw effluent, with 4.05 mL/mg and 0.34 mL/mg for 10% and 100% wastewater concentrations respectively. This may have been due to experimental error, since the results obtained for the second run using raw effluent as well as the run using pre-treated effluent, showed that for every milligram COD reduced, 0.395 mL methane was produced.

4.4.5 Results of Fed-Batch Digestion

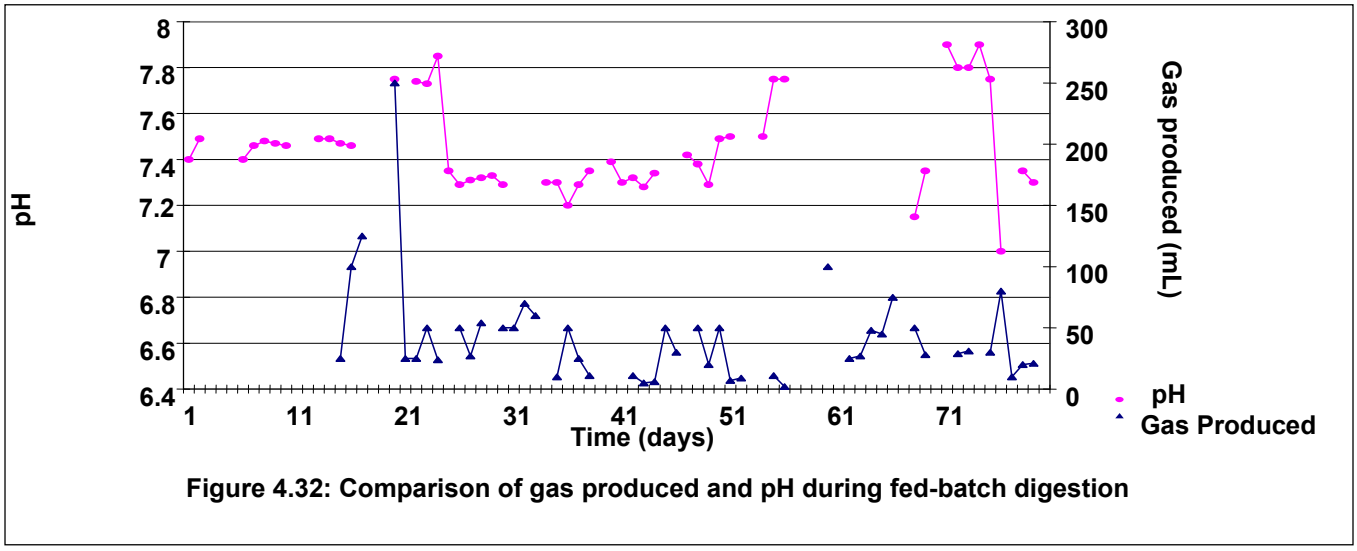


Figure 4.32: Comparison of gas produced and pH during fed-batch digestion

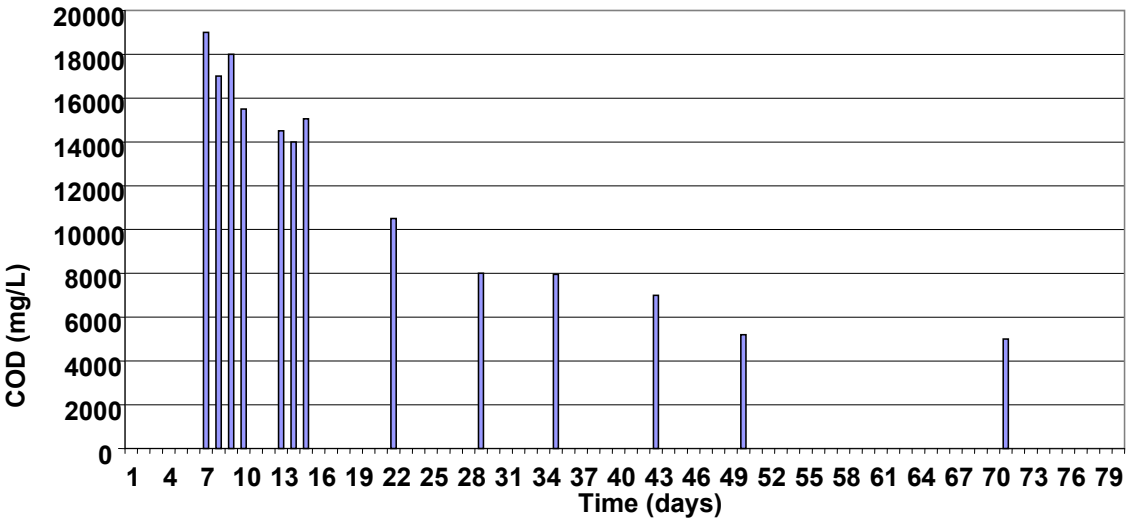


Figure 4.33: Graph illustrating COD (mg/L) during fed-batch digestion

The digester was initially fed artificial effluent to aid in acclimatization of the microorganisms to the environmental conditions. Gas production, pH and COD were monitored to ensure proper digester operation. Figure 4.32 illustrates the comparison of pH and gas production of the fed-batch digester. During the first 19 days, with HRT of 20 days, the graph indicates steady pH readings between 7.35 and 7.48. This suggests that the buffering capacity of the digester was sufficient to maintain the pH within normal working range. Thereafter, the working capacity of the digester was reached and the contents drained, leaving 950 mL sludge. The substrate was then changed to 50 mL effluent with a COD content of 2000 mg/L. Looking at Figure 4.32, the graph indicates an increase in pH to 7.78 after day 20. This could be attributed to adaptation of the microorganisms to the new substrate. Thereafter, the pH stabilized between 7.2 and 7.35. Gas production was low (average 25 mL) but stable. This suggests that the methanogenic bacteria were not severely inhibited by the increase in pH. The best pH range for the anaerobic bacteria is between 6.8 and 7.2 (Ross *et al*., 1992, Switzenbaum *et al*, 1990).

After 55 days, the digester had reached its working capacity and the contents drained, leaving 1 L of sludge. At this point, the pH of the digester contents was 7.78, alkalinity 1759 mg/L as $CaCO_3$ and COD content of 5700 mg/L. Although the pH seemed high, alkalinity concentration suggested sufficient buffering capacity of the digester. The feed

rate was then increased to 100 mL/d, consisting 90% effluent and 10% artificial effluent. An increase in pH was observed, initially, but stabilized between 7.3 and 7.4. Gas production averaged at about 45 mL per day. The increase in gas production with feed rate of 100 mL per day in comparison with a feed rate of 50mL per day suggests that the increased feed volume increased the readily degradable organics which resulted in an increase in gas production.

After day 88, the digester contents were drained, again leaving 1 L of anaerobic sludge. The feed rate was continued at 100 mL per day. The pH remained stable, between 7.25 and 7.30. Gas production increased to an average of 62 mL per day (not shown on graph). This indicates that the microorganisms had acclimatized to the effluent at a feed rate of 100 mL per day. After day 98 days, the reactor was drained, leaving 1 L of anaerobic sludge. The feed rate was increased to 150 mL per day. The pH was stable, between 7.25 and 7.3 and gas production increased to an average of 95 mL per day (not shown on graph).

It is clear that with an increase in feed volume, there is an increase in gas production. Therefore, the increasing feed volumes did not cause an organic overload. An organic overload causes increased acid production, with a decrease in pH and little or no gas production.

Figure 4.33 illustrates the COD profile of the digester, which indicates a gradual decrease in total COD of the digester contents. This suggests that the microorganisms were capable of degrading the available organic material present in the wastewater.

Results show that anaerobic fed-batch digestion is a promising method of treatment of edible oil effluent.

4.4.6 Results of the Anaerobic Baffled Reactor

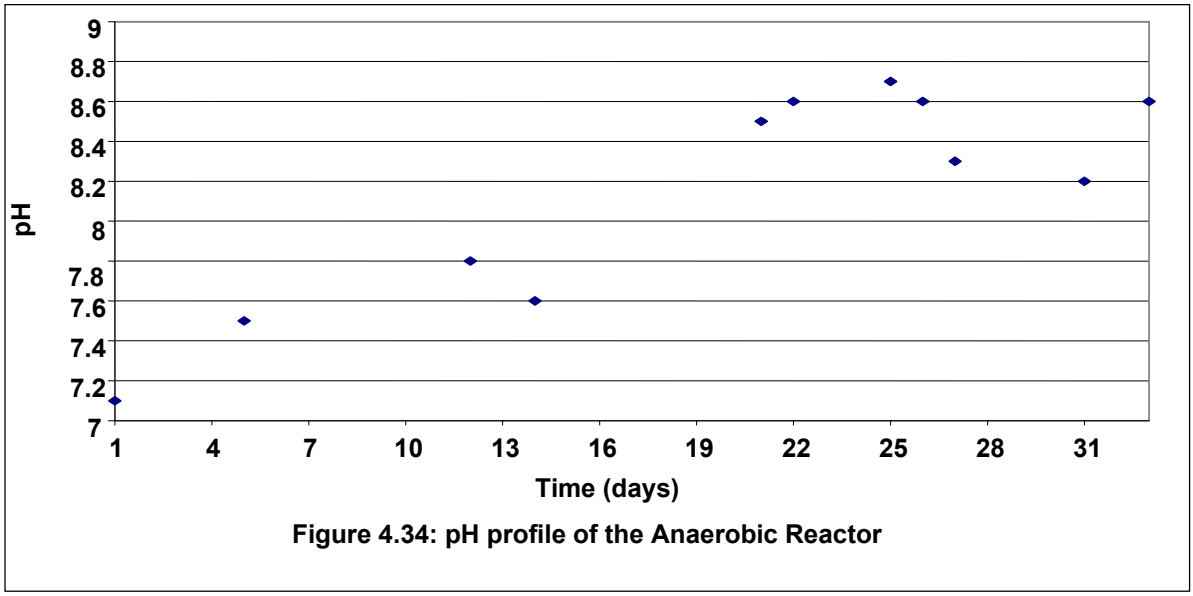


Figure 4.34: pH profile of the Anaerobic Reactor

The reactor was fed a combination of artificial effluent (10%) and effluent (90%), with pH ±7 and HRT of 30 days. The initial pH of the digester contents was within the normal working range of anaerobic digestion i.e., 6.5 and 8.2 (Ross *et al*., 1992), with alkalinity at 2370 mg/L as $CaCO_3$. Figure 4.34 illustrates the pH profile of the ABR during digestion. It can be seen that the pH gradually increases over time up to 8.7 on day 25. A gradual decrease in pH was seen after day 25 as the substrate fed to the digester was decreased to 6.5. However, the pH continued to increase thereafter. This suggests that the digester was unable to maintain adequate buffering capacity. It is possible that high sulphate content of the effluent, promoted growth of SRB producing H_2S which can be toxic at elevated concentrations to sulphate reducers as well as other microbial activity (Speece, 1996). Inhibition caused by H_2S is dependant on the pH of the environment. At alkaline levels the inhibitory effect of H_2S is higher than at neutral or acidic pH levels (Hilton and Oleszkiewicz, 1989).

It was observed that the first few compartments (closest to the influent port) contained more settled biomass than the other compartments. This is not characteristic of an ABR, as biomass within the reactor tends to rise and settle with gas production and move horizontally down the reactor (Nachaiyasit and Stuckey, 1995). Also, the different levels of

liquid were observed in various compartments. It is possible that the fats and oils contained in the substrate formed a lipid barrier, disrupting normal circulation of the liquid.

After day 33, a decline was observed in pH of the digester (not shown in graph), with no gas production. The experiment was terminated, since no gas production indicated that the methanogenic bacteria were no longer active. It was therefore concluded that the ABR is not suitable for treatment of this type effluent.

4.5 CONCLUSIONS

From this investigation of anaerobic digestion of vegetable oil effluent, the following can be concluded,

- Gravitational separation of lipids is not a recommended method for reducing the lipid content of effluent due to the long settling time required. Settling times below 24 hr showed no significant difference in lipid content.
- The use of barium chloride is an effective method to reduce content in the effluent
- Effluent contains approximately 30% biodegradable fraction that under anaerobic conditions has the potential to produce 0.395 mL of CH_4 mg COD mineralized.
- Anaerobic toxicity of edible oil effluent indicates that the effluent is not harmful to the existing anaerobic process if added at a COD concentration of 2000 mg/L.
- Anaerobic digestion using fed-batch digestion configuration can achieve only 30% biodegradation of COD present in edible oil effluent.
- Experiments show that the ABR configuration is unsuitable for anaerobic digestion of edible oil effluent.

CHAPTER 5
PRETREATMENT METHODS TO ENHANCE SECONDARY BIOLOGICAL TREATMENT OF THE EDIBLE OIL EFFLUENT

5.1 INTRODUCTION

The effluent, when collected from Company X, was milky white in colour with a red-orange layer on the surface – indicating the presence of oil. The pH of the effluent was either very acidic, resulting in a strong odour that was an irritant to the nasal passages and the eyes, or the effluent was alkaline. The alkaline nature of the effluent was due to the presence of caustic soda, which results in extensive foaming.

Raw effluent cannot be directly added into the system, as the high COD and FOG concentrations would result in shock loadings and the death of the bacteria. To avoid this, the effluent was often pretreated, to lower both the COD and FOG concentrations, prior to biological treatment, by coagulation and flocculation. In the case of the effluent from Company X, the addition of a chemical coagulant, called C40 (Chemserve Trio, SA), together with mechanical stirring causes the FOG's, present in the effluent, to agglomerate or flocculate. These oil particles then settle and it was the clear supernatant above the settled flocs that was more suitable for introduction into an activated sludge unit.

Pretreatment may be defined as, the process or processes that prepares wastewater to a condition where it can be further treated in a conventional secondary biological process (Kiely, 1997). The present method of pretreatment involves the coagulation and flocculation of the edible oil effluent with chemical flocculent Compound C40.

5.1.1 Alternate Pre-Treatment Methods Used in This Study

Alternate pretreatment methods used in this study included the use of various coagulants with DAF, Ozonation, Peroxone and Ultrasonication.

5.2 AIMS AND OBJECTIVES

The aim of this study was to evaluate different pretreatment technologies to produce an effluent with optimum environmental discharge requirements. The objectives are as follows:

1. Evaluation of the most efficient coagulant at optimum concentration
2. To determine the efficiency of coagulation followed by DAF
3. To evaluate the efficiency of ozonation, peroxone and ultrasonication

5.3 MATERIALS AND METHODS

5.3.1 Coagulation & Flocculation Using the Standard Jar Test (Coagulation Test) (Pryor & Freese, 1998)

An 800 mL sample of the C40-coagulated effluent was taken and poised at the discrete pH values of 5.5, 7.5 or 9.5 by the addition of either sulphuric acid or sodium hydroxide. Following this the sample was placed on a magnetic stirrer and stirred at 300 rpm while the different coagulants were added at concentrations of 5, 10, 15, 20, 25 and 30 ppm (parts per million). The following coagulants were used: Aluminum sulphate (alum), ferric chloride and various commercially available, polymeric coagulants: DMDAAC/PAC - Z553D, DMDAAC/PAC - LP526, PA/PAC – 735 and PA – U5000. Preparation of coagulants can be seen in Appendix 14.

Following the addition of the coagulant the stirring speed was reduced to 40 rpm for 15 min, after which, the sample was transferred to the DAF unit where it was allowed to settle for an additional 15 min. After settling, a sample was taken from the supernatant above the settled flocs, for COD analysis (see Appendix 3a).

5.3.2 Dissolved Air Floatation Subsequent To Coagulation & Flocculation

The pressure cylinder was filled with water up to the halfway mark and the inlet valve on the pressure cylinder opened to allow compressed synthetic air to enter to a final pressure of 400 kPa (4 Bar). The pressure cylinder was then shaken vigorously to allow an even distribution of air throughout the water. Following this, 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 5 min to allow the bubble-floc agglomerates to rise to the surface of the sample and, thus, to form the float. A sample for subsequent COD and FOG analyses was taken from the liquid below the float.

Each of the six coagulants prepared were used to treat the C40-coagulated effluent by additional coagulation and flocculation followed by DAF at each of the concentrations from 5 ppm to 30 ppm at the poised pH values of 5.5, 7.5 and 9.5, respectively.

In order to determine the total amount of organics removed (total COD reduction) after coagulation and flocculation and DAF, the following calculations were done using the COD values

The average COD values for coagulation & flocculation were subtracted from the original COD values of the C40-coagulated effluent. The values obtained give an indication of the amount of organics removed after coagulation & flocculation.

1. The average COD values for DAF were subtracted from the average COD values for coagulation and flocculation. The values obtained give an indication of the amount of organics removed after DAF.
2. The values obtained in 1 and 2 were then added to obtain the total COD reduction after coagulation & flocculation and DAF.

5.3.3 Comparison of the Alternative Pretreatment Method to the Current Method

Pretreatment of the Raw Effluent Using Both Pretreatment Methods

From a batch of raw effluent, 800 mL was decanted into a 1000 mL conical flask. This sample was pretreated using the coagulation and flocculation and DAF combination, which showed the greatest COD removal under optimum operating conditions.

From the same batch of raw effluent, 800 mL was decanted into a 1000 mL beaker. This sample was pretreated using the current pretreatment method of coagulation & flocculation with C40 only.

5.3.4 Ozonation

The ozone generator (Ozonox) was attached to a pump to ensure a steady supply of air, which in turn, allowed for a steady supply of generated ozone to be introduced into the sample. Gas exiting the sample was allowed to bubble through two potassium iodide (20 g of KI crystals in 5 L) traps, connected in series.

5.3.4.1 Preparation of a Calibration Curve

A standard sodium thiosulphate solution ($Na_2S_2O_3.5H_2O$) was prepared according to the Iodometric Method I (See Appendix 15). This standard solution, 25 g of sodium thiosulphate was added to 1 L of freshly boiled distilled water, was used to determine the amount of ozone produced by the ozone generator over a specific time period. The sample bottles and traps were filled with 400 ml and 800 ml of KI solution respectively. Following this the sample was ozonated for 1 min, inducing a colour change form clear to yellow. The ozonated sample was titrated against the $Na_2S_2O_3.5H_2O$ until the end point was reached where the sample turned from a yellow colour to clear. The amount of $Na_2S_2O_3.5H_2O$ used to induce the end point was recorded. Similar experiments were done at increased time intervals from 2 - 10 min. The concentrations of ozone produced were calculated and plotted on a calibration curve (See Appendix 15).

5.3.4.2 Ozonation Procedure (Pryor & Freese, 1998)

The pH of 800 ml of flocculated sample was adjusted using either sodium hydroxide or sulphuric acid. Following this the sample bottle was connected to the KI filled bottles acting as gas traps. The sample was ozonated for 3 min, thereafter; residual ozone was calculated using the Indigo Colorimetric Method (See Appendix 16). From this the total amount of ozone produced as well as concentrations used by the sample can be calculated. Similar sets of experiments were made at discrete pH values of 7, 9 and 11.

5.3.5 Peroxone Procedure

The pH of 660 ml of flocculated samples was poised at pH 7, 9 and 11 by using either NaOH or H_2SO_4. To these samples 140 ml of 50% hydrogen peroxide ($H_2O_{2)}$ was added to produce a final sample concentration of 10%. The samples were, subsequently, ozonated for 3 min and the residual ozone and total ozone consumed were calculated. The results were evaluated using TLC with the original effluent sample serving as a control. See Appendix 17 for TLC determination.

5.3.6 The Effectiveness Of Ultrasonics As A Pretreatment Method Of An Edible Oil Effluent

40 ml of the C40 flocculated sample was placed in a plastic centrifuge tube which was then placed in a beaker of ice and sonnicated by the use of a Virtis Virsonic 100 (50 Hz).

The procedure was carried out for 10 min on different samples using output wattages of either10, 15, 20 and 25 W, respectively (See Appendix 18)

For the experiments using a sonnicator bath, UMC5 (220 V, 50 Hz) the samples (100 ml) were sonnicated for 15 min. As in the previous experiments, TLC was used to evaluate the results while the original sample was used as a control.

5.3.7 Evaluation of the Effectiveness of the Pretreatment Methods

5.3.7.1 TLC

Procedure

The water insoluble fractions from the experimental samples (40 ml) were extracted using hexane (10 ml) in a separation funnel and spotted, using Micro haematocrit tubes, on silica gel that was coated on aluminium plate (Merck, 60E254). The sample was run using a mobile phase of 20% ethyl acetate in hexane. The mobile phase was allowed to run until it was approximately 1 cm away from the top edge of the plate and then the process was then stopped and the solvent front marked. After completion, the plate was viewed under a CAMAG Universal UV lamp (wavelength of 252 nm) and the Rf and H_{Rf} values were calculated and intensities were recorded.

In all experiments the relative effectiveness of the treatment method was determined by comparing the results of a control sample to the treated sample using analytical methods such as, TLC (Appendix 17), FOG (Appendix 10) and COD analysis (Appendix 3a).

5.4 RESULTS AND DISCUSSION

5.4.1 Optimum Conditions for Enhanced Coagulation and Flocculation

Alum, $FeCl_3$ and four polymeric coagulants were used to further treat the C40-coagulated effluent by additional coagulation, flocculation and DAF, at discrete concentrations from 5 to 30 ppm and at different pH values of 5.5, 7.5 and 9.5. The effectiveness of the additional treatment procedure was tested by comparing the COD for the original C40-coagulated effluent samples with the samples following additional coagulation and flocculation. Likewise, the effectiveness of the DAF procedure was tested by determining COD and FOG concentrations of the samples following coagulation, flocculation and DAF

At the concentrations of 5, 10, 20 and 30 ppm, and at a pH of 5.5, $FeCl_3$ had the highest total COD reduction in comparison with the other coagulants. Therefore, at these concentrations and pH values, $FeCl_3$ functions the best in removing organics from the effluent. Further, the highest overall total COD reduction was found to be at a $FeCl_3$ concentration of 10 ppm. The initial COD concentration was 1455 mg/L at 10 ppm and after coagulation and DAF the effluent COD was 1030 mg/L. (Graph not shown)

Similar results were recorded at the concentrations of 5, 10, 15 and 20 ppm, and at a pH of 7.5, $FeCl_3$ again performed the best in removing organics from the effluent compared with the other coagulants. At this pH value $FeCl_3$ had the highest overall total COD reduction at a concentration of 20 ppm. The initial COD concentration was 1445 mg/L and following coagulation and DAF the effluent was 970 mg/L. (Graph not shown)

At pH values of 9.5 and at concentrations of 5 and 25 ppm, coagulant C performed optimally while at concentrations of 10 and 15 ppm, coagulant A performed the best. At concentrations of 20 and 30 ppm, on the other hand, $FeCl_3$ showed the greatest COD removal. The initial concentration at 20 ppm was 1150 mg/L and following pretreatment the effluent was 870 mg/L. At 30 ppm the initial COD was 1065 mg/L and following

pretreatment the effluent was 662 mg/L. The best coagulant to use, at pH 9.5, cannot clearly be identified. However, it must be noted that the overall effectiveness of ferric chloride, in the removal of organics from the effluent, has decreased at pH 9.5 as compared with the pH levels of 5.5 and 7.5 (Graph not shown).

At both pH 5.5 and pH 7.5, $FeCl_3$ recorded the highest COD removal rates although the overall greatest removal was recorded at the more acidic pH of 5.5 with decreased effectiveness noted as the pH increased. A possible reason for the decreasing effectiveness of $FeCl_3$ as pH increases may be due to the inorganic structure of $FeCl_3$ that is altered with pH increase. Alteration of the structure of $FeCl_3$ results in the prevention of this coagulant from binding to organics in the industrial edible oil effluent. Subsequently, few or no flocs are formed and the organics are not removed from the effluent by settling. The total efficiency and effectiveness of the DAF process is, likewise affected. Pryor and Freese (1998) observed a similar effect, in that inorganic coagulants such as $FeCl_3$ and alum were generally more effective during coagulation and flocculation than polymeric coagulants at lower pH values. It can, therefore, be concluded that as an alternative pretreatment method, $FeCl_3$ at a concentration of 10 ppm and at a poised pH of 5.5 should remove the greatest amount of organic compounds from the effluent.

5.4.2 The most Effective Pretreatment Method for the Industrial Edible Oil Effluent

Two samples of the raw industrial effluent were pretreated. The one sample was pretreated using the currently used method of coagulation & flocculation using C40 while the other sample was pretreated using $FeCl_3$ at optimum operating conditions. The raw effluent acted as the control, and the results obtained are detailed below (Table 5.1).

Table 5.1 Results for COD

Sample	COD Values (mg/L)		Average COD Values (mg/L)
Raw effluent sample	3940	3850	3895
Sample pretreated using C40	1040	1100	1070
Sample pretreated using $FeCl_3$	2880	2880	2880

From the three samples, the raw effluent clearly had the highest COD value of 3895 mg/L while the two pretreated samples showed reduced COD values. However, of the two, the C40 pretreated sample recorded the lowest (1070 mg/L) COD value and, thus, the greatest organics removal capabilities. Similarly, the amount of FOG's, in the raw effluent

sample and samples pretreated with C40 and $FeCl_3$ were determined. Like the COD removal rates the greatest FOG removal efficiencies were recorded with the C40 pretreated sample, again, indicating the effectiveness of this pretreatment method (Table 5.2)

Table 5.2 FOG Concentrations

Sample	Total Amount of FOG's (mg/L)
Raw effluent sample	2432
Sample pretreated using C40	164
Sample pretreated using $FeCl_3$	570

This, overall, greater removal of organic compounds from the effluent using C40 was also recorded in the TLC plates run with these samples. The TLC plates indicated that C40 was able to remove some of the organic compounds that were not removed by any of the other coagulants tried during this study. This was indicated by fewer spots when compared with the other samples, as well as lower colour intensity.

5.4.3 Subjecting the Effluent to Specific Treatments at pH 7

During this phase of the study the pretreatment methods of ozonation and ultrasonication were investigated. The samples, and a raw effluent sample as control, were subjected to the respective treatments, at specific pH values, and the organic removal efficiency determined by TLC. The results following the respective pretreatments at a pH of 7 are detailed below (Table 5.3)

Table 5.3 The TLC Data of the Effluent Sample Treated at pH7.

SAMPLE		COMPOUND			
		A	B	C	D
ORIGINAL	R_f VALUE	0.39	0.45	0.64	0.68
	H_{Rf} VALUE	39%	45%	64%	68%
	INTENSITY	6	5	10	2
pH 7	R_f VALUE	0.39	0.45	0.62	0.66
	H_{Rf} VALUE	39%	45%	62%	66%
	INTENSITY	3	2	8	2
OZONATION	R_f VALUE	0.45	0.58	0.7	0.75
	H_{Rf} VALUE	45%	58%	70%	75%
	INTENSITY	4	3	5	1
PEROXONE	R_f VALUE	0.42	0.51	0.66	0.72
	H_{Rf} VALUE	42%	51%	66%	72%
	INTENSITY	4	2	7	1

Ozonation: **Residual Ozone:** 9.570×10^{-3} mgO_3/L **Total Ozone consumed:** 2.4384 mgO_3/L

Peroxone: **Residual Ozone:** 9.159×10^{-3} mgO_3/L **Total Ozone consumed:** 2.3564 mgO_3/L

By comparing the results for any of the pH values, it can be seen that there was no significant change between the R_f values of the compounds in the sample when it was subjected to the ozonation and the peroxone procedures. Intensities showed very little reduction, indicating that some of the substance had been removed, although, the reduction appears to be insignificant.

A similar set of experiments were made at a pH of 9, which indicated that (results not shown) the ozonation procedure resulted in no change in the R_f values and intensities when compared to the original sample. The sample, subjected to the peroxone process, showed a decrease in the intensities indicating that, although insignificant, a small amount of compound had been converted to other compounds.

At a pH of 11 (results not shown) there was no significant differences between the different samples. However, there were changes in the colour of the sample subsequent to treatment. The effluent turned more yellow after ozonation and following peroxone, the sample lost its yellow haze and became colourless.

Additional tests were made to determine the relative effectiveness of the ozone pretreatment method compared with currently used coagulation with C40. Following the tests the samples were analysed by TLC and the results are given below (Table 5.4). From these results it can be seen that the R_f values and intensities of the compounds did not show any significant change. Also, from the results shown it can be seen that the chemical coagulant, C40, removes compound C that is present in the raw effluent while ozonation does not. This shows that coagulation with C40 is a more effective pretreatment method of the oil effluent than ozonation

Table 5.4: The TLC Data of the Samples Treated by Ozonation and Coagulation.

SAMPLE		COMPOUND				
		A	B	C		E
ORIGINAL	R_f VALUE	0.49	0.58	0.64	0.73	0.8
	H_{Rf} VALUE	49%	58%	64%	73%	80%
	INTENSITY	4	6.5	6.5	10	5
COAGULATION C40	R_f VALUE	0.49	0.6	-	0.76	0.82
	H_{Rf} VALUE	49%	60%	-	76%	82%
	INTENSITY	4	4	-	7	2
OZONATION	R_f VALUE	0.49	0.6	0.67	0.76	0.82
	H_{Rf} VALUE	49%	60%	67%	76%	82%
	INTENSITY	4	6	6	9	1

Following the treatment of the samples the results were, also, evaluated using infra red (IR) techniques although the results are not shown From the IR spectra achieved it was seen that the sample contains low concentrations of alkanes and alkenes. This can be seen by the absorbance of these compounds at a specific wavelength. Alkenes absorb infra red at 2000 – 1500 cm^{-1} (Faust, 1995). There was little absorbance at this wavelength. Alcohol functional groups and aromatics also appeared to be in small quantities. No carbonyl groups could be detected. Further, there appeared to be no significant difference between the spectra achieved from the treated and untreated effluent. This showed that what complex molecules present in the effluent is not broken down into simpler and more biodegradable ones. This indicated that ozonation is an unsuitable method of pretreatment of the oil effluent.

The test indicated that there is very little FOG present in the original flocculated sample, as C40 removes most of these substances. Coagulation is therefore is the best method of pretreatment as compared to the $FeCl_3$ coagulant.

5.4.4 Effluent Pretreatment by Sonnication

Although the complete experiment included running samples at various watts (10, 15, 20 and 25 W) only the results of the 10 W experiments is shown (Table 5.5) due to the conclusions reached being very similar despite the increased output. After subjecting the effluent sample to ultrasonic treatment, it can be seen that the R_f values were relatively similar and that there was no decrease in intensities for each compound. It therefore appears as if ultrasonics was not effective in pretreating the edible oil effluent.

Table 5.5 The TLC Data of the Effluent Sample Sonnicated Using an Output of 10 W.

SAMPLE		COMPOUND			
		A	B	C	D
ORIGINAL	R_f VALUE	0.42	0.53	0.67	0.73
	H_{Rf} VALUE	42%	53%	67%	73%
	INTENSITY	9	7	10	4
SAMPLE (10W)	R_f VALUE	0.42	0.52	0.67	0.73
	H_{Rf} VALUE	42%	52%	67%	73%
	INTENSITY	9	6	10	4

5.4.4.1 The results achieved by sonnicating using a sonnicator bath

After subjecting the effluent sample to ultrasonic treatment, it can be seen that the R_f values were relatively similar and that there was no decrease in intensities for each compound. It, therefore appears as if ultrasonics, using the more powerful sonication bath, was not effective in pretreating the edible oil effluent (Table 5.6)

Table 5.6 The TLC Data of the Effluent Sample Sonnicated Using a Sonnicator Bath.

SAMPLE		COMPOUND				
		A		C	D	E
ORIGINAL	R_f VALUE	0.39	0.48	0.52	0.63	0.69
	H_{Rf} VALUE	39%	48%	52%	63%	69%
	INTENSITY	9	8	3	1 0	7
SONNICATION	R_f VALUE	0.39	0.48	0.56	0.67	0.72
	H_{Rf} VALUE	39%	48%	56%	67%	72%
	INTENSITY	9	8	3	10	7

5.5 DISCUSSION

All the results, viewed collectively, seem to indicate that the techniques of ozonation, peroxone and ultrasonics are ineffective methods for the pretreatment of an edible oil effluent.

The lack of effectiveness of ozonation can be due to the low presence of alkenes in the effluent, which was shown by the IR scan (Graph not shown). It has been shown that ozonation performs optimally in the presence of unsaturated bonds (Kloos, 2000). Therefore, with these present in low concentrations, optimum ozonation proved unsuccessful.

Hydrogen peroxide also proved incapable of accelerating the ozonation procedure. Part of this relative inefficiency of peroxone could be due to the time of peroxide addition. During the present investigation peroxide was added prior to ozonation, which occurs in two stages. If peroxide is added prior to the first stage, as was the case, the hydroxyl radical competes with the ozone-reactive molecule for the ozone (Arce Systems, 2000). This could slow the process and, subsequently, hinder the peroxone process. However, the colour change of the sample at pH 11 shows that peroxone is efficient in removing colour. Peroxone may prove effective if the peroxide is added after the first stage although this can only be done using specialised pumps.

As recorded for the other methods, ultrasonics, likewise, proved non-effective. The ultrasonics did not appear able to break the strong bonds of the complex molecules found in the edible oil effluent. One reason for this could be due to the low frequency of the instrument used. Ultrasonics may prove effective if attempted at a higher frequency.

5.6 CONCLUSION

The effectiveness of the $FeCl_3$, alum and the polymeric coagulants proved to be poor as compared to the commercial coagulant C40. The pretreatment methods of ozonation, peroxone and ultrasonics are ineffective methods of pretreatment of an edible oil effluent.

Hence from all of the results obtained it can therefore be concluded that the alternative pretreatment methods were not as effective as the current pretreatment method. Thus, the method of chemical coagulation and flocculation with C40 appears to be the most effective pretreatment for the edible oil effluent investigated in this case study.

CHAPTER 6
LABORATORY SCALE AEROBIC BIOLOGICAL TREATMENT PROCESS

6.1 INTRODUCTION

Current method of effluent treatment in the in this case study was restricted to either physical separation of oil and grease via a gravity fat trap or dissolved air floatation and subsequently pH correction before discharge. Even after application of these physico-chemical techniques, the remaining emulsified grease tends to clog sewer pipes and pumps and often does not subscribe to municipal discharge standards (Eroglu *et al.*, 1990; Mkhize *et al.*, 2000; Mkhize and Bux, 2001).

Globally, trends are currently focusing on applying biological processes for treatment and final polishing of the effluent. Eroglu *et al*. (1990) conducted research on the comparative evaluation of treatment alternatives for wastewater's from an edible oil refining industry. Results showed that activated sludge proved successful in removing most of the fats, oils and greases.

6.2 AIMS AND OBJECTIVES

Therefore, the emphasis of this aspect of the research focused on developing and optimizing biological treatment process i.e. activated sludge in order to successfully treat sunflower oil effluent. The objectives included:

- Wastewater characterization and large-scale pretreatment of the oil effluent
- Development of laboratory scale activated sludge process
- Operation of the activated sludge process
- Optimization of the process for maximum COD removal

The investigation subscribed to using effluent from a single factory (Company X), since previous research (Orhon *et al.*, 1999) has shown that developing remediation technology in the edible oil waste sector has to be subjected to a case by case approach. In addition, the effluent used was from the refinery process, since it comprised of a major portion of the final effluent.

6.3 MATERIALS AND METHODS

6.3.1 Laboratory scale pretreatment process

6.3.1.1 Collection of effluent

Composite effluent samples were collected from the drain at the end of the refinery process. The volume collected comprised of 22 x 25 L containers, giving a total volume of 550 L. The company was situated approximately 90 km north west of Durban. The samples were transported immediately to the laboratory for processing which included a battery of chemical test to characterize the influent. The balance of the samples was stored in the cold room at 4^{o}C to prevent further biological activity. It should be noted that collection of sample was ultimately dependent on the regular operation of the refinery process. However, during the course of this investigation, the problems that hampered regular supply of effluent were as follows:

- Regular interruptions in the refinery process due to problems in obtaining crude oil
- Alternating between refining soya, cotton seed and sunflower oil without prior notification which was problematic since the investigation was limited to effluent of the sunflower oil refinery process.
- An explosion at the company had disrupted the refinery process for 3 months.

The above hurdles are quite common when investigating industrial problems as the industries rarely place priority on research needs of the project, and therefore progress is subjected to conditions at the industry. The effluent collection period spanned during the entire period of the laboratory trials i.e. May 2000 until October 2001.

6.3.1.2 Wastewater characteristics determined

On receipt of the effluent, samples were immediately obtained and the following chemical analysis was conducted:

COD: The COD of the effluent after pretreatment was still high and therefore was diluted with distilled water in order to satisfy the objectives of the research i.e. subjecting the treatment process to incremental increase in COD from 500 mg/L to 1800 mg/L (at full

strength after pretreatment i.e. undiluted). A colorimetric method was used using the Merck SQ 118 Photometer (Method No. 14690) (Appendix 3a)

FOG: Since the effluent was comprised primarily of fats and oils, it was essential to determine the FOG and manipulate it i.e. pre-treat and reduce content in order to prevent organic shock load of the system. The procedure involved applying Standard Methods (1989) (Appendix 10).

Nutrients: Due to changes in the refinery process, nitrogen and phosphorus became limiting in the effluent during the early stages of the treatment process. Therefore, the TKN and TP of the effluent were determined. In order to maintain the integrity of the biological system, nitrogen and phosphorus was added to the effluent at a C: N: P ratio of 100: 10: 1. These salts, ammonium chloride (NH_4Cl) for N and Potassium dihydrogen phosphate (KH_2PO_4) for phosphorus was added directly to the feed tank after the flocculation procedure.

- **Total phosphate (TP)**: The method involved using the Merck SQ 118 photometer (Method No. 14842) (Appendix 4).
- **Total Kjeldahl nitrogen (TKN)**: The TKN is indicative of the total nitrogen content of the wastewater. The Spectroquant analysis method 14537 was employed using the Merck photometer (SQ 118) (Appendix 5).

pH: The pH of the effluent was corrected to pH $\pm$ 7 prior to biological treatment by the addition of acid in the feed tank. The volume of acid added to neutrality was ultimately dependent on the pH of the batch of effluent collected.

6.3.1.3 Large scale pretreatment procedure

The effluent was processed immediately on receipt. The batch was separated into 2x 275 L batches. One batch was stored at 4^{o}C for future use. A volume of 275 L was dispensed into a 300 L vessel in order to facilitate the flocculation and coagulation process at room temperature (20^{o}C). The initial pH of the effluent was acidic but on addition of the flocculent, the pH became basic (approximately pH 9). The pH was further adjusted to pH 7 by the addition of sulphuric acid. The flocculent (C40) was added to the reactor with

slow stirring. The approximate weight of C40 that was added was 2210 g for 275 L of effluent, giving a final C40 concentration of 8 g/L. However, the amount of C40 required for complete flocculation did vary amongst the batches. The latter was due to fluctuation in the FOG component of the oil effluent, which was depended on the refinery process. The time taken until clarification was reached was approximately 10 min. The supernatant (flocculated effluent) was allowed to remain for 24 - 48 hr in the vessel to facilitate efficient removal of the emulsified FOG's. On completion of the flocculation process, the supernatant was transferred to a 300 L feed vessel situated in the cold room.

6.3.2 Laboratory scale unit description

6.3.2.1 Unit set-up and configuration

The pilot scale activated sludge process was manufactured by the Department of Civil Engineering, University of Cape Town. The unit was set up next to the cold room, thus facilitating easy supply of influent situated in the cold room at 4^{o}C (Feed Tank). The temperature of the room in which the pilot plant was located was maintained at 20^{o}C. The laboratory scale process was operated for approximately 18 months (May 2000 - October 2001).

During the initial period of the investigation, the pilot plant was operated based on the 3-stage Phoredox process used for biological phosphate removal. However, due to a change in the oil refinery process i.e. phosphoric acid was replaced with citric acid; the total phosphate concentration of the effluent was within the discharge standards. The pilot plant configuration was subsequently changed to the modified Ludzak- Ettinger configuration focusing primarily on carbon and nitrogen removal. A schematic design of the pilot plant is given in Fig. 7.1. The reactor configuration comprised of the following: an anoxic (AX) zone (V = 8 L); first aerobic (AE1) zone (V = 10 L); and second aerobic (AE2) zone (V = 10 L). The settler (2.5 L), was positioned downstream of the reactors at an angle of 60^{o} to the perpendicular. Although the latter configuration follows that of an MLE process, an additional aerobic zone was present. Therefore the process can be regarded as an adapted MLE process (Fig. 6.1).

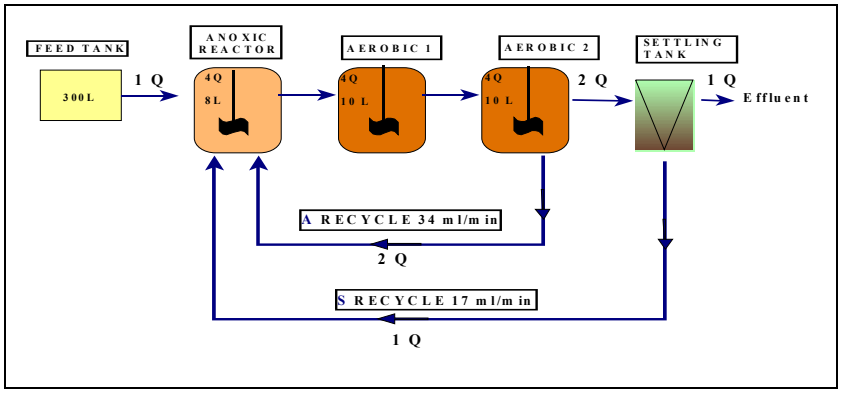


Figure 6.1 Schematic representation of the laboratory scale unit modeled upon the modified

After the flocculation and coagulation process, the influent was transferred to the Perspex feed tank (V = 300 L) which was located in the cold room (4°C) in order to limit any biological activity prior to treatment. Target influent flow rate (Q_i) was set at 17 mL/min (24.48 L/d). The S-recycle is the flow that is pumped from the clarifier (settling tank) to the AX reactor, with a set ratio of 1:1 with respect to Q_i. The A-recycle, was pumped from AE2 to the AX reactor at a set ratio of 2:1, with respect to Q_i.

The system sludge age (R_s) was maintained at 16 days i.e., the unit volume capacity = 28 L; sludge wasted = 1. 75 L. However, the R_s did vary during the course of the investigation. The calculation of sludge age is as follows:

$$R_s = V/Q_i$$

where, V = total volume in the reactor and Q_i = flow rate.

The oxygen utilization rate (OUR) was measured in AE2 using a dissolved oxygen (DO) probe and meter. Air supply to the aerobic reactors (AE1 and AE2) was facilitated using the OUR meter that controlled the aquarium pump, which was switched on at a DO concentration of 2 mg/L and switched off when the DO concentration of the reactors reached 5 mg/L. The meter was operated in OUR mode, which recorded the OUR over a 24 hr period.

6.3.3 Pilot plant operation

6.3.3.1 Acquisition of seed inoculum

Mixed liquor was obtained from the aeration tank of Darvill wastewater works situated in the Pietermaritzburg region of KwaZulu- Natal. The reason for selecting the latter wastewater works was that most of edible oil industries were localized in this region, and their final effluents were discharged into Darvill. Therefore the sludge would have already been exposed and acclimatized to edible oil effluents. The MLSS of the Darvill seed sludge was approximately 3500 mg/L. The desired MLSS concentration of the system was 2000 mg/L. Therefore, 16 L of Darvill mixed liquor was decanted into the pilot plant and 12 L of water was added giving a total volume of 28 L. This rendered a final suspended so concentration of 2 000 mg/L. The inoculum was fed diluted edible oil effluent (COD $\pm$ 300 mg/L) for a period of 2 weeks in order to further acclimatize the microorganisms in the sludge to the edible oil effluent.

6.3.3.2 Basic approach to treatment process

The design, operation and optimization of a laboratory scale process to treat the edible oil effluent was dependent on nature and regular supply of effluent and manipulating the process to achieve maximum organic removal. This approach necessitated the compartmentalization of the laboratory scale process into phases that had common operating conditions during the course of the investigation. Other factors that supported this approach included inherent problems with the laboratory scale process resulting in system failure, varying nature and strength amongst effluent batches and disruption in effluent supply. The laboratory scale treatment process was initiated on the 18 May 2000 and continued until October 2001. The period under investigation was divided into five phases as explained in Table 6.1. (See Appendix 19).

6.4 RESULTS

6.4.1 Pilot plant process

6.4.1.1 Wastewater characterization

Some of the notable features of the characteristics measured are as follows:

1. The pH of the raw effluent is acidic on collection and upon flocculation it becomes basic. The pH is corrected to neutral prior to being used as feed influent.
2. The raw wastewater has a high organic pollution load. However, approximately 74% is removed upon flocculation. The prepared influent was diluted in accordance with the concentrations required for incremental COD increase during the course of the investigation.
3. Large portion of the organic pollution load (COD) comprised of FOG. A large amount of FOG's was removed during flocculation (± 92%).
4. The wide ranges of the total phosphate results were due to high phosphate concentration of the raw wastewater during Phase 1 and 2 and limiting phosphorus conditions for the rest of the investigation. The above explanation also applies to the TKN. Both nitrogen and phosphorus were limiting nutrients in the raw wastewater and had to be supplemented in the prepared influent during Phase 3 - 5.

For the sake of creating a clearer understanding of the results, the duration of operation the laboratory scale process and the results were divided into phases that encompassed common operating conditions. For Phases 1, 2, and 3 the plant was operated as Phoredox system. The first two phases experienced operational problems as new procedures were tried out. By Phase 3, the nature of the effluent from the industrial plant had changed; citric acid was now used in place of phosphoric acid in the refinery process. This obviated the need for phosphorus removal, which had been poor up to that time. The process was then changed (Phase 4) to an adapted Modified Ludzak Ettinger process, with an added aerobic reactor, the focus now being on COD removal. Only the details of the optimum fourth and fifth phases are in this report.

6.4.1.2 Phase 4E (1-12-00 – 1-2-01)

During phase 4E the influent COD concentration was increased from 1500 mg/L to 1800 mg/L. The COD removal efficiencies improved with the highest being 1550 mg/L COD removed. There was a substantial variation in the COD removal efficiency during the mid and latter portions of this phase. The decrease in COD removal between days 165 and 170 could be attributed to experimental error since high standard deviations were recorded (Fig 6.2).

The industry shut down on the 27-1-01 and therefore termination in the supply of influent feed for a period of 5 weeks. The process was shut down and no analysis was conducted during this period. However, in order to sustain the biomass, final effluent from another edible oil company was obtained and served as feed influent. The viability of the microorganisms in the activated sludge was monitored by the OUR and remained within the range of 20 - 25 mgO/L/h.

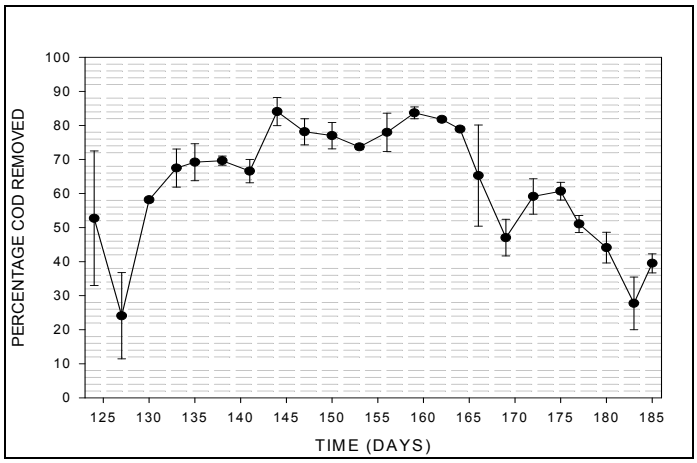


Figure 6.2 Percentage COD removed during Phase 4E

6.4.1.3 Phase 5C (18-4-01 – 21-5-01)

The influent COD concentration was maintained at 1800 mg/L. The percentage COD removal during the phase peaked at 90 % removal and was consistent throughout most of the phase until day 72 when there was a decrease (Fig 6.3) The MLVSS/MLSS ratio was mostly consistent averaging at 0.8 mgVSS/mgTSS.

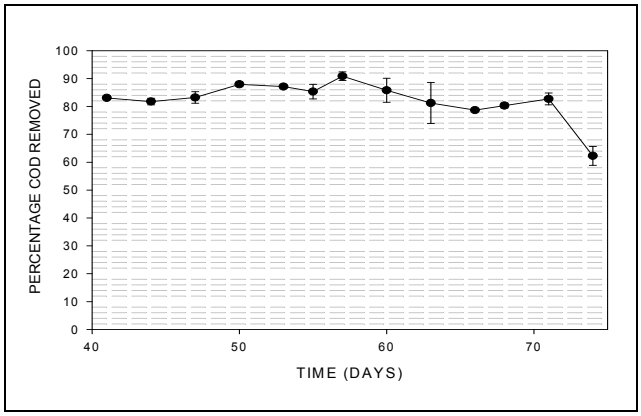
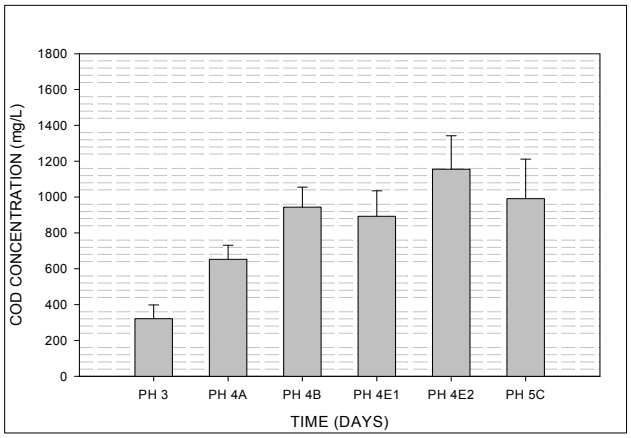


Figure 6.3 Percentage COD removal during Phase 5C

6.4.2 Process Performance

6.4.2.1 Performance with increase in COD concentration

One of the aims of the research was to assess process performance with increase in influent COD concentration. This was subjective to the COD of the raw effluent obtained from industry and operational limitations experienced during the treatment process. The influent concentration ranged from 500 to 1800 mg/L COD (undiluted after pretreatment). It also should be noted that the values reflected in Fig 6.4 are an average within the phase and not the actual values, which in some cases are higher. However, it reflects a trend of COD removed with increase in influent COD concentration for the entire treatment process.



* each bar reflects the mean value of COD removed during the respective phases

Figure 6.4 Increase in COD removed with increase in influent COD concentration

Table 6.1 Increase in COD concentration across phases and F/M ratios

PHASE	PERIOD	INFLUENT COD CONCENTRATION (mg/L)	MEAN F/M RATIOS
3 (PH 3)	06/06/00 - 21/07/00	500	0.5476 (0.19)
4 A (PH 4A)	28/07/00 - 16/09/00	800	0.2740 (0.13)
4 B (PH 4B)	17/09/00 - 19/10/00	1200	0.4838 (0.14)
4 E1 (PH 4E1)	01/12/00 - 11/12/00	1500	1.3363 (0.31)
4 E2 (PH 4E2)	12/12/00 - 01/02/01	1800	0.9302 (0.42)
5C (PH 5C)	18/04/01 - 21/05/01	1800	0.6133 (0.14)

(The F/M ratios are expressed as mean values with samples standard deviations in parenthesis)

With reference to Fig 6.4, the results show that as the influent COD concentration (S_{ti}) is increased, the amount of COD removed by the process also increases. However, there was a slight decrease in concentration of COD removed during phase 4E. A similar trend was noticed during phase 5C when compared to phase 4E. Maximum COD removal was achieved during phase 4E and although the mean influent concentrations during phase 5C were the same, the concentrations removed were slightly lower. It should be noted that 1800 mg/L COD was the maximum strength of the influent i.e. effluent from industry which is undiluted after pretreatment. The steady state performance of the process is presented in the graphs of the individual phases where the operating conditions were similar.

6.4.3 Associated problems

During the period of operation of the laboratory scale treatment process, some of the major problems encountered were as follows:

- Disruption in the supply of effluent from industry which resulted in having to recycle the process final effluent to sustain the system.
- Blockages in the tubes due to high FOG (Phase 3) resulting in loss of MLSS and subsequent diminished process efficiency.
- Associated bulking problem also resulting in poor settling during phase 3 which recorded a DSVI of 244 mL/g.
- Inconsistency in the industrial effluent strength (COD) amongst batches thus prevented achieving the desired process influent concentrations i.e. after pretreatment the S_{ti} was sometimes lower than desired for the phase.
- Occasional mechanical problems associated with peristaltic pumps thus effecting daily flow rates and infrequent disruption of electricity thus temporarily shutting down the process.

- Fractionation of the S_{ti} was not possible due to the variable nature and chemistry amongst the different batches.

6.5 DISCUSSION

The laboratory scale process, when used to treat edible oil effluent, clearly showed up the problems involved with disposing of these effluents. In the first phase of the process operation the FOG inhibited the organisms to such an extent that the process failed. This indicated a need for pretreatment of the effluent in order to reduce the fat and grease load. This was achieved by flocculation. On a full-scale process this sort of pre-treatment may also be required, if the effluent is to be successfully treated. The importance of the nature of the effluent is also illustrated by the problems encountered during Phase 2, when high phosphorus loads proved to be a problem.

Once a consistent effluent had been obtained and adjusted to a suitable standard, the process showed reasonably high rates of COD removal overall, apart from perturbations caused by slight changes in the nature of the feed. Changes in concentration of the feed resulted in reductions in efficiency, as the system microbial population would have to adjust and the system would take a while to reach steady state again. This can be seen by the results obtained in Phase 4 and 5 of the investigation, where COD removal fluctuated when the organic load decreased or increased.

Subsequent to large-scale pretreatment of the wastewater to reduce the high FOG content and remove COD, the effluent was characterised. The pH of the refinery wastewater on collections was acidic i.e. pH 3.8 and basic i.e. pH 9.2 after pretreatment and adjusted to neutral prior to serving as influent feed. The current findings substantiated previous research by Saw *et al.,* (1987) that showed raw effluent pH measurements of pH 2 and COD range of 1010 to 8200 mg/L, which compared favorably with the COD of the effluent collected that ranged between 6190 and 8470 mg/L. It should be noted that the wide range was ultimately due to varying strengths of the effluent collected from industry and was therefore dependent on the refinery process. The pretreatment process using flocculent removed approximately 74% and 92% of the COD and FOG's respectively (Table 6.1). Previous research by Sengul, (1990) using coagulants such as poly-

electrolytes when treating sunflower oil effluent showed COD and FOG removal rates of 76 and 77%, respectively. Therefore, pretreatment has proven to be necessary and highly effective when adopting a two-stage treatment approach. Biological treatment serves to remove the resident COD and satisfy municipal discharge standards.

Phase 4E

Phase 4E was initiated with the influent COD concentration increased to 1500 mg/L. however, the system responded with a sharp decrease in COD removal efficiency (Fig 8.2). The reason for the above behavior could be associated with one of various factors. Analyzing this trend which showed recovery in COD removal capacity within few days of the episode, the most likely cause could have been the presence of inhibitory compounds in the influent which when dissipated within a few days allowed the system to return to normal performance. On recovery of the process, the influent COD concentration was increased to 1800 mg/L, which was basically the pre-treated effluent from industry without any dilution, i.e. full strength. There were odd occasions after pretreatment, that the concentrations were lower or much higher depending of the effluent batch, but these were more exceptions than the rule and the influent COD concentrations were adjusted accordingly. As the influent COD concentration increased, the concentration removed also increased, with the highest being 1550 mg/L. The concentrations removed during the mid to latter portions of this phase were high averaging greater than 1000 mg/L COD. However, towards the end of the phase the COD removal efficiency decreased indicating that they system was experiencing a certain degree of stress.

Phase 5C

This batch of effluent after pretreatment had an influent feed COD concentration >1400 mg/L. the process efficiency was high recording 90% removal rates (Fig 6.3) and COD concentrations removed at 1324 mg/L. The performance of the system during the phase was typical of steady state behavior with consistent removal rates, except towards the end (day 72). An observation that required mentioning was that with change in effluent batch and increase in influent COD concentration, the process reacted with immediate higher COD concentration removed e.g. In phase 5B (701 mg/L) and 5C (1465 mg/L) with 88% and 90% removed, respectively. This trend was also evident during phase 4. Possible justification for the above behavior could be that the capacity of the activated sludge

system to remove oil effluent COD exceeded the pretreated influent COD concentrations during the current research. Ozturk *et al*., (1990) showed higher maximum removal rates of 1800 mg/L.

Having achieved the desired objectives and reproducibility of the system results to changing parameters, the process was terminated.

One of the primary objectives of the research was to ascertain the response of the system to an increase in the influent COD (organic loading). The treatment process was partitioned into phases based on varying operational conditions, one of which was the increase in S_{ti} during each phase. The overall trend was clearly evident that with the phasic increase in the S_{ti}, the concentration of COD removed by the treatment process also increased (Fig 6.4). It should be noted that these observations were made across phases where the operational parameters were not always the same. Therefore Fig 6.4 was useful for observing the overall trend. However, minor deviations did occur e.g. there was a slight decrease in the COD concentration removed during Phase 4E. A similar pattern was observed during Phase 5C. Although graphically it appears that the system had reached a plateau phase from phase 4E onwards, it was not exposed to higher concentrations than 1800 mg/L (Fig 6.4). Analyzing the response of the system to increase in COD, it was highly likely that if S_{ti} of greater than 1800 mg/L was used, the system would have removed higher concentrations of COD. Current findings substantiate research by other workers in the field with Ozturk *et al*., (1990) achieving removal capacity of 1800 mg/L using an S_{ti} of 2500 mg/L. Mkhize, (2002) showed average COD removal capacity of 1976 mg/L when exposed to oil effluent with an S_{ti} of 2600 mg/L. The concentrations reflected in fig 6.4 are not the actual concentrations removed during the phases, since the means were used to construct the graph.

The F/M ratio is an important parameter for the operation of activated sludge processes and describes the degree of starvation and potential food availability to the microbial population (Droste, 1997). The F/M ratio, sludge age and settling velocity index (SVI) are all interrelated and impact on the process performance and the ratios reflected in the current investigation varied from 0.27 to 1.33 mgCOD/mgMLSS.d. Theoretically, an increase in the S_{ti} should result in an increase in the F/M ratio from 0.54 to 0.27 (Table

6.2). This was attributed primarily to an increase in the MLSS during Phase 4. A similar trend was noticed when the F/M ratio decreased from 1.33 to 0.93 COD/mg MLSS.d. Mkhize, (2002) showed a reduction in the COD removal efficiency and attributed it to the increased operational F/M ratio that was maintained at 1.5 kgCOD/kgMLSS/d. However, increasing the F/M ratio of the system is reported to have an overloading effect to the biomass, which results in the overall poor system substrate metabolism performance (Casey *et al*., 1995).

6.6 CONCLUSIONS

The conclusions that were derived from the laboratory scale treatment studies were as follows:

- The treatment process was conducted in phases that encompassed common operational parameters and therefore allowed accurate critical analysis of the data.
- Large scale pretreatment achieved the desired objectives and removed sufficient COD including inhibitory compounds such as high concentration FOG's to facilitate biological treatment.
- The COD parameter was used as measurement of the organic removal capacity of the system, as has been common practice by other researchers in the field.
- The influent COD concentration was gradually increased from 500 to 1800 mg/L (undiluted) and the system responded accordingly with improved COD removal and maximum COD removed at 1550 mg/L during steady state conditions.
- The F/M ratio varied and ranged from 0.27 to 1.33 mg COD/mg MLSS.d during the phases and the system responded accordingly.
- More than 90% of the influent COD was biodegradable.

In conclusion, this method of treatment would be practical as a polishing step in the treatment of edible oil effluents. A pre-treatment stage would probably be necessary, however, in order to remove excessive troublesome components present in the effluent, such as FOG's. It might be necessary to supplement the effluent feed to the plant with nutrients and adjust the pH of the stream. In practice this may be achieved by blending the effluent with a domestic waste stream. The COD removal and reduction of compounds in

the product effluent indicate that this sort of treatment might be suitable for pre-treatment of edible oil effluents, prior to their release to a wastewater works for further treatment. It is important to note that theses types of effluents differ considerably from one another for a variety of reasons, and any process would have to be adapted to these differences.

CHAPTER 7
ALTERNATE TREATMENT TECHNOLOGY USING FUNGI, YEASTS AND BACTERIA FOR DEGRADATION OF EDIBLE OIL EFFLUENT

7.1 INTRODUCTION

Most trade effluents containing FOG would have received some form of preliminary physico-chemical treatment before passing to the biological stage. In its simplest form, this would consist of a fat trap or an oil interceptor. Although these processes can provide effective protection (through FOG removal) to subsequent biological stages, they are often not effective due to overloading and poor maintenance. In situations where the FOG is not removed the wastewater can have serious impacts on receiving wastewater plants. One such impact is the reported association between FOG, usually in municipal sewage, and the formation of stable foams on the aeration tanks of activated sludge plants. This foam becomes a problem when it accumulates in the final settlements tank and is then discharged with the treated effluent (Forster, 1992).

7.1.1 The Fungal Degradation of Lipids

The degradation of lipids by fungi is predominantly an enzymatic process. Fungal lipases are considered to play a vital role in lipid degradation and are present in a variety of fungi. Those belonging to the genera *Mucor*, *Rhizopus* and *Geotrichum* are considered to be extremely effective in the hydrolysis of triglycerides. Lipases belong to a group of enzymes known as hydrolases which catalyse the degradation of triglycerides by hydrolysing the ester bond between the glycerol and acyl moiety. Lipase activity is affected by pH, temperature and the availability of essential growth nutrients but it has been reported that highest lipase activity occurs at the oil-water interface in the presence of hydrophobic substrates (Lazar and Shröder, 1992).

Studies suggest that the utilisation of fatty acids by various microorganisms is determined not only by fatty acid toxicity and species of the microorganism but also the composition and pH of the growth medium, and the chain length of the fatty acid.

7.1.2 Yeasts

Yeast are fungi that grow as single cells, producing daughter cells either by budding or by binary fission and they, therefore, 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 a hyphal phase depending on environmental conditions. The classification of yeasts is a specialized field using cell, ascospores, colony characteristics for distinguishing genera, and physiological characteristics, particularly the ability to ferment individual sugars, to identify species. Yeasts are heterotrophic, lack chlorophyll and are characterized by a wide dispersion of natural habitats (SGD, 2001). To a considerable extent the morphology exhibited by a particular yeast is directly associated with the mechanism it employs for asexual reproduction. Yeasts are able to grow at pH values of between pH 3 - 8 and at temperatures ranging from 20°C – 30°C. (Walker, 1998).

7.1.3 Bacteria

Bacteria are the most numerous and diverse of the microorganisms. They are unicellular and considerably smaller than protozoa, algae, or fungi. Most, but not all bacterial cells are enclosed by a cell wall. Bacteria are a heterogenous group anatomically, physiologically and biochemically. Primarily differentiated on the basis of their morphology (shape and structure) their growth, physiological and biochemically characteristics. Bacteria occur in one of 3 shapes - spherical, rods, or spirals. Groups of bacteria that exhibit other morphologies include filamentous, ensheathed, and appendaged stalked bacteria. Most bacteria reproduce by binary fission which is an asexual process giving rise to two equal daughter cells, others by budding, or fragmentation. Some are motile, have helical hair like appendages called flagella, others by gliding type motion (Atlas and Bartha, 1993).

7.1.4 Physical Factors Affecting Oil Degradation by Yeast and Bacteria

Rates of oil degradation depend greatly on the composition, state and concentration of the oil, environmental temperature, pH, oxygen availability and concentrations of mineral nutrients (for this study purposes only pH and temperature were tested) (Atlas and Bartha, 1993). Degradation of oil is accomplished primarily by bacteria and fungi and some yeasts (Leahy and Colwell, 1990).

7.2 YEAST AND BACTERIAL DEGRADATION OF EDIBLE OIL EFFLUENT

7.2.1 Aims and Objectives

The aim of this study was to evaluate the degradative capabilities of the yeast and bacterial cultures for edible oil effluent treatment by optimising growth parameters. The objectives are as follows:

1. Determining the optimum pH at a temperature of 21°C and 31°C
2. Determining the growth capabilities on solid media containing fatty acids as sole carbon sources.

7.2.2 Materials and Methods

7.2.2.1 Isolation

A soil sample was obtained from the area around the deodorizer effluent pipeline at the edible oil industry (Pietermaritzburg). One gram of soil was suspended in 9 mL of saline solution for 24 hr. A serial dilutions series, ranging from 10^{-1} - 10^{-8} was performed, after which a loopful of each dilution was streaked on malt extract agar (Appendix 20) and nutrient agar (Appendix 21) for yeast and bacteria isolation, respectively. An activated sludge sample from the pilots scale bioreactor at the Center for Water and Wastewater Technology was also obtained and likewise inoculated on to malt extract agar and nutrient agar. Nutrient agar plates were incubated for 24 hr and malt extract plates for 48 hr (longer depending on the growth) at 25EC. Colonies were restreaked until monocultures of bacteria and yeast were achieved. A purity check (Appendix 22) was done to differentiate yeast from fungus and those that morphologically resemble bacteria.

7.2.2.2 Preliminary Screening Tests

The screening procedure was divided into two parts:

Oleic acid batch test

Oleic and stearic acid plate test

These tests were done to reduce the number of isolates to be tests.

A. Oleic Batch Tests

This experiment was done to determine which of these isolates are able to utilize oleic acid. Conical flasks (250 mL) were used and contained 100 mL of nutrient solution (Appendix 23) and oleic acid. Oleic acid (10 mL) was added after the nutrient solution had been autoclaved, as the high temperature would have changed the structure of the fatty acid. Cupric chloride added as an indicator was dissolved into the oleic acid turning it blue. This facilitated visual observation of the oleic acid degradation as of the blue stained acid could be followed. Following inoculation of the flasks with the respective isolates (15) they were agitated on a Labcon shaker at room temperature of 22EC. The flasks were monitored visually every day for 10 days and isolates that were found to degrade the acid were that chosen for the next step.

B. Oleic and Stearic Acid Plate Test

This experiment was done to determine at which pH values and temperatures the isolates were able to grow. Oleic acid (Appendix 24) and stearic acid (Appendix 25) plates were poised at pH values of 5, 7 and 8. The isolates were streaked on duplicate plates of each of the fatty acids at the pH values mentioned. One set of plates was incubated at 21EC and the other at 31EC for 10 - 14 days. from these plates two bacteria and two yeast cultures were chosen for further experimentation.

7.2.2.3 Identification of Isolates

Bacteria were identified using the API 50I utilizing APILAB software. Yeast cells were identified using microscopic analysis, sugar tests (glucose, sucrose, inositol, arabinose, mannitol and lactose) and morphological observations, in conjunction with Barnett *et al*, (1990).

7.2.2.4 Edible Oil Effluent Batch Test

Edible oil effluent was taken from the deodorizer effluent pipe at Sealake Oil Industries. Conical flasks (1 L) were used containing 250 mL of edible oil effluent, together with 250 mL of double strength nutrient solution (Appendix 23) and poised at pH values of 5, 7 and 8 using either 1M HCl or NaOH. Following this the flasks were inoculated with the isolates obtained from the agar plates (see section 3.2.2) and incubated at either 21EC or 31EC

for 7 days. An identical set of flasks was made but not inoculated which acted as the control.

7.2.2.5 Analysis

The pH was monitored using the Beckman ¯50 pH meter. Initial FOG concentrations (Appendix 10) and COD (Appendix 3a) was measured prior to inoculation. Thereafter, FOG and COD was measured every 2 days for 7 days.

7.2.3 Results

7.2.3.1 Isolation of Bacteria and Yeast

From the soil sample eight bacteria and six yeast cultures were obtained while only one bacterial culture was obtained from the activated sludge. All of the bacteria isolated were gram positive rods.

7.2.3.2 Preliminary Screening

A. Oleic Batch Tests

From Table 7.1 it can be seen that the bacterial cultures 3,4, 5 and isolate F removed a large amount of the added oleic acid after 24 hr (day 2) while culture no. 1 showed similar removal after 48 hr. The remaining bacteria cultures did not show any oleic acid removal even after 10 days. Therefore only bacteria 3, 4, 5 and F were retained for additional screening and experiments. Likewise, yeast cultures 3, 4 and 5 showed a rapid utilization of the oleic acid during the first 24 hr and after 3 days the remaining cultures, also recorded good oleic acid removal. Bacterial cultures 3, 4, 5 and F and yeast cultures 3, 4 and 5 were chosen for further experimentation.

Table 7.1 Oleic acid utilization by bacteria and yeast isolates using batch tests

	Isolate No.	Day 2	Day 3	Day 4	Day 5	After 10 days
BACTERIA	1	* * *	* *	* *	* *	n
	2	* * *	* * *	* * *	* * *	n
	3	* *	* *	*	0	n
	4	*	*	*	*	n
	5	*	*	0	0	n
	6	* * *	* * *	* * *	* * *	n
	7	* * *	* *	* *	* *	n
	8	* * *	* * *	* * *	* * *	n
	F	* *	* *	*	*	n
YEAST	1	* * *	*	*	*	n
	2	* * *	* *	* *	* *	n
	3	*	*	*	*	n
	4	*	*	*	*	n
	5	*	*	*	*	n
	6	* * *	*	*	*	n

Key:

0 - no oil remaining
* - little oil remaining
* * - moderate oil remaining
* * * - almost all initial oil remaining
n - no change from day 10

B. Oleic Acid Plates

Table 7.2 Growth of bacteria and yeast on oleic acid plates at pH values of 5, 7 and 8 and at 21C° and 31°C

pH	BACTERIA (B)				YEAST (Y)			TEMP
	3	4	5	F	3	4	5	
5	0	*	* * *	* * *	* * *	* * *	* * *	21EC
	*	*	* *	* *	* *	* *	* * *	31EC
7	*	0	* *	*	*	* *	* *	21EC
	0	0	0	0	0	* *	* *	31EC
8	0	0	0	0	*	* *	* *	21EC
	0	0	0	0	0	0	*	31EC

Key:

0 - no growth
* - little growth
* * - moderate growth
* * * - excellent growth

At a pH of 5 it can be seen that at a temperature of 21°C cultures B3 and B4 showed poor growth while the remaining organisms showed extremely rapid growth. On the other hand, B3 and B4 showed poor growth at 31°C. For the yeast cultures only Y5 showed good growth while the remaining organisms grew moderately well.

For a pH of 7 and at 21°C cultures B5, Y4 and Y5 grew moderately well while the other cultures grew poorly or not at all. At the higher temperature of showed poor growth at 31° C cultures Y4 and Y5 again grew moderately while the remaining organisms showed no growth.

At an elevated pH of 8 only the yeast cultures Y4 and Y5 showed moderate growth at 21°C. The remaining organisms did not grow or grow poorly and at a temperature of 31ºC only Y5 showed little growth and the rest none.

C. Stearic Acid Plates

Table 7.3 Growth of bacteria and yeast on stearic acid on plates at pH values of 5, 7 and 8 and 21°C and 31°C

pH	B A C T E R I A (B)				Y E A S T (Y)			TEMP
	3	4	5	F	3	4	5	
5	0	0	*	0	*	*	* *	21°C
	0	0	*	0	0	0	* *	31°C
7	0	0	* *	* *	*	* * *	* * *	21°C
	*	*	* *	* *	0	*	* * *	31°C
8	0	0	* *	* * *	* *	* * *	* * *	21° C
	*	*	*	* * *	*	* *	* * *	31° C

Key:

0 - no growth
* - poor growth
* * - moderate growth
* * * - excellent growth

At pH of 5 it can be seen at temperature 21°C Y5 grew moderately well while Y3, Y4 and B5 showed poor growth. B3, B4 and F did not show growth. At 31°C Y5 again grew moderately. B5 grew poorly while the remaining organisms did not grow.

For a pH of 7 and at 21°C, Y5 and Y5 grew excellently at B5 and F grew moderately, but B3 and B4 did not grow while Y3 grew poorly. At 31°C Y5 only showed excellent growth. B5 and F showed moderate growth. B3, B4 and Y4 grew poorly while Y3 did not grow.

At a pH of 8, Y4, Y5 and F showed good growth at 21°C. B5 grew moderately. Remaining organisms grew poorly or not at all. At 31°C only Y5 and F grew extremely well. *Candida* spp grew moderately. Remaining organisms grew poorly.

7.2.3.3 Identification

The identifications of the isolates were not 100% correct. Therefore only tentative identification was done. The results from the API identification indicated that the bacterial culture was *B. lactosporus* (96.8% certainty). The other cultures showed very low correlation factors with less than 80% assurance; therefore these organisms were not identified with this method. The yeast cultures were identified as a *Candida succiphila* and a *Rhodosporidium sp.*

7.2.3.4 FOG Degradation

The graphs for FOG degradation at pH's 5, 7 and 8 at 21 and 31°C can be seen in Appendix 26.

7.2.3.5 COD Removal

The graphs for COD degradation at pH's 5, 7, 8 at 21 and 31°C can be seen in Appendix 27.

The FOG removal rates have been detailed below (Table 7.4). The following results were recorded:

pH 5: At 21°C F (90%) showed high FOG removal, followed by Candida sp (53%), *Bacillus sp* (42%) and lastly by *Rhodosporidium sp* (11%). At 31° C a high percentage of FOG was utilized except by Candida sp (27%). *Rhodosporidium sp* removed 91%, F removed 86% and *Bacillus sp* removed 81%.

pH 7: Only *Candida sp* (87%) removed high percentage of FOG while the remaining organisms removed an average percentage at 21°C. FOG percentage removal was 56% (F), 52% (Bacillus sp) and 52% (*Rhodosporidium sp*). At 31°C *Rhodosporidium sp* was only able to remove 68% FOG. F, *Bacillus sp* and *Candida sp* were only able to remove 49%, 40% and 42% respectively.

pH 8: F removed 59% of FOG which was just above average at 21°C. Excellent removal was observed by *Bacillus sp* (74%), Candida sp (75%) and *Rhodosporidium sp* (75%). At 31°C both Candida sp and *Rhodosporidium sp* were able to remove 85% FOG. *Bacillus sp* removed 47% while F had the lowest FOG removal at 9%.

Table 7.4 Final FOG removal percentage of F, *Bacillus sp*, *Candida sp* and *Rhodosporidium sp* in batch tests

pH	BACTERIA		YEAST		TEMP
	F	*Bacillus sp*	*Candida sp*	*Rhodosporidium sp*	
5	90	42	53	11	21°C
	86	81	21	91	31°C
7	56	52	82	52	21°C
	49	40	42	68	31°C
8	59	74	75	75	21°C
	9	47	85	85	31°C

7.2.4 Discussion

7.2.4.1 Preliminary Screening

Preliminary screening was performed in order to:

1. To reduce the number of isolates.
2. To determine which of the isolates were able to degrade or utilize fatty acid.
3. To determine which of the isolate were able to grow in the presence of fatty acids under the designated pH and temperature parameters

A. Oleic Acid Batch Test

Oleic acid was used for preliminary screening as they form part of the FOG triglyceride structure in edible oil effluent. Table 7.1 shows the results of the yeast and bacteria isolates to utilize oleic acid in batch tests. Yeast cultures were better able to utilize or grow in oleic acid than the bacterial cultures. Although bacteria B3, B4, *Bacillus* sp and F performed extremely well by removing almost all of the oleic acid, B1, B2, B6 and B7 did not utilize oleic acid. Y3, *Candida* sp and *Rhodosporidium* sp performed just as well has B3, B4, *Bacillus* sp and F. But Y1, Y2 and Y3 did not initially utilize oleic acid or utilized it poorly. But as days progressed these cultures were able to utilize almost all of the oleic

acid. Eventually only Y2 performed as well as the Y3, Y4 and Y5. The organisms able to utilize oleic acid may have been able to induce lipase activity under these conditions. A possible reason for the slow start of the other organisms could be due to:

- pH and temperature were not conducive for the organisms to grow (not tested or maintained);
- Limited nutrient availability;
- Oleic acid concentration may have been to low or too high. This may have inhibited growth of the organism;
- Oleic acid was not able to be used as a carbon source by a particular organism;
- External environmental conditions or nutrient concentration may have not been conducive for lipase production or activity; and
- Organisms may have an inefficient lipase system.
- *Bacillus sp*, isolate F and Y2, *Candida* sp and *Rhodosporidium* sp were chosen for further study because they demonstrated very good acid degradation rates

B. Oleic and Stearic acid Plates

Table 7.2 and 7.3; show the growth of the isolates on oleic and stearic acid agar plates, respectively. Oleic and stearic acid plates were used to determine which of the chosen isolates were able to grow at pH values of 5, 7, 8 and temperatures 21°C and 31°C.

Oleic acid plates showed that *Candida sp* and *Rhodosporidium sp* were able to grow at pH 5, 7, and 8 and at both temperatures, except at a pH of 8 and at 31°C. *Bacillus sp*, F and Y3 were only able to grow up to pH 7 (21°C). B3 and B4 grow poorly or not at all. Stearic acid plates showed growth of *Rhodosporidium* sp on all the plates. *Candida* sp, F and *Bacillus sp* seemed only to grow from pH 7 to pH 8. The organisms showed fairly different growth rates on oleic and stearic acid plates. A possible explanation could be due to the state or form of the fatty acids at these temperatures. Oleic acid was liquid while stearic acid was almost in a solid form at room temperature. It was reported that fatty acids in a more solid form are more difficult to degrade (Ratheledge, 1994). From this experiment *Bacillus* sp, F, *Candida* sp and *Rhodosporidium* sp were chosen for further experimentation.

7.2.4.2 FOG's

Most organisms cannot tolerate extreme pH values. Under extreme alkaline or acidic conditions to which organisms do not function, microbial cell components may be hydrolyzed or enzymes may be denatured. Amino acids are zwitterions, having both basic and acidic portions. The pH affects the dissociation of these functional groups on proteins molecule. In order to perform enzymatic activity, enzymes must be in a particular state of dissociation. Certain pH values will be optimal for activities of specific enzymes. The pH of an environment affects microorganisms and microbial enzymes directly and also influences the dissociation and solubility of any molecules that indirectly influence organisms. The availability of required nutrients, such as ammonium and phosphate, which limit microbial growth and mobility of toxic heavy metals (Atlas and Bartha, 1993). In addition to affecting survival and growth, temperature influences the metabolic activities of microorganisms.

The results of this study indicated the capabilities of isolates *Bacillus sp*, F, *Candida sp* and *Rhodosporidium* sp to utilize and degrade FOG in sunflower oil effluent under pH values of 5, 7 and 8 and at 21°C and 31°C (Table 7.4). It was assumed that yeast and bacteria are able to reduce FOG levels in edible oil effluent. This assumption was based on early works by Huss, (1908) and Harrisson, (1927; cited Ratheledge, 1994). They found that bacteria and yeast were able to be cultivated on oils and fats. Earlier works by Pan *et al* (1959; cited Ratheledge, 1994) also showed that it was possible that vegetable oil could be utilized as a sole carbon source of carbon and energy.

From the result obtained it can be clearly seen that the yeast and bacterial isolates were able to reduce FOG levels in the sunflower oil effluent under the different pH and temperature values. However, there were no definitive trends, as organisms behaved differently under different conditions. The bacteria showed more constant FOG degradation abilities than the yeast with the highest FOG removal being observed at pH 5 21°C and 31°C which ranged between 90% and 81%, with the exception of *Bacillus* sp at 21°C where only 42% FOG was removed. However, a high removal of FOG, by *Bacillus sp,* was observed at pH 8 at 21°C with 74%.

It appears that pH values have a greater physiological effect on bacteria than temperature, which was also concluded by Ratheledge, (1994). He stated that the attainment of optimum initial pH and the maintenance of the pH during growth of culture were of great importance. He also stated that maintenance of a constant pH during growth of a culture is especially important for those organisms that produce acid but are not acid tolerant (in this study isolates were not tested for acid tolerance). This could possible explain why the bacteria removed FOG so erratically. In this study the pH was not maintained in the batch culture as to prevent the risk of contamination by the pH probe and pH chemicals. It also seems that the temperature affects the bacteria differently at different pH values.

Yeasts are known to grow very well when the initial culture medium pH is between 4 - 6, but many yeast are capable of growth at lower pH than bacteria, but do not generally grow well at alkaline pH values (Walker, 1998). From this study it was observed that the percentage FOG utilization increased as pH increased (although *Rhodosporidium* sp did remove 95% FOG at pH 5, 31° C). This supported the works of Jeffery *et al* (1999), who observed that this range (pH 8) appears to favor both emulsification of oil and its cleavage by fungal lipase activity, as well as their utilization for cell growth. They also observed that an increase in pH alone could be responsible for most of the enhanced sunflower oil utilization and biomass growth. Although it has been stated that yeasts do not perform well under alkaline conditions the highest FOG removal occurred at pH 8 by both *Candida* sp and *Rhodosporidium* sp (75% and 85% respectively) while the lowest FOG removed was 11% by *Rhodosporidium* sp at pH 5 (21°C). Another possible explanation for this occurring could be that actively growing yeasts acidify their growth medium through a combination of differential ion uptake proton secretion during nutrient transport (Walker, 1998). Most laboratory and industrial yeast tend to grow well at between 20°C and 30°C (Winkelmann, 1998). For *Rhodosporidium* sp at 31°C, the FOG removal rates increased from 21% (pH 5) to 42% (pH 7) to 85% (pH 8). This seemed to indicate that temperature enabled the yeast to remove FOG at a greater rate as the pH increases.

From these batch tests it was also observed that in negative control the edible oil effluent seemed to degrade itself. The reasons for this are unclear but could be related to extraction efficiency, solvents used and the change of organic fraction over time due to hydrolysis and photo-degradation. These factors could also explain why the FOG values

appeared to increase above initial values. Furthermore placing the microbial population under stress could have caused cells to undergo lysis. Cells that lysed could have released their internal lipid components that may have added to the external FOG.

Poor FOG utilization could be explained by the following:

- Failure of yeast to perform well at pH 5 could be attributed to the possibility that the pH inhibits lipase activity. Koh *et al* (1983; cited Ratheldge) found that out of 200 isolates only a few were effective in the degradation palm oil. These authors seemed to have had their media pH at 5.5, but most yeast lipase activity occurs at between pH 6.5 and 7.0 (Ratheledge, 1994).
- Poor microbial degradation could be attributed to concentration, level or quantity FOG present. It is stated that if FOG concentration are too high or too low it may inhibit the microorganism (as in the oleic batch tests).
- Tan and Gill (1985b; cited Ratheldge, 1994) observed that for successful growth of a microorganism to occur on a lipid, the culture medium had to be maintained close too the optimum pH of the lipase that was needed to hydrolyze the oil. During this study pH was not maintained which may have contributed to some of the organism's low performance.
- Another factor to be taken into account for successful microbial growth is the dispersal and viscosity of oil and fats within the vessel (Tan and Gill, 1985b; cited Ratheledge, 1994). Poor removal of FOG could have been attributes to poor agitation. As different agitation rates may be needed for different organisms (Ratheledge, 1994). High viscosity of FOG compounds could have attributed to poor FOG utilization. This was found by Wakelin and Forster, (1997). They found that the high viscosity of castor oil resulted in this oil being less effectively dispersed in the culture medium than other FOG substrates.
- Nutrient availability or depletion could also be a factor. Once growth is established an oil, all other nutrient must continue to be present otherwise only partial utilization will ensue (Ratheledge, 1994). As this experiment was done under batch tests no nutrients were added. Therefore nutrient concentrations were not maintained. This could have resulted in some of the average rates of FOG removal.
- Poor removal by the organisms could also be caused by those organisms being in the lag phase, due to slower growth rates (growth rates were not determined in this

study). Wakelin and Forster (1997) observed that poor FOG was removed when the activated sludge was under a lag phase.

7.2.4.3 FOG Removal in Association with COD

FOG is a lipid, which, is component of organic compounds and, thus, measured as COD (Raunkjaer *et al,* 1994; cited Dueholm *et al.*, 2001). However, COD has many other separate fractions such as readily biodegradable COD and soluble COD (Wentzel *et al.*, 1999). Although COD consist of many fractions, each fraction is not measured separately but as a whole.

The COD concentrations recorded during this study showed a general trend of an initial COD decrease within the first three days and then, subsequent, increases until day 7. The COD elevation could be attributed to cell shock due to the new environment. This could have lead to cell lysis, resulting in release of internal organic matter into the external environment. The control was also observed to show such early increases at pH 5, 21°C. The reason for this occurrence is unclear. However, it is noticed that these increases seem to have occurred at pH 5. Therefore, it could be possible that certain organisms may not initially tolerate an environment at pH 5. The decrease of COD, on the other hand, could be due to organisms utilizing FOG and other organic matter.

Although COD concentrations, generally, increased by the day 7, FOG was still being removed. This could prove that FOG forms a small component of COD. Because even though FOG was removed COD increases still occurred.

7.2.5 Conclusion

From this study it can be concluded that pH and temperature affect the ability of organisms to utilize FOG. These factors seem to affect the organisms simultaneously or individually. The effect of pH and temperature on the organism's ability to utilize FOG was not conclusively quantified. Further studies need to be done in order to determine the exact relationship or effect that pH, temperature and COD have on FOG utilization. It can also be concluded that microorganisms may only enhance FOG degradation rather that be totally responsible for FOG degradation (this was indicated by the spontaneous

degradation of the effluent in the control batch). It is possible that pH control may have an effect on FOG degradation.

7.3 FUNGAL (MOULDS) DEGRADATION OF EDIBLE OIL EFFLUENTS

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

The powerful degrading capabilities of many fungi 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).

Therefore similar experiments were made to determine the effectiveness of using fungi (specifically moulds) to degrade the triglyceride component (i.e. the long chain fatty acids) found in the effluents discharged from edible oil industries. The possibility of using fungi to biologically treat lipid waste effluents offers an environmentally sound and cost-effective method to produce better quality effluents (Anke, 1997).

7.3.1 Aims and Objectives

The aim of this study was to evaluate the degradative capabilities of the fungal cultures for edible oil effluent treatment by optimizing growth parameters. The objectives were as follows:

1. To determine the optimum pH at a temperature of 21^{o}C and 31°C
2. To determine the growth capabilities on solid media containing fatty acids as sole carbon sources.

7.3.2 Materials and Methods

7.3.2.1 Collection of soil samples:

Soil samples surrounding the effluent discharge pipe at the edible oil industry in Pietermaritzburg, was collected in a sterile universal. Samples were processed immediately on arrival back to the laboratory.

7.3.2.2 Isolation and Identification of fungi from soil samples:

Exactly 1 g of soil sample was added into 9 mL of an osmotically neutral saline solution (0.9% NaCl) for 24 hr to maintain microbial cell stability and viability. Thereafter, 1 mL of the saline mixture was serially diluted from 10^{-1} to 10^{-8} concentrations to reduce the initial amount of microorganisms. Inoculum from the samples with lower concentrations (i.e. 10^{-4} to 10^{-8}) was point inoculated onto Sabouraud Dextrose Agar (SDA) plates (Appendix 28) to obtain fungal monocultures. These agar plates were then incubated in an incubator (Scientific Series 9000) for 5 to 6 days at 25^{o}C to promote successful fungal growth. Frequent sub-culturing of the evident fungal growth on these plates was then performed until fungal monocultures (specifically moulds) were obtained. Purity checks of these isolates were determined by morphological and microscopic appearances. Identification of these pure isolates was performed by staining the cultures of interest with Lactophenol cotton blue solution (Appendix 29) and viewing their appearances microscopically. These microscopic appearances were stored onto computer and various books containing fungal pictures and related literature were employed to identify these fungal monocultures.

7.3.2.3 Growth potential of isolated fungi on fatty acids:

A growth medium containing the essential fungal growth nutrients (i.e. potassium, vitamins, trace minerals, trace elements, iron and zinc sources), used by fungi for growth and activation of enzymes required for organic compound degradation were obtained from the University of Natal in Pietermaritzburg. Aliquots (1 mL) of these growth nutrients which came in 3 separate sterile universals, potassium, magnesium and nitrogen sources, as well as bacteriological agar and commercially available long chain fatty acids (oleic and stearic acids as an organic source) were used to produce long chain fatty acid containing agar plates (Appendix 24/25) at discrete pH values of 5, 7 and 8. Studies suggest that

sunflower oil does contain significant quantities of linoleic and oleic acids. The fungal monocultures, obtained previously, were point inoculated onto the plates and incubated (Scientific Series 9000) separately at either 21°C or 31°C respectively for 7 to 8 days. Following incubation the growth potential was determined by visual observation of fungal growth.

Fungal degradation of fatty acids from an edible oil effluent in batch cultures:

Effluent was obtained from the deodorizer at the refinery of Company X, as this effluent contains significant amounts of FFA's and other organic compounds. Mixtures containing the fungal growth nutrients (i.e. growth medium) and the raw edible oil effluent at discrete pH values of 5, 7 and 8 were produced (Appendix 23) to determine the ability of the fungal monocultures in degrading the long chain fatty acid components. Thereafter, 500 mL of these mixtures at the poised pH values were decanted into sterile 1 L conical flasks for further experimentation. Loopfuls of the fungal monocultures, freshly cultured, on SDA plates were used to aseptically inoculate the 1 L conical flasks that contained. Duplicate sets of the batch culture flasks were aerated for 10 days at either 21°C or 31°C respectively. Over the period of experimentation daily samples were taken for COD analysis (Appendix 3a) and every second day FOG's (Appendix 10) analysis. Also, uninoculated 1 L conical flask's containing the above mentioned mixtures poised at the different pH's of 5, 7 and 8 and at either of the temperatures served as the controls.

7.3.3 Results and Discussion

From these results (Table 7.5) it can be seen that both the *Alternaria* sp. and *Mucor* spp. were capable of good growth on oleic acid agar plates at a pH of 5 and at 21°C. Furthermore, growth declined for both *Mucor* spp. as the pH was increased to pH 7 and pH 8 respectively while the *Alternaria* sp. was unable to grow at the elevated pH value.

Table 7.5: Fungal growth on oleic acid agar plates, at 21°C and pH's 5, 7 and 8, respectively.

PH	Temperature (°C)	*Alternaria sp.*	*Mucor sp. 1*	*Mucor sp. 2*
5	21	++++	++++	++++
7	21	O	++	+++
8	21	O	++	2

Key: ++++ = Excellent growth +++ = Good growth
++ = Moderate growth + = Little growth O = No growth

Table 7.6: Fungal growth on oleic acid agar plates at 31°C and at pH's of 5, 7, and 8 respectively:

pH	Temperature (°C)	*Alternaria sp.*	*Mucor sp. 1*	*Mucor sp.2*
5	31	O	++++	++
7	31	O	+	++
8	31	O	O	O

Key: ++++ = Excellent growth +++ = Good growth
++ = Moderate growth + = Little growth O = No growth

Similarly to the previous temperature the two *Mucor* spp. showed good to moderate growth at lower pH values (Table 7.6) with decreasing growth potential as the pH increased. The *Alternaria* sp., On the other hand did not grow at any of the pH poised plates at 31°C.

Using stearic acid as a carbon source the *Alternaria* sp. was again unable to grow at any of the pH poised plates. *Mucor* sp. 1 grew well at a pH of 5 but as the pH increased, growth declined almost to a minimum. On the other hand *Mucor* sp. 2 showed relatively good initial growth and as the pH increased, growth increased significantly. (Table 7.7)

Table 7.7: Fungal growth on the stearic acid agar plates at 21°C and pH's 5, 7 and 8 respectively:

pH	Temperature (°C)	*Alternaria sp.*	*Mucor sp. 1*	*Mucor sp. 2*
5	21	O	++++	++
7	21	O	+++	+++
8	21	O	+	++++

Key: ++++ = Excellent growth +++ = Good growth
++ = Moderate growth + = Little growth O = No growth

Table 7.8: Fungal growth on stearic acid agar plates at 31°C and at pH's 5, 7, and 8 respectively:

pH	Temp (31°C)	*Alternaria sp.*	*Mucor sp. 1*	*Mucor sp. 2*
5	31	O	++++	++
7	31	O	++++	+++
8	31	O	+++	+++

Key: ++++ = Excellent growth +++ = Good growth
++ = Moderate growth + = Little growth
O = No growth

For the plates incubated at 31°C the *Alternaria* sp. was incapable of growth on any of the agar plates (Table 7.8). The *Mucor* sp. 1, however, recorded excellent growth initially with a decrease in pH value, while *Mucor* sp.2 showed an increase with increasing pH.

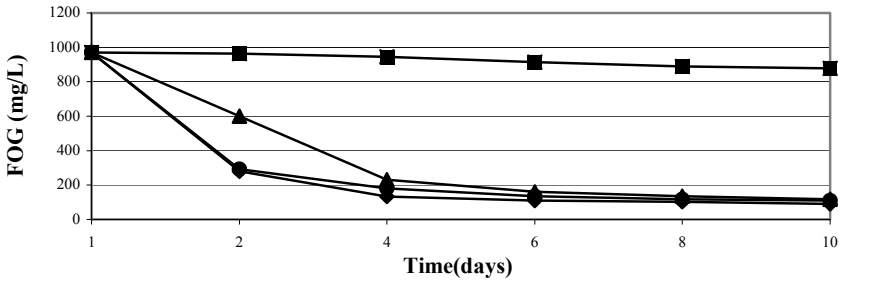


Figure 7.1: Total change in FOG concentration over time at a temperature of 31°C and pH of 5

Rapid FOG utilisation was observed by the fungal isolates during this 10-day experimentation (Fig 7.1). FOG concentrations decreased from initially 970 mg/L to 90 mg/L by the *Alternaria* sp., 110 mg/L and 117 mg/L by the *Mucor* sp. 1 and *Mucor* sp. 2, respectively. Graphic illustrations for the FOG and COD removal for pH 5 at 21°C, pH 7 and 8 at 21°C and 31°C can be found in Appendix 30.

Fats and oils are considered as triglyceride components that are "difficult" substrates for various microorganisms to metabolise. Recent studies have shown that vegetable oils could be used as an energy and carbon source by many microorganisms for growth provided that the concentration of the oils and fats are kept below a critical level and culture conditions suitably optimised (Ratledge, 1994).

Fatty acid components of edible oils differ largely in chain length, being saturated or unsaturated and may contain odd and even number of carbon atoms. This shows its diversity in containing different types of fatty acids. It is therefore clear that the effluent will contain a complex mixture of the different forms of fatty acids. It is thought that fungal growth and degradation of fatty acid components is dependent upon the efficiency of lipase activity, as well as, the fatty acid critical inhibitory concentrations properties of the fungi (i.e. moulds and yeasts). Studies also suggest that lipases could be specific (i.e. metabolise triglycerides containing specific fatty acids) or non – specific (i.e. metabolise triglycerides containing different fatty acids), (Wakelin and Forster, 1997).

Considering the above mentioned properties or characteristics, the following observations were noticed in tables 7.1 to 7.4. The *Alternaria* species grew relatively well at a pH of 5 at 21^{o}C on the oleic acid agar medium but or no growth was observed on the stearic acid agar medium. This probably occurred due to the lipase produced by this fungal isolate as been relatively specific under the prevailing conditions. The overall poor growth observed by the *Alternaria* sp. on both the fatty acid containing agar media could be attributed to either the concentration of the fatty acid incorporated in the agar medium had a toxic effect on this fungal isolate, or, alternatively, the solid media did not allow for enzyme expression and/or activity. The other fungal isolates both belonging to the *Mucor* spp. were capable of growing successfully on both the oleic and stearic acid agar media under varying pH and temperature conditions. These successes in growth by both the *Mucor* spp. can be largely attributed to the presence of a non – specific lipase producing system.

Microorganisms utilise various industrial pollutants or natural occurring substances as sources of carbon, nitrogen, phosphorus, sulphur or other elements that maybe required by the cells to sustain growth. However organic components such as carbonaceous materials that are oxidised by microorganisms to provide energy for efficient growth are of the utmost 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 between 30% and 40% of the total organic matter (expressed as COD), (Dueholm *et al*., 2001). Studies have shown that various microorganisms (i.e. moulds and yeasts) are capable of effectively using long chain fatty acids as sole 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).

Carbonaceous material can be divided into two principle forms, i.e. biodegradable and non-biodegradable. Each form has two principle fractions, namely soluble and particulate. The readily 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 or microbial populations (Lilley *et al*., 1997).

Referring to Figure 7.4, (Appendix 31), the rapid fungal (i.e. moulds) metabolism of the soluble readily biodegradable fraction is shown by the initial decrease in the COD values. Those isolates belonging to the *Mucor* spp showed a slow but steady decline in the total organic matter present which was shown by the gradual decrease in COD values. The presence of relatively unfavourable conditions (a pH of 5 at 21^{o}C) for organic substrate utilisation resulted in less than 50 % reduction of the total COD fraction present in the edible oil effluent, by both the *Mucor* spp. Previous studies show that for particular microorganisms to grow on a specific compound, it must be converted to intermediate products that characterise major metabolic sequences. If these compounds cannot be modified enzymatically to yield these intermediates, then these compounds will not be able to serve as energy and carbon sources and hence the energy yielding and biosynthetic processes will not function (Alexander, 1999). The lack of growth and poor organic substrate utilisation observed by both the *Mucor* spp. could indicate that the organic constituents in the edible oil effluent were unable to be enzymatically modified to yield these intermediates under the prevailing conditions. However the fungal isolate belonging to the *Alternaria* spp*.,* showed higher metabolic activity under the prevailing conditions and resulted in a 59% reduction in the total COD fraction.

Similar experiments made i.e. a pH 5 of at 31^{o}C also showed initial rapid decrease in COD values observed for the *Alternaria* sp*.* was probably largely attributed to the efficient utilisation of the soluble readily biodegradable fraction. Constant COD values thereafter represent the presence of a readily biodegradable fraction. Other organisms take a bit longer but get to the same constant period eventually. This indicates that the *Alternaria* sp*.* expresses lots of lipases and exhibits higher metabolic rates at this higher temperature. Reports suggest that the percentage of carbon substrate that is converted into cells is an indication of biological efficiency of converting the substrate into biomass (Alexander, 1999). The efficiency of utilising the organic constituents present in the edible oil effluent as growth and energy substrate's by the fungal isolates under these prevailing conditions was indicated by the extremely high COD reduction rates that were observed (i.e. 93% by the *Alternaria* sp. and 91% by both the *Mucor* spp).

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). This phenomenon is probably observed by the *Mucor* spp 1 in some of the experiments whereby although initial COD utilisation rates were slow and erratic, rapid utilisation of the organic matter was observed after the forth day (i.e. shown by rapidly decreasing COD values and an overall COD reduction of 87%). The *Alternaria* spp. however, did not require an acclimation period as it grew efficiently during many of the mentioned conditions resulting in a 92% COD reduction.

A compound not readily dissolved in water is considered as been not readily accessible for microbial utilisation. The rapid uptake of this compound by the microbial cell from the aqueous phase and subsequent rapid utilization of it as a carbon and energy source for growth is inhibited, unless the microbe has a special mechanism for uptake and assimilation of the organic substrate using special degradative enzymes (Alexander, 1999). Recent studies have shown that the aerobic removal of fats, oils and greases (i.e. FOG's) by various microorganisms involves the initial extracellular attack on triglycerides via the hydrolysis of the ester bonds by microbial lipases. The term FOG used in this study collectively represents the triglyceride component (i.e. the long chain fatty acids) that would be present in the vegetable oil effluent (Wakelin and Forster, 1997).

From the results mentioned and shown it can be seen that the fungal isolate belonging to the *Alternaria* sp. has unique capabilities to utilise and breakdown the FOG components of the edible oil effluent very efficiently under the varying pH and temperature conditions. This was observed by an 88% reduction (in Fig. 7.3, Appendix 31), and up to 90% reduction in the other experiments. This probably, also, indicates the ability of this isolate to utilise the available long chain fatty acids as sources of carbon and energy for efficient growth. Possibly attributing to this unique capability is that the *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 in it (e.g. linoleic acid). 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). Owning to this property it is clearly seen that the *Alternaria* sp. was able to efficiently grow in the presence of long chain fatty acids in an aqueous system. However this organism grew poorly on the fatty acid agar plates possibly due to the lack of an increased surface area of exposure to the fatty acid constituents and a lack of nutrient diffusion on the fatty acid agar plates. Studies have revealed that among that the many extracellular lipase producing fungi that have been isolated from sunflower seed, those belonging to the *Alternaria* sp. were one of the strongest extracellular lipase producers (Roberts *et al*., 1987). The lipase producing system of the isolate from the *Alternaria* sp. could also be regarded as been specific, in that little to no growth was observed on the fatty acid agar plates that separately contained oleic and stearic fatty acids respectively. Although it can be speculated that the organism was probably capable of utilising the long chain fatty acids such as linoleic acid which would have been abundantly available in the edible oil effluent.

Many microorganisms are capable of excreting biosurfactants into the extracellular medium for the conversion of the water insoluble substrates into sizes of less than 1*u*m, which can then be easily assimilated by these microbes (i.e. Pseudo - solubilization**)**, (Alexander, 1999). This phenomenon could also probably attribute largely to the efficiency of the *Alternaria* spp. in reducing the FOG component present in the edible oil effluent and it can probably, also, be considered that lipase production in this fungal isolate is constitutive, i.e. does not require to be grown on an oil containing medium, prior to experimentation, to induce lipase producing activities.

Previous 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 and rather than because of its toxicity. However, growth by some microorganisms in the presence of long chain fatty acids has been observed when the pH of the growth medium is maintained between ranges 5.5 and 7. It is considered that the composition of the growth medium, its pH, chain length of the fatty acid and the inhibitory concentration of the fatty acids in the aqueous growth medium largely determines the microbial utilisation or breakdown of the fatty acid component (Ratledge, 1994). Considering the above-mentioned properties and the differing FOG removal capabilities of all the fungal isolates, it can be suggested that FOG removal is likely to be affected by substrate specificity of the

induced extra-cellular lipase, the physical and chemical characteristics of the substrate and the pH of the culture medium.

Many fungi belonging to the *Mucor* spp. have strong lipolytic activity when grown in the presence of triglyceride components, or oils, as an organic substrate (Akhtar *et al*., 1980). Reports have also shown that the extracellular lipase produced by the *Mucor* spp. 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). Lipase productivity by fungi belonging to the *Mucor* spp. depends largely upon prevailing culture conditions such as pH and temperature of the growth environment. The optimum pH range of pH 6 to pH 8 is considered essential for lipase excretion by this fungal genus. Also significant lipolytic activity has been evident at pH 5 provided that the required substrate is available (Lazar and Schröder, 1992). Studies show that a pH of 8 has appeared to favour both emulsification of sunflower oil and it's cleavage by fungal lipase activity for utilisation for cell growth and production of fungal lipids (Jeffery *et al*., 1999). This activity probably explains the high FOG removal rates observed in some of the experiments, where FOG removal rates ranged from 74% to 80% for both the *Mucor* spp. Enhanced sunflower oil degradation was observed in other studies by the those belonging to the *Mucor* spp. in the presence of increasing pH's and adequate biomass formation (Jeffery *et al*., 1999). Considering the above properties, it is evident that excellent lipase production (i.e. shown by an 89% reduction in the FOG content in the edible oil effluent) was observed at a pH of 5 at 31^{o}C by both *Mucor* spp. Significant FOG removal was also observed as the pH parameters were increased from pH 5 to pH 8.

Among various physical, chemical and biological factors that influence microbial growth and destruction of the organic pollutant, essential nutrient availability, aeration, pH and temperature are considered of the utmost performance for efficient degradation. Oil degradation is considered strongly temperature dependant and both growth and degradation rates increase with an increase in temperature (Alexander, 1999). Rapid COD and FOG removal rates were observed at a temperature of 31^{o}C when compared to the removal rates observed at a temperature of 21^{o}C. However, *Mucor* spp. 1 showed lower COD and FOG removal rates at a pH of 8 at 31^{o}C (Appendix 31) possibly due to the temperatures been too sensitive for efficient growth. Extremes of alkalinity or acidity tend

to decline microbial growth and lipolytic activity (Ratledge, 1994), however it was observed that both the varying pH and temperature parameters did largely affect the overall performance of the *Alternaria* spp. Also, better COD and FOG removal rates were observed at pH's of 8 than pH's of 7 pertaining to both *Mucor* spp, hence showing the ability of these isolates to grow and have exhibit increased levels of lipolytic activity at this highly alkaline condition.

Although many microbes are frequently the major and occasionally the sole means for degradation of a particular compound, the absence of a microorganism often does result in the partial disappearance or breakdown of the compound or substance rather slowly over a period of time (Alexander, 1999). This phenomenon was observed in all the controls (i.e. uninoculated flasks) that were also subjected to the varying pH and temperature parameters. This was observed by slow declining COD and FOG removal rates.

7.3.4 Conclusions

It can be concluded from this study that different fungi (i.e. specifically moulds) have different patterns of lipolytic activity and that lipase production by these fungal isolates were largely influenced by varying pH and temperature parameters. Also, differences in FOG hydrolysis could be attributed to differences in the substrate specificity of the extracellular lipases produced by the isolates. The use of the *Alternaria* spp. to breakdown the long chain fatty acids in the sunflower oil effluent seems to be best option as it performed more efficiently and effectively then both *Mucor* spp. It can be concluded that the parameters of 31^{o}C and pH 5 offers the best controlled conditions for the removal of the FOG constituents in an edible oil effluent, which was shown by a 90% FOG removal rate by the *Alternaria* spp. and 89% removal rates by both *Mucor* species. Lastly it can be concluded that lipase activity in both the *Mucor* spp. are non-specific and those produced by the *Alternaria* spp. can be considered specific and lipase production by all the isolates can be considered constitutive.

CHAPTER 8
GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 CONCLUSIONS

- Bench scale activated sludge treatment process has shown that balancing of the TKN/COD ratio, P/COD ratio and RBCOD/S_{ti} ratios of the influent facilitated favorable biological remediation of the oil effluent.
- Good COD and FOG removal rates were obtained using the bench scale process.
- Phosphorus removal capacity of the biomass was good. The possible limit in phosphorus removal of the process could be attributed to the limited availability of RBCOD.
- Findings have shown that Prefermentation served to increase VFA production thereby enhancing biological phosphorus removal.
- Using anaerobic digestion technology to evaluate edible oil effluent treatment the following was concluded:
    - The use of barium chloride to reduce the sulphate content in edible oil effluent was effective
    - Anaerobic toxicity studies showed that the maximum influent COD that could be treated successfully was 2000 mg/L.
    - Anaerobic digestion using fed-batch configuration can achieve 30% biodegradation of COD present in edible oil effluent.
    - The experiments showed that ABR configuration was unsuitable for anaerobic digestion.
- Current research findings conclusively showed that in order for edible oil effluent to be successfully treated biological, pretreatment is essential.
- Comparison of a variety of pretreatment methods showed that chemical coagulation and flocculation with C40 proved to be the most effective pretreatment method for edible oil treatment.
- Comparison in costs to dispose of the various effluents by Durban Metro Wastewater, showed that the biologically treated edible oil effluent will be the cheapest to dispose of costing R0.50/kL, while the raw effluent and flocculated effluent will cost R23.88/kL and R3.84/kL respectively.

- Findings from investigations on using monocultures of yeast, bacteria and fungi showed that pH and temperature impacts on the organisms' ability to utilize the organic content.
- When comparing the degradative capabilities of the individual monocultures to activated sludge to treat the oil effluent, findings show that activated sludge treatment process would be a method of choice.
- Up-scaling of the aerobic treatment process from bench scale to a larger laboratory scale activated sludge treatment process focusing on organic removal only presented the following findings:
    - Due to the high FOG content of the edible oil effluent, successful biological treatment using activated sludge necessitated pretreatment with C40 (commercial flocculent), which presented the best results.
    - The activated sludge showed successful removal of COD from the effluent with the maximum concentration removed of 1550 mg/L and percentage removed was greater than 80% at steady state.
    - However, fluctuation of process performance with regards to process COD removal efficiencies could undoubtedly be attributed to the variability of the organic strength of the effluent from industry and regular disruptions in supply.
    - The F/M ratio varied and ranged from 0.27 to 1.33 mg COD/mg MLSS.d during the phases and the system responded accordingly.
    - More than 90% of the influent COD was biodegradable.

8.2 RECOMMENDATIONS

- Conventional pretreatment technology for wastewater treatment in the edible oil sector has been limited to the use of ferric chloride, alum, and other polyelectrolytes and more commonly used is DAF. Future studies should focus on the chemical characterization of the commercial flocculent C40 to further reduce the cost and enhance accessibility. The successful application of C40 will support the premise that large scale application on site at the industry will be comparatively more efficient, cheaper and most important it requires far less space than DAF and therefore more practically.

- Pretreatment technology complimented activated sludge treatment and can be used in combination for the treatment of high FOG edible oil effluents.
- An alternate approach to biological treatment could involve primary treatment of the effluent by anaerobic digestion followed by aerobic treatment as a polishing step. However this approach would require more space and therefore not as favorable as combined pretreatment with activated sludge.
- The sludge that is produced from the pretreatment process comprises a mixture of coagulant and edible FOG that could be used as a supplement to animal feed, thus further minimizing waste production and generating income.
- Parallel investigations showed that the raw effluent comprised of valuable essential oils that could be extracted and purified for commercial purposes. (Results not presented in current investigation).
- Future research will include constructing a mass balance, to distinguish whether the biomass in solution is indeed degrading the effluent, or whether the effluent is merely adhering to the surface of the activated sludge flocs and vessel walls

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APPENDICES

APPENDIX 1: VOLATILE FATTY ACID DETERMINATION

Equipment:

3 10 mL centrifuge tube with caps

4 2 mL vials with caps

5 1 mL pipette

6 Pasteur pipettes

7 Gas chromatograph (GC) (Pye Unican 4 500)

8 Centrifuge

Reagents:

9 Di-Ethyl Ether

10 98% Sulphuric acid

Preparation of samples:

11 1 mL of sample was pipetted in to a 10 mL centrifuge tube

12 2 mL of Di-ethyl ether was added

13 3 drops of sulphuric acid was added for preservation of samples

14 the sample was then centrifuged at 2000 rpm for 3 min

15 the di-ethyl ether layer was then pipetted in to a 2 mL vial and stored in the fridge until use.

Gas Chromatography configurations

- Gas Flows
  - Hydrogen Gas 30 mL/min @ 86.161 kPa
  - Nitrogen Gas 80 mL/min @ 103.393 kPa
  - Air 136.4 mL/min @ 68.929 kPa
- Column Type: SP 100, using Flame Ionization Detector (FID)
- Column Temperature: 120°C
- Injector and Detector Temperature: 200°C

APPENDIX 2: ALKALINITY AS $CaCO_3$ DETERMINATION

INTRODUCTION

The methods for the determination of alkalinity in natural or treated waters are also used for wastewaters. Although methods for the relative proportions of hydroxide, carbonate and hydrogen carbonate alkalinity are given, they are rarely required in the case of wastewaters. Colour and turbidity in the sample make use of indicators difficult or impossible, hence the simplest alternative is to use an electrometric method.

Principle of method:

The alkalinity of natural or treated waters is usually due to the presence of hydrogen carbonate, carbonate and hydroxide compounds of calcium, magnesium, sodium and potassium. The total alkalinity is determined by titration of the sample to the end-point of a suitable indicator having a colour change at pH 4.5 titration to an end-product of pH 8.3 determines approximately the alkalinity contributed by hydroxide and half of carbonate present (pH 8.3 is approximately that of a dilute hydrogen carbonate solution). The use of these two titrations enables an approximate calculation to be made of the concentrations of the three forms of alkalinity.

Method:

The samples were analysed in duplicate. 100 mL of filtered sample was placed in a conical flask. The conical flask was placed over a white surface. 3 drops of phenolphthalein indicator were added to the solution. The pink sample was titrated with 0.1 N sulphuric acid solution to a colorless end-point. Few drops of methyl orange indicator were added to the solution. The yellow colored solution was titrated with the standard acid until the first perceptible colour change towards orange was achieved.

Calculation of results:

Alkalinity for 100 mL sample as mg $CaCO_3$/L = Volume of 0.1 N sulphuric acid (mL) x 50.

APPENDIX 3: (a) COD DETERMINATION USING THE SPECTROPHOTOMETER

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^{o}C or 120EC)
- Merck SQ 118 Spectroquant
- Photometer tubes

For higher range (500 - 10000 mg/L) (Method Number 024):

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

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

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

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

Sample	:	3 mL
Solution A:		0.3 mL
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 hr at 100EC. Remove from thermoreactor and let tubes cool to room temperature before measuring COD concentration in Merck SQ 118 spectrophotometer.

(b) COD DETERMINATION USING OPEN REFLUX METHOD

Reagents:

- **Standard Potassium Dichromate Solution: 0.0417 M**

  12.259 g of $K_2Cr_2O_7$ was dissolved in distilled water and diluted to 1L

- **Sulphuric Acid Reagent**

  Silver sulphate was added to concentrated sulphuric acid at the rate of 5.5 g $AgSO_4$ per liter of H_2SO_4 and allowed to stand for 2 days.

- **Ferroin Indicator**

  1.485 g if 1,10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ was dissolved in 100 mL distilled water.

- **Standard Ferrous Ammonium Sulphate (FAS): 0.25M**

  98 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in distilled water. 20 mL concentrated sulphuric acid was added to the mixture and diluted to 1L.

Standardization of FAS

- 10 mL of standard $K_2Cr_2O_7$ was diluted to 100 mL with distilled water
- 30 mL of concentrated sulphuric acid was added and cooled
- This solution was titrated against FAS using 2 to 3 drops of ferroin indicator.
- Molarity FAS = Volume $K_2Cr_2O_7$ titrated (mL) x 0.25/ Vol. FAS used (mL)

- **Mercuric Sulphate**

  $HgSO_4$ crystals or powder

- **Potassium Hydrogen Phthalate (KHP)**

  425 mg of KHP was dissolved in distilled water and diluted to 1L. KHP has a theoretical COD of 1.176 mg O_2/mL and this solution has a theoretical COD of 500μg O_2/mL.

Procedure

- Samples with COD > 50 mg O_2/mL sample were used and those with COD > 900 mg O_2/L smaller sample volumes were used and diluted to 50 mL.
- Samples were placed in 500 mL refluxing flasks and 1 g of $HgSO_4$, glass beads and 5 mL sulphuric acid reagent added.
- Samples were cooled to prevent loss of volatile materials
- 25 mL of $K_2Cr_2O_7$ solution was added. The flask was attached to a condenser and cooling water turned on.
- The remaining sulphuric acid reagent (70 mL) was added through the open end of the condenser.
- The open end of the condenser was covered and the sample refluxed for 2 hr.
- Thereafter, the condenser was disconnected and the sample cooled to room temperature. Excess $K_2Cr_2O_7$ was titrated against FAS using Ferroin indicator
- The endpoint of the titration was taken as the first sharp colour change from blue-green to reddish brown.

APPENDIX 4: ORTHO-PHOSPHATE (PO_4-P) DETERMINATION

INTRODUCTION

Phosphate in water and wastewater may be present in two main forms: the organically bound phosphate, and the inorganic phosphate. The inorganic phosphate is further divided into two main forms based on its reactivity and its availability for utilization as a nutrient source by plants. The polyphosphate which is a polymeric form is inert in water and is not available as a plant nutrient. Yet the orthophosphate form on the other hand, is readily available in water and wastewater as a plant nutrient. This is the form of phosphate that needs to be monitored and controlled to prevent eutrophication of water resources.

Principle of Method:

The sample is mixed with a reagent that reacts with the orthophosphate group in water, which produces a distinct yellow solution the absorbance of which can be measured using a spectrophotometer.

Method:

The samples were first diluted with deionised water to a desired concentration range. Spectroquant analysis method 14842 for 1.0 – 30 mg/L PO_4-P using SQ 118 Merck photometer.

APPENDIX 5: TOTAL NITROGEN (TKN) DETERMINATION

INTRODUCTION

Nitrogen in wastewater appears both as oxidized nitrogen and reduces forms. Both the ammonium (free and saline) and the organic forms are present in wastewater and constitute the total nitrogen.

Principle of the Method:

The sample is mixed with reagent 1 in the presence of a catalyst. After heating in a thermal block, the sample is mixed with the second reagent, which results in immediate colour development. The resultant colored solution is measured using a photometer

Method:

Spectroquant analysis method 14537 for 0.5 – 15 mg/L N using SQ 118 Merck photometer.

APPENDIX 6: FREE AND SALINE AMMONIA (NH_4-N)

INTRODUCTION

The free and saline ammonium nitrogen is readily available in wastewater as a plant nutrient that is responsible for eutrophication phenomenon. Free and saline ammonia may result in wastewater from biodegradation of organic nitrogen. Hence it is important to determine both organic and ammonium nitrogen in wastewater in order to control and prevent the mineralization of water resources.

Principle of the method:

Ammonia is quantitatively separated from other forms of nitrogen in wastewater through sample distillation under alkaline conditions. The ammonia is then determined colorimetrically after addition of Nessler's reagent.

Method:

Spectroquant analysis method, 14739 for 0.01 – 2.0 mg/L NH_4-N, using SQ 118 Merck photometer.

APPENDIX 7: NITRATES (NO_3^-N) DETERMINATION

INTRODUCTION

Nitrate is the oxidized form of nitrogen. It is not usually found in domestic wastewater influents. But industrial wastewater may contain appreciable amount of nitrates that results from the oxidation of the total nitrogen in wastewater due to chemical processes and harsh environmental conditions that industrial water may be subjected to during manufacturing and processing. As a result it is important to test for this form of nitrogen from industrial effluents although it is not necessary to do the same for domestic wastewater.

Principle of method:

The sample is reacted with a colour-forming reagent in the presence of a strong oxidizing acid. The resultant colored solution is measured against the blank using a photometer.

Method:

Spectroquant analysis method 14542 for 2.0 – 80 mg/L NO_3^- using SQ 118 Merck photometer.

APPENDIX 8: SULPHATE (SO_4^{2-}) DETERMINATION

INTRODUCTION

Sulphate is widely distributed in nature and may be present in natural water in concentrations ranging from a few to several thousand milligrams per liter. Mine drainage waste and some selected chemical industrial wastewaters may contribute large amounts of point source sulphate deposition.

Principle of method:

The sample reacts with Barium chloride at low pH that results in the formation of Barium sulphate. At high pH excess Barium reacts with Methylthymol blue to produce a chelate. The uncomplexed Methylthymol blue is gray. The gray uncomplexed Methylthymol blue indicates the concentration of sulphates.

Method:

The samples were first diluted with deionised water to desired concentration ranges. Spectroquant analysis method 14564 for 100 – 1000 mg/L SO_4^{2-} using SQ 118 Merck photometer.

APPENDIX 9: 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^{o}C to 105^{o}C. The increase in weight of the filter represents the total suspended solids (Standard Methods, 1989).

Apparatus:

- Glass Fibre Filters (pore size of 0.45□m)
- Desiccator
- Drying oven, for operation at 103^{o}C to 105^{o}C
- Analytical balance, capable of weighing 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 20 mL 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^{o}C to 105EC for 1 hr. Cool in dessicator to balance temperature and weigh. Store in dessicator until needed.

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 50 mL of this to measuring cylinder. Add to filter. Wash filter with three successive 10 mL 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°C to 105°C for 1 hr. Cool in dessicator 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 10: FATS, OILS AND GREASES (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^{o}C
- Sodium sulfate, Na_2SO_4, anhydrous crystal

Procedure:

A sample volume of 30 mL 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 30 mL of hexane and added to the separatory funnel. The funnel was shaken vigorously for 2 min. 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 10 g Na_2SO_4, both have been solvent rinsed**,** into a clean, tared bulb flask. The extraction was done twice more with 30 mL of solvent each time. The

extracts were drained in the tared flask and the filter and Na_2SO_4 were rinsed with 10 to 20 mL 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 dessicator. 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 11: pH DETERMINATION

Principle:

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.

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 12: PREPARATION OF THE NUTRIENT MEDIUM ACCORDING TO OWEN *et al*., (1979).

The defined nutrient medium containing trace elements, minerals and vitamins were prepared according Owen *et al*., (1979). The stock solutions for preparation of the nutrient medium are presented in Table 12.1 and the method for the preparations is presented in Table 12.2.

Table 12.1: Stock solutions for preparation of mineral salts medium

STOCK SOLUTION	COMPOSITION	CONCENTRATION (g/L)
S2	Rezazurin	1
S3	$(NH_4)_2HPO_4$	26.7
S4	$CaCl_2.2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na_2MoO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2.4H_2O$	20
	EDTA	20
S6	Cysteine	50
S7	Biotin	0.002
	Folic acid	0.002
	Pyridoxine HCl	0.01
	Riboflavin	0.005
	Thiamin	0.005
	Nicotinic acid	0.005
	Pantothenic acid	0.005
	Vitamin B12	0.0001
	p-aminobenzoic acid	0.005
	Thiotic acid	0.005

Table 12.2: Preparation of the defined mineral salts solution

STEP	METHOD	VOLUME (mL)	MASS (g)
1	1 L of deionised water was added to a 2 L vessel		
2	The following were added:		
	Stock solution S2	1.8	
	Stock solution S3	5.4	
	Stock solution S4	27	
3	Deionised water was added up to 1.8 L		
4	Boiled for 15 min		
5	Cooled to room temperature		
6	The following were added:		
	Stock solution S7	1.8	
	Stock solution S5	1.8	
	Stock solution S6	1.8	
7	$NaHCO_3$ was added as powder		8.4

APPENDIX 13: MIXED LIQUOR SUSPENDED SOLIDS (MLSS) AND VOLATILE SUSPENDED SOLIDS (VSS) DETERMINATION

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 (50 mL)
- Centrifuge (capable of 3 000 rpm)
- Crucibles
- Drying oven (operating at 103^{o}C to 105^{o}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^{o}C to 105^{o}C and left overnight to dry. After 24 hr it was removed from oven and placed in dessicator. 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

VSS DETERMINATION:

Principle:

The residue from the MLSS is ignited to a constant weight at 550^{o}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^{o}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^{o}C for 1 hr. The crucible is then place in a dessicator and cooled. The crucible is then re-weighed.

Calculation:

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

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

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

APPENDIX 14: PREPARATION OF COAGULANTS

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 (1 ppm) of $FeCl_3$.
- This solution was prepared fresh whenever required, since occurred causing a color change from yellow to cloudy orange.

Aluminum Sulphate (Alum)

- 4 mL of 43.7% alum (density – 1.29) was pipetted into a 1000 mL volumetric flask and was made up to the 1000 mL mark with distilled water.
- When added to an 800 mL effluent sample, 1 mL of the alum solution yielded 2 mg (2 ppm) of alum.

Polymeric coagulants

- The following polymeric coagulants were prepared: dimethyldiallylamonium chloride (DMDAAC/PAC – Z553D); DMDAAC/PAC – LP526, unblended polyamines/PAC (PA/PAC – 735) and PA – U5000.
- 0.8 mL of the coagulant was pipetted in to a 1000 mL volumetric flask and was made up to the 1000 mL mark with distilled water.
- When added to an 800 mL effluent sample, 1 mL of the coagulant solution yielded 1 mg/L (ppm) of coagulant.

APPENDIX 15: IODOMETRIC METHOD I

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

Sodium Thiosulphate

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

The Standardization 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:
    1. Sodium thiosulphate: 25 g in 1 L
    2. $K_2Cr_2O_7$: 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 x 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.

    3. 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.

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

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.

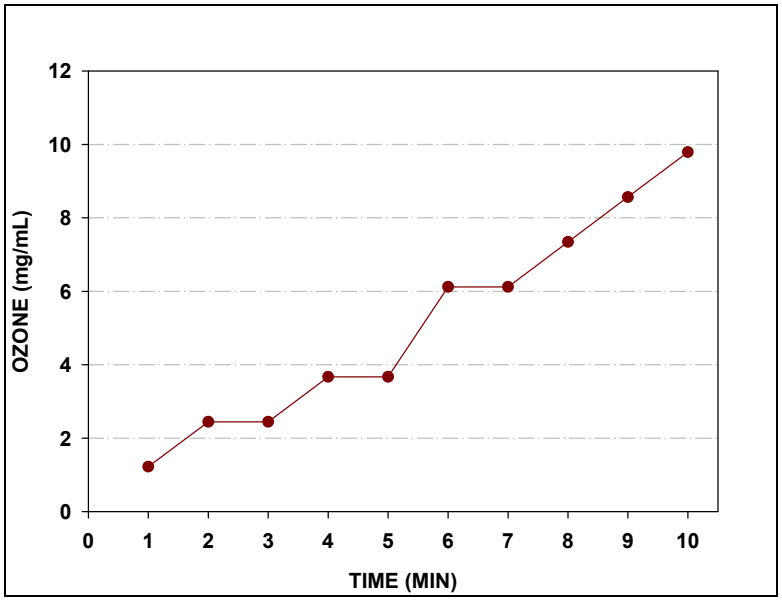

Figure 15.1: Ozone calibration curve

APPENDIX 16: INDIGO COLORIMETRIC METHOD

- **Preparation of Reagents**

1. 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.

2. 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.

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:

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

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.

RESIDUAL OZONE CALCULATION

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

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

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

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

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

= 0.1246 mg O_3/L

APPENDIX 17: THIN LAYER CHROMATOGRAPHY (TLC)

Preparation of Reagents

- Mobile Phase

  20% ethyl acetate in hexane was prepared by adding 40 mL of ethyl acetate to 160 mL hexane.

Procedure;

Extraction

- 40 mL of sample was added to a separating funnel
- 10 mL of hexane was added to the funnel and shaken vigorously
- The funnel was held in an upright position with the aid of a bosshead attached to a clamp.
- The layers were allowed to separate.
- The top hexane layer was collected and stored in a polyvial while the bottom layer was discarded.

Plating

- A microheamatocrit tube was used to transfer the sample to the TLC plate.
- The origin was marked 1 cm away from the bottom of the plate using a pencil. The sample spots were marked.
- The sample was applied and the plate was placed in the tank, which contained approximately 20 mL of the mobile phase.
- The mobile phase was allowed to run until it was approximately 1 cm away from the top edge of the plate.
- The process was then stopped and the solvent front marked.

Detection and Evaluation

- The plates were viewed under UV lamp, compounds marked and their intensities recorded.
- R_f and H_{Rf} values were calculated in the following way:
- R_f = distance sample moved from the origin
  - Distance solvent front moved from origin

$H_{Rf} = R_f \times 100$

APPENDIX 18: ULTRASONICS

Use of a Virtis Virsonic 100

- The machine mode was adjusted to continuous.
- The probe was placed in a beaker of ice and the output power adjusted until the required output was achieved
- The probe was placed in the sample for the required time.

Use of a Sonnicator Bath

- Water was filled in the UMC5 and the sample contained within a glass bottle was placed inside
- The lid was placed on top and the timer set to the required time
- The machine was then switched on.

APPENDIX 19: PHASES CONDUCTED DURING LABORATORY SCALE TREATMENT

Table 19.1 Activity during the phases of the laboratory scale treatment process.

PHASE	PERIOD	ACTIVITY
1	18/5/00 - 24/5/00	Pilot plant operated as a Phoredox Process for biological nitrogen & phosphorus removal. Effluent was not pre-treated and system failed after five days due to very high FOG loading (approximately 8 200 mg/L)
2	25/5/00 - 05/6/00	Due to high FOG, an efficient pre-treatment protocol was developed and optimized. The pilot plant was operated as Phoredox process. Although, the FOG was low, the phosphorus removal capacity was poor and COD removal efficiency decreased. Process was terminated.
3	06/6/00 - 21/7/00	Refinery process was changed, i.e. citric acid was used instead phosphoric acid. Therefore the effluent contained minimal amount of phosphorus and there was no need for nutrient removal. The process configuration was changed to facilitate carbon removal only. In order to maintain the biological integrity of the system the effluent was supplemented with nitrogen and phosphorus. However an erroneous setting of the dissolved oxygen meter i.e. limit 6 mg0_2/L instead of 5 mg 0_2/L. This resulted in a bulking problem and subsequent loss of MLSS and poor overall COD removal efficiency. Influent COD concentration was maintained at 500 mg/L.
	21/5/00 - 27/ 5/00	The process was shut down completely and completely and the system including tubing was cleaned thoroughly
4 A	28/7/00 - 16/9/00	The process was restarted with fresh Darvill seed inoculum. The MLSS was 2546 mg/L. The sludge age was maintained at 16 days. The COD of the influent feed was increased to 800 mg/L. The free and saline ammonia (FSA) concentration in the effluent was high. In order to solve the latter problem, the feed supplementation ratio was changed from 100:10:1 to 100:5:1 (C:N:P). In addition, the system configuration was also changed to introduce an anoxic zone and the process was operated as a modified MLE process. Sustaining constant influent feed concentration was problematic due to precipitation caused by resident flocculent in the feed tank and variation amongst the different batches of effluent collected. Therefore in order to determine the efficiency of the process, COD removal rates were reflected as percentages (result section). The pH was maintained at pH ± 7.
4 B – 4F	17/9/00 - 02/2/01	Process remained as an MLE configuration. The influent COD increased to approximately 1200 mg/L. The MLSS was set at 3500 mg/L. On the 21/10/00, there was a shut down of the refinery process at the industry and subsequently there was no supply of feed influent. The final effluent had to be recycled and served as influent in order to sustain the system. The effluent supply normalized on 3/11/00 On 01/12/00 the effluent feed concentration was increased to approximately 1500 mg/L. On 12/12/00 the influent concentration was increased to approximately 1800 mg/L. However, it was difficult to maintain exact influent feed concentration (reasons mentioned previously) There was a disruption in influent supply for 1 month. However, the final effluent was recycled to sustain the system during this period. No analyses were conducted.
5	06/3/01 - 07/10/01	The process remained as an MLE configuration. However there was a decrease in the influent COD which was uncontrollable since the quality and strength of the influent was dependent on the supply from industry. The process parameters remained the same.

APPENDIX 20: MALT EXTRACT AGAR

Composition	g/L
Malt Extract	17.0
Agar	15.0

Preparation

- Suspended 17 g in 1 L of demineralized water.
- Bring to the boil with frequent stirring until completely dissolved
- Autoclave under mild conditions (10 min at 115°C)

APPENDIX 21: NUTRIENT AGAR

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

Preparation:

- Suspend 31 g in 1 L distilled water
- Bring to the boil
- Sterilize by autoclaving at 121EC for 15 min.
- When almost cooled, pour in to petri dishes and allow gel to form.

APPENDIX 22: PURITY CHECK

- Prepare methylene blue solution by adding 1 g methylene blue powder to 100 mL distilled water.
- Place one drop methylene blue to a clean slide
- With the aid of an inoculating loop transfer the yeast to the droplet on the slide. Spread yeast. Place cover slip over the slide.
- Examine under the microscope using 100 x objective (oil immersion).

APPENDIX 23: NUTRIENT SOLUTION

- 1 L of distilled was added to a conical flask
- The following constituents were weighed out on an analytical balance and then added to the distilled water:
- 2g 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 $MgSO_4.7H_2O$.
- The medium was transferred to a schott bottle and sterilized by autoclaving at 121^oC for 15 min.

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

The solution was then autoclaved at 121^oC for 15 min. The cooled solution was then used to dilute the effluent as required.

APPENDIX 24: OLEIC ACID PLATES

Composition:

Nutrient Solution

- Macro nutrients 1 mL/L
- Micro nutrients 1 mL/L
- Vitamins 1 mL/L
- Agar 15 g/L
- Oleic acid 50 mL/L
- Distilled water 1000 mL/L

Preparation

- Mix nutrient solution and agar with distilled water
- Autoclave at 121°C for 15 min.
- Add oleic acid when media is about 45°C.
- Cool to room temperature.
- Shake vigorously before pouring on to Petri dish.

APPENDIX 25: STEARIC ACID PLATES

Composition:

Nutrient Solution

- Macro nutrients 1 mL/L
- Micro nutrients 1 mL/L
- Vitamins 1 mL/L
- Agar 15 g/L
- Stearic acid 25 g/L
- Distilled water 1000 mL/L

Preparation

- Mix nutrient solution and agar with distilled water
- Autoclave at 121°C for 15 min.
- Add oleic acid when media is about 45°C.
- Cool to room temperature.
- Shake vigorously before pouring on to Petri dish.

APPENDIX 26: FOG DEGRADATION GRAPHS FOR BACTERIA AND YEAST ISOLATES

The reduction in FOG concentrations following batch tests inoculation with *Bacillus sp*, F, *Candida* and *Rhodosporidium* sp. are shown in Figures 7.1 to 7.6. Likewise the COD reduction and percentage removal is illustrated in Figures 7.7 to 7.12.

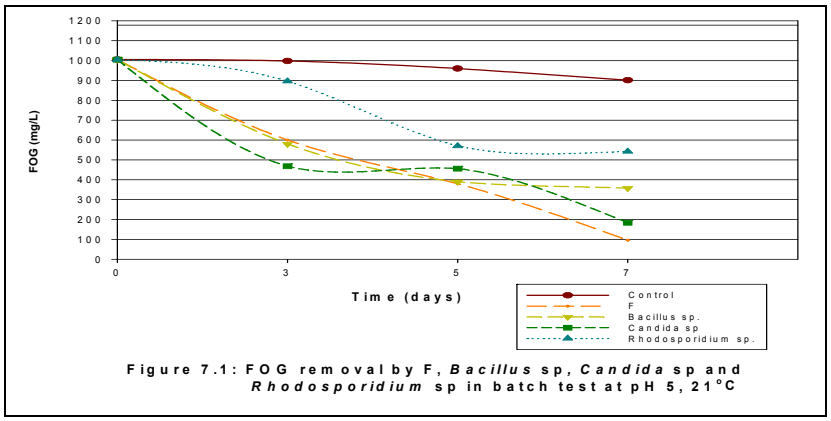

Figure 7.1: FOG removal by F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 5, 21°C

Figure 7.1: pH 5, 21°C

In Fig. 7.1 initial FOG concentration was 1005 mg/L. The control remained relatively stable but still showed slight FOG reduction. Final FOG was 901 mg/L (day 7). F reduced rapidly from initial to day 3 (600 mg/L) and day 5 (380 mg/L). However, between day 5 and day 7 FOG decreased to 96 mg/L. *Bacillus sp* also reduced FOG from initial to day 3 (580 mg/L). By day 7 the FOG concentration had elevated to 358 mg/L. *Candida sp* diminished FOG from initial to day 5 (468 mg/L). But FOG increased to 184 mg/L by day 7. *Rhodosporidium sp* showed worst FOG reduction. Between initial and day 3 (896 mg/L), but between day 3 and day 7 the FOG escalated to 542 mg/L.

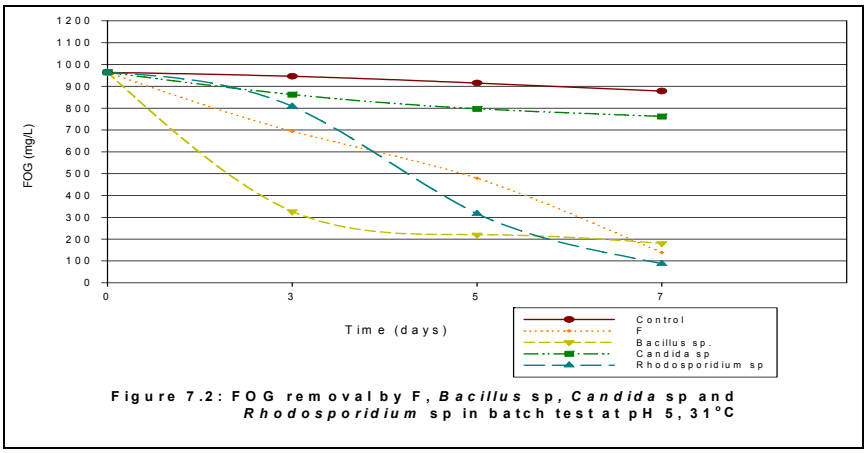

Figure 7.2: FOG removal by F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 5, 31°C

Figure 7.2: pH 5, 31° C

Initial FOG concentration was 964 mg/L (fig. 7.2). Control slowly degraded to 878 mg/L by day 7. F slowly degraded FOG to 694 mg/L (day 3) and 479 (day 5). Between day 5 and day 7 FOG was rapidly utilized to 138 mg/L. Initially *Bacillus sp* reduced FOG instantly to 326 mg/L (day 3). *Bacillus sp* slowed down between day 3 and day 5 (220 mg/L) to finally reduce FOG to 180 mg/L (day 7). FOG was not utilized very well by *Candida sp* at this parameter. By day 7 FOG was only diminished to 762 mg/L. Between initial and day 3 (808 mg/L) *Rhodosporidium sp* degraded slowly. By day 5 and day 7 utilization increased quickly as FOG was measured at 316 mg/L and 87 mg/L respectively.

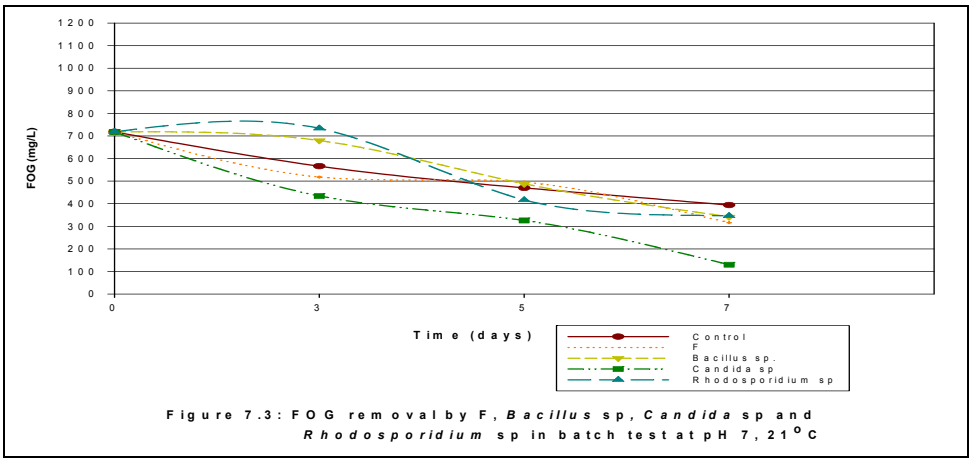

Figure 7.3: FOG removal by F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 7, 21°C

Figure 7.3: pH 7, 21°C

FOG concentration, in Fig. 7.3, was initially measured to be 718 mg/L. The control was observed to degrade itself to a final concentration of 394 mg/L (day 7). F degraded FOG at a moderate pace throughout this test. FOG concentrations were measured to be 518 mg/L (day 3), 496 mg/L (day 5) and 316 mg/L (day 7). *Bacillus sp* also performed at relatively the same pace as F to reduce FOG to a final concentration of 342 mg/L (day 7). *Candida sp* also showed moderate rate of FOG utilization between day 1 and day 5 but thereafter decreased FOG fairly quickly. On day 3, day 5 and day 7 FOG was measured to be 434 mg/L, 326 mg/L and 130 mg/L respectively. *Rhodosporidium sp* performed the best. Degradation began instantly as FOG concentration was measured to be 734 mg/L on day 3. By day 5 (416 mg/L) *Rhodosporidium sp* slowed down. But by day 7 the FOG concentration was measured at 316 mg/L.

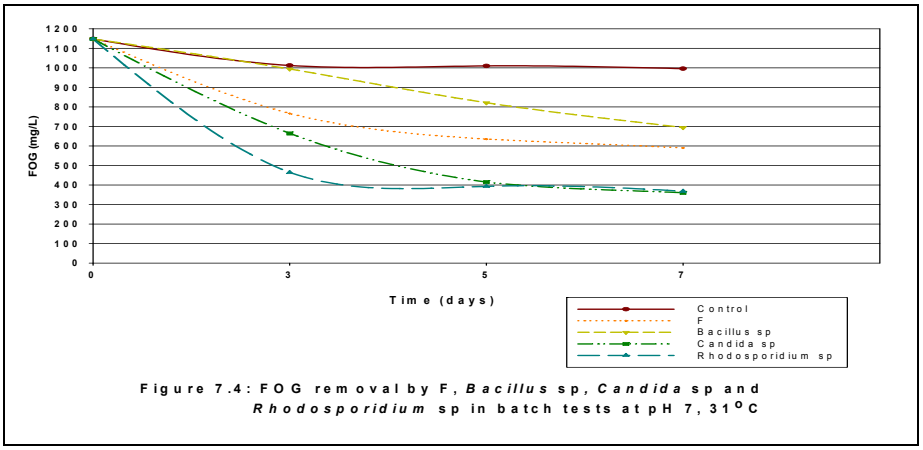

Figure 7.4: FOG removal by F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch tests at pH 7, 31°C

Figure 7.4: pH 7, 31°C

In Fig.7.4: FOG concentration was 1148 mg/L. Control was measured at 1012 mg/L, 1010 mg/L and 996 mg/l on the respective days. F decreased FOG from 766 mg/L on day 3 to 635 mg/L and 590 mg/L on day 5and day 7 respectively. *Bacillus sp* also performed as well as F. FOG was reduced to 994 mg/L, 821 mg/L and 694 mg/L on respective days. *Candida sp* performed very well. FOG concentration was reduced from 664 mg/L on day 3 to 415 mg/L on day 5, eventually to 360 mg/L on day 7. *Rhodosporidium sp* rapidly decreased FOG to 464 mg/L by day 3 to reduce to a final concentration of 368 mg/L (day 7).

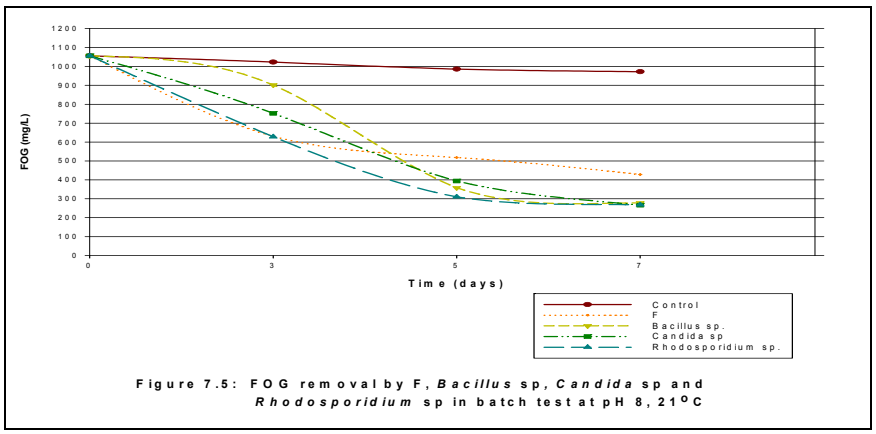

Figure 7.5: FOG removal by F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 8, 21°C

Figure 7.5: pH 8, 21°C

Initial concentration was 1056 mg/L. Control was relatively stable to have a final concentration of 972 mg/L. F utilized FOG rapidly by day 3 (630 mg/L). By day 5 F slowed down reduction to 428 mg/L. *Bacillus sp* started degradation slowly but by day 7 it had accelerated its pace. FOG was measured at 902 mg/L, 358 mg/L and 278 mg/L on the respective days. *Candida sp* also started slowly. By day 3 FOG was only lessened to 752 mg/L. By day 5 (394 mg/L) utilization

occurred rapidly but by day 7 (266 mg/L) it had slowed down again. *Rhodosporidium sp* performed relatively the same as *Candida sp.* FOG was 628 mg/L (day 3), 310 mg/L (day 5) and 268 mg/L (day 7).

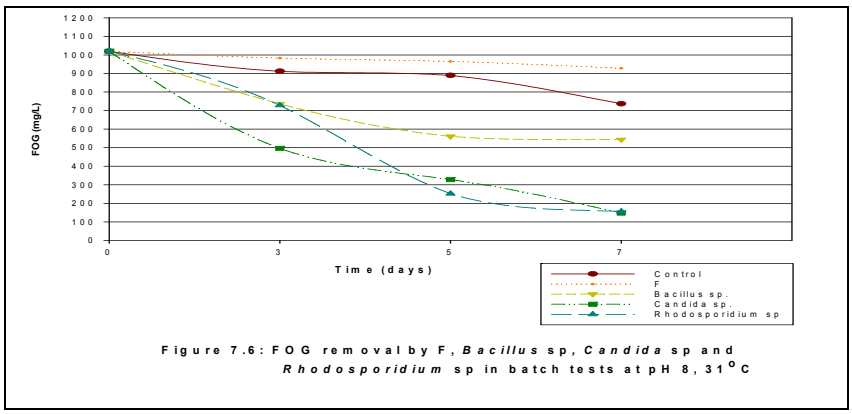

Figure 7.6: FOG removal by F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch tests at pH 8, 31°C

Figure 7.6: pH 8, 31°C

Initial FOG was 1020 mg/L. Control gradually deceased from 912 mg/L to 889 mg/L and finally to 737 mg/L. F utilize FOG poorly only reducing it to 983 mg/L (day 3), 965 mg/L (day 5) and 928 mg/L (day 7). *Bacillus sp* performed little better than F, by reducing FOG to 786 mg/L, 561 mg/L and 544 mg/L on the respective days. *Candida sp* reduced FOG quickly by day 3 (328 mg/L), by day 5 (496 mg/L) it had slowed down. But by day 7 utilization had increased again to reach final concentration of 148 mg/L. *Rhodosporidium sp* did not show much capability for the first 3 days (728 mg/L). By day 5 (252 mg/L) and day 7 (156 mg/L) *Rhodosporidium sp* rapidly utilized FOG.

APPENDIX 27: COD DEGRADATION GRAPHS FOR BACTERIA AND YEAST ISOLATES

(N.B.: The increase in COD could be attributed to Soluble Microbial Presence (SMP)).

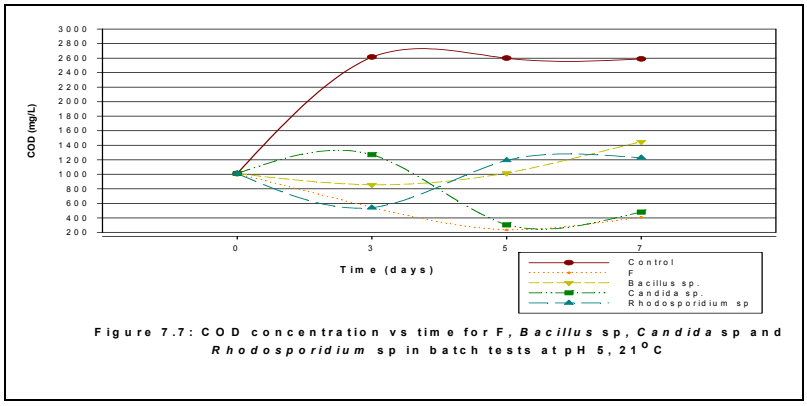

Figure 7.7: COD concentration vs time for F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch tests at pH 5, 21°C

Figure 7.7: pH 5, 21° C

Initial COD concentration was 1010 mg/L. Control COD concentration increased to 2615 mg/L (day 3) and eventually decreased slightly to 2599 mg/L (day 5) and to 2589 mg/L (day 7). F reduced COD to 545 mg/L (day 3) and 234 mg/L (day 5). By Day 7 COD increased to 407 mg/L. *Bacillus sp* reduced COD to 855 mg/L (day 3). But by day 5 and day 7 COD had increased to 1016 mg/L and 1448 mg/L respectively. *Candida sp* initially increased COD to 1271 mg/L (day 3) but COD decreased drastically to 302 mg/L by day 5. On day 7 COD measured at 479 mg/L. *Rhodosporidium sp* rapidly decrease COD to 539 mg/L (day 3). But by day 5 and 7 the COD concentration increased to 1192 mg/L and 1227 mg/L respectively.

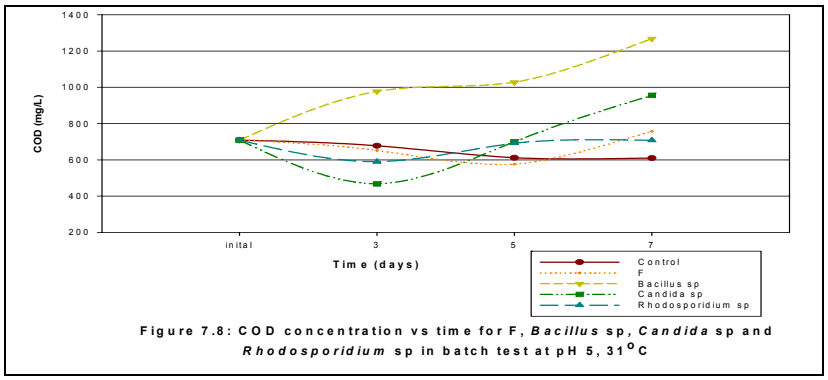

Figure 7.8: COD concentration vs time for F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 5, 31°C

Figure 7.8: pH 5, 31°C

In Fig. 7.8 initial COD concentration was 709 mg/L. COD for control decreased gradually from 678 mg/L (day 3), to 612 mg/L (day 5) and to 610 mg/L (day 7). F slowly decreased COD to 652 mg/L, 576 mg/L and 758 mg/L on the respective days. *Bacillus sp* did not decrease the COD concentration. COD increased to 978 mg/L on day 3, 1028 mg/L on day 5 and 1286 mg/L on

day 7. COD was drastically reduced by *Candida sp* by day 3 (468 mg/L). But began increasing by day 5 (700 mg/L), to reach a final concentration of 956 mg/L by day 7. *Rhodosporidium sp* reduced COD to 509 mg/L (day 3) but increased it to 692 mg/L and 708 mg/L by day 5 and 7 respectively.

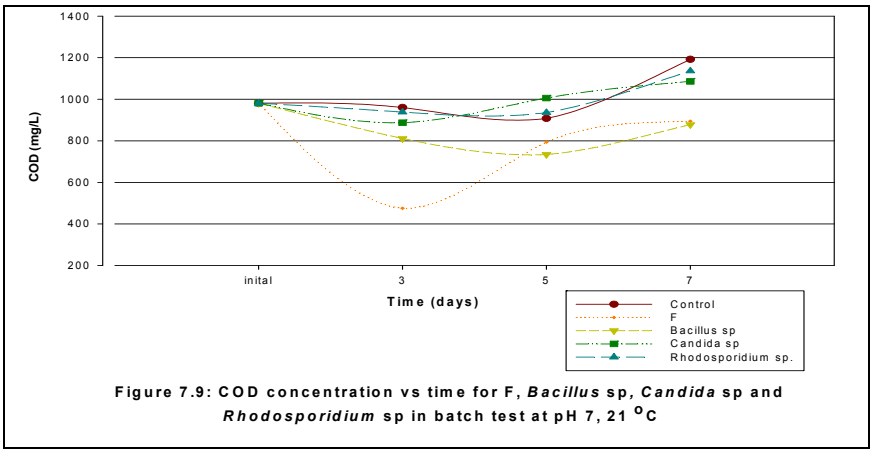

Figure 7.9: COD concentration vs time for F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 7, 21 ^{o}C

Figure 7.9: pH 7, 21° C

In Fig. 7.9 COD concentration on day 1 was 981 mg/L. Control COD remained fairly stable up to day 5 but increased to 1192 mg/L by day 7. F decreased COD to 475 mg/L (day 3). But increases were observed on day 5 (794 mg/L) and day 7 (894 mg/L). *Bacillus sp* slowly decreased COD to 811 mg/L and 734 mg/L by day 3 and 5 respectively. But an increase was measured on day 7 (878 mg/L). *Candida sp* reduced COD to 887 mg/L. But showed large increases on day 5 (1006 mg/L) and day 7 (1086 mg/L). COD was initially reduced very slowly by *Rhodosporidium sp* for the first three days (887 mg/L). By day 5 and 7 the COD elevated to 986 mg/L and 1136 mg/L respectively.

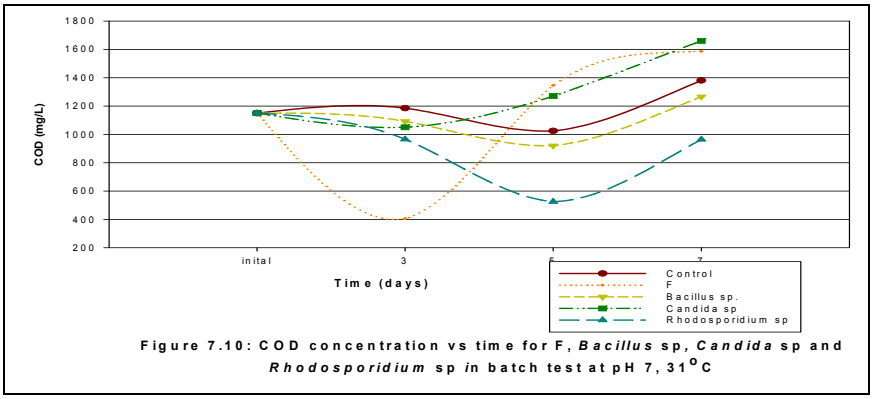

Figure 7.10: COD concentration vs time for F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 7, 31°C

Figure 7.10: pH 7, 31° C

Initial COD concentration was 1150 mg/L (Fig. 7.10). Control showed COD increasing to 1185 mg/L by day 3, decreasing to 1024 mg/L by day 5 and again an increase to 1380 mg/L by day 7. F reduced COD to 405 mg/L (day 3). But between day 3 and day 7 COD increased to 1346 mg/L (day 5) and 1586 mg/L (day 7). *Bacillus sp* slowly decreased COD between day 3 (1092 mg/L) and day 5 (920 mg/L). By day 7 it had increased to 1266 mg/L. *Candida sp* showed a little change in COD concentration by day 3 (1050 mg/L). After day 3 COD concentration began increasing to 1270 mg/l (day 5) and 1660 mg/L (day 7). *Rhodosporidium sp* decreased COD to 965 mg/L (day 3) and 525 mg/L (day 5). By day 7 COD elevated to 964 mg/L.

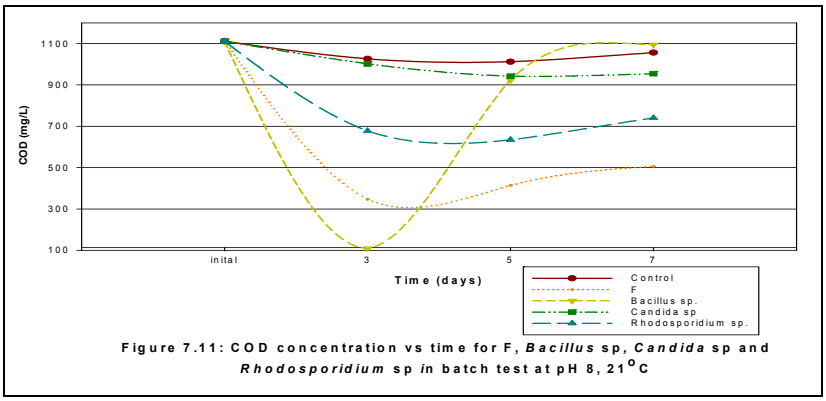

Figure 7.11: COD concentration vs time for F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch test at pH 8, 21°C

Figure 7.11: pH 8, 21° C

COD concentration was initially measured at 1111 mg/L. Control remained relatively stable with decrease in concentration from day 1 to day 5 (1012 mg/L), but increased slightly to 1056 mg/L by day 7. COD concentrations for F decreased drastically to 347 mg/L by day 3. On day 5 and 7 the COD was measured at 414 mg/L and 506 mg/L respectively. *Bacillus sp* COD was measured to be 109 mg/L, 924 mg/L and 1094 mg/L on the respective days. *Candida sp* slowly

reduced COD by day 3 (1002 mg/L) and day 5 (942 mg/L), to then increase it slightly by day 7 (954 mg/L). *Rhodosporidium sp* reduced COD rapidly to 677 mg/L (day 3). Thereafter COD was measured at 634 mg/L on day 5 and 740 mg/L on day 7.

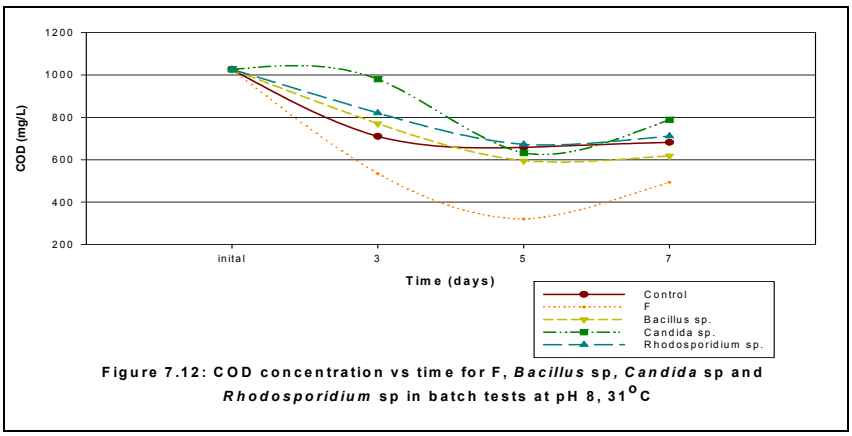

Figure 7.12: COD concentration vs time for F, *Bacillus* sp, *Candida* sp and *Rhodosporidium* sp in batch tests at pH 8, 31°C

Figure 7.12: pH 8, 31° C

Initial COD concentration was 1026 mg/L. Control showed a decrease from 710 mg/L (day 3) to 658 mg/L (day 5). On day 7 it was measured at 682 mg/L. F decreased COD to 534 mg/L (day 3) and 320 mg/L (day 5). By day 7 COD had increased to 493 mg/L. *Bacillus sp* started slower by reducing COD to 770 mg/L (day 3) and 595 mg/L (day 5), but increased to 618 mg/L by day 7. *Candida sp* only reduced COD to 980 mg/L, 631 mg/L and 788 mg/L by the respective test days. *Rhodosporidium sp* also barely reduced COD. COD was reduced to 820 mg/L (day 3), 672 mg/L (day 5) and slightly rose to 711 mg/L by day 7.

APPENDIX 28: SABORAUD DEXTROSE AGAR (SDA)

- 60 g of Biolab Saboraud Dextrose Agar powder was weighed out using a top balance
- This was then put in to a 1 L conical flask and 1 L of distilled water was added
- The contents of the flask was then boiled until the agar dissolved
- The contents was then poured in to Schott bottle and sterilized by autoclaving at 121^{o}C for minutes.
- When almost cooled the media was then poured in to the petri dishes and left to solidify.

APPENDIX 29: LACTOPHENOL COTTON BLUE SOLUTION

Composition

- Lactic acid 20 mL
- Phenol 20 mL
- Glycerol 40 mL
- Distilled water 20 mL
- Aniline blue 0.05 g

Procedure:

- Mix the above contents in to a beaker (except the Aniline blue dye) and heat gently using a hot plate/magnetic stirrer (Agimatic) until completely dissolved. Then add the Aniline blue dye. Lactophenol cotton blue solution, results.

APPENDIX 30: COD AND FOG GRAPHS FOR THE FUNGAL ISOLATES

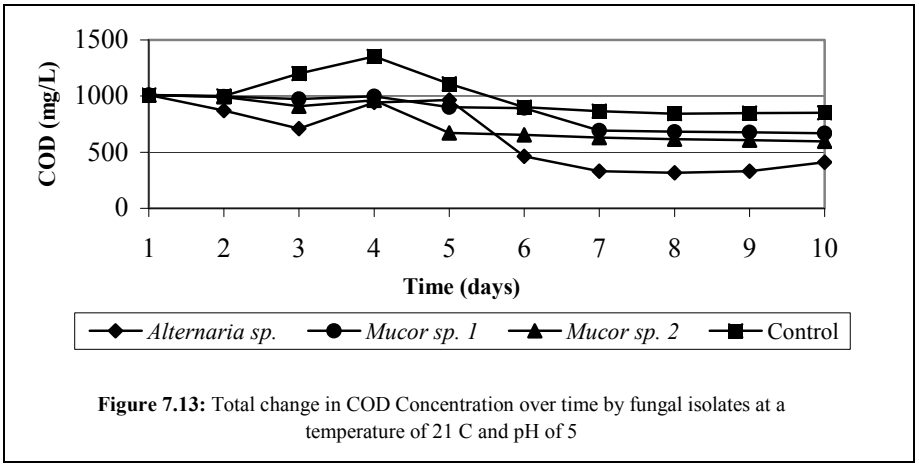

Figure 7.13: Total change in COD Concentration over time by fungal isolates at a temperature of 21 C and pH of 5

Figures 7.13 and 7.14 21°C, pH 5

For the *Alternaria sp.* the total COD concentrations showed an overall decrease from 1010 mg/L to an eventual 410 mg/L. Also, moderate decreases in total COD concentrations were observed for both *Mucor spp.,* i.e. *Mucor sp. 1* showed a decline from 1010 mg/L to 669 mg/L while *Mucor sp. 2* recorded a decrease from 1010 mg/L to 597 mg/L. The control showed a slow decrease in concentrations from 1010 mg/L to 851 mg/L by the end of the tenth day.

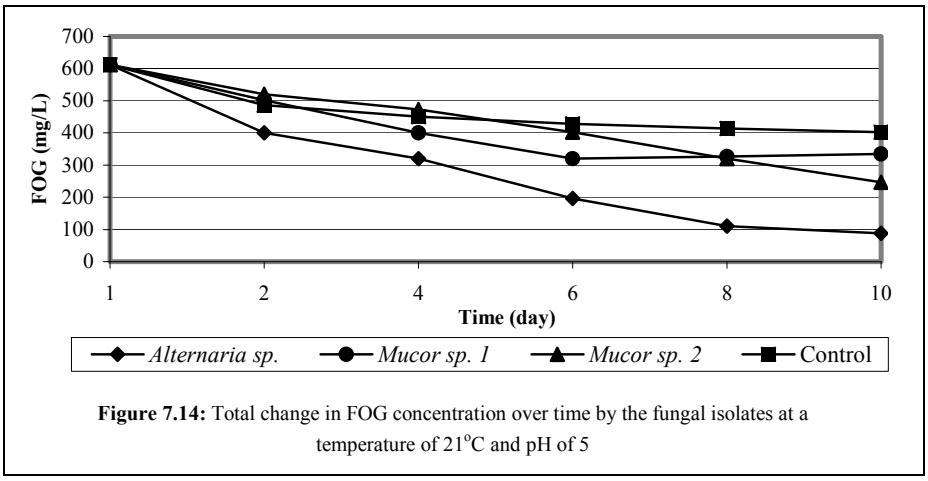

Figure 7.14: Total change in FOG concentration over time by the fungal isolates at a temperature of 21°C and pH of 5

An overall decrease in the FOG from initial concentrations in the edible oil effluent was observed by all the fungal isolates. Decreases in FOG concentrations from 612 mg/L to 88 mg/L were observed by the *Alternaria sp*. Isolates belonging to the *Mucor spp.*, i.e. *Mucor sp.* 1 and *Mucor sp.* 2, showed a decrease in FOG concentrations from 612 mg/L to 335 mg/L and 247

mg/L respectively. It was also observed that the FOG concentration in the control steadily decreased from 612 mg/L to 402 mg/L by the end of the tenth day.

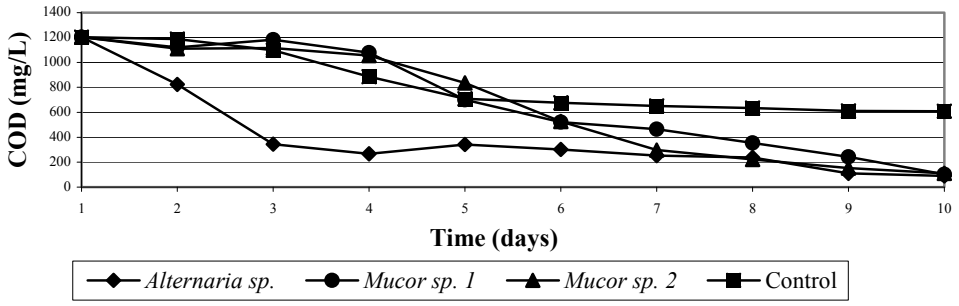

Figure 7.15: Total change in COD concentration over time at a temperature of 31°C and pH of 5

Figures 7.15 and 7.16. pH 5, 31°C

As expected overall rapid decreases in COD concentrations by all the isolates and the control was observed. Decreases in COD concentrations ranged initially from 1204 mg/L to 90 mg/L by the *Alternaria* sp., 103 mg/L by the *Mucor* sp. 1 and 110 mg/L by *Mucor* sp. 2. The control showed a gradual decline in concentrations from 1204 mg/L to 610 mg/L.

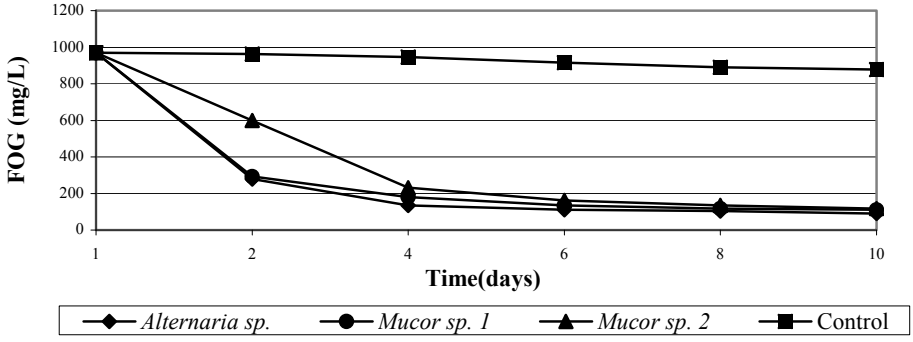

Figure 7.16: Total change in FOG concentration over time at a temperature of 31°C and pH of 5

Rapid FOG utilisation was observed by the fungal isolates during this 10-day experimentation. FOG concentrations decreased from initially 970 mg/L to 90 mg/L by the *Alternaria* sp., 110 mg/L and 117 mg/L by the *Mucor* sp. 1 and *Mucor* sp. 2, respectively

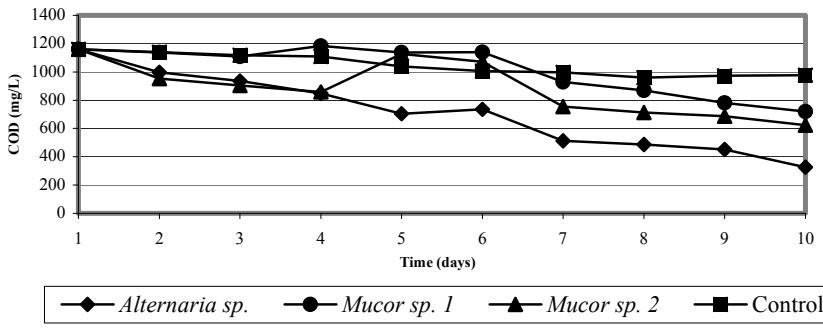

Figure 7.17: Total change in COD concentration over time by the fungal isolates at a temperature of 21°C and pH of 7

Figures 7.17 and 7.18. pH 7, 21°C

Initial COD concentrations were 1160 mg/L and thereafter fluctuated during the 10 day period, especially when considering the *Mucor* spp. However, overall reductions in COD concentrations were observed for example decreases to 325 mg/L by the *Alternaria* sp., 719 mg/L by *Mucor* sp.1 and 623 mg /L by *Mucor* sp. 2. A minor reduction to 978 mg/L of the COD concentration was observed in the control.

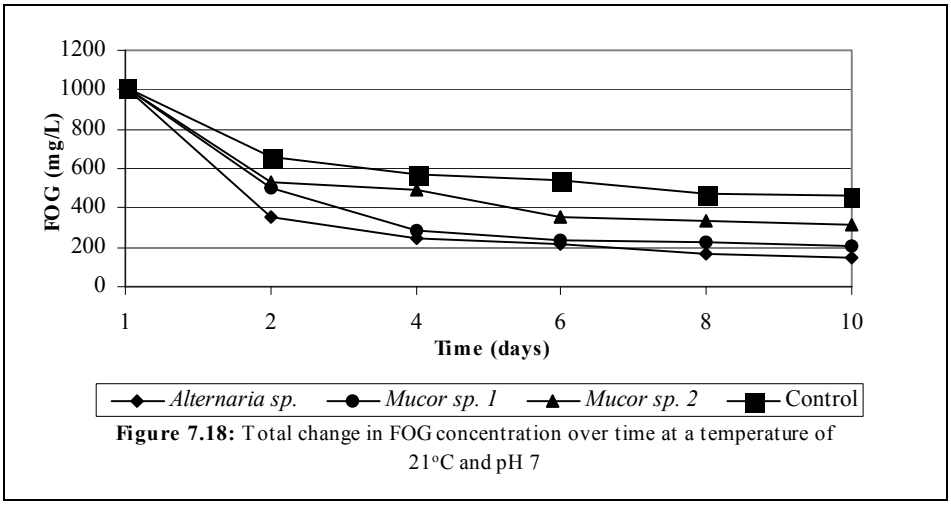

Figure 7.18: Total change in FOG concentration over time at a temperature of 21°C and pH 7

Overall decreases in FOG concentrations from an initial concentration of 1010 mg/L to eventual concentrations of 146 mg/L by the *Alternaria* sp., 211 mg/L by *Mucor* sp. 1 and 319 mg/L by the *Mucor* sp. 2 respectively were recorded. Likewise the COD concentrations of the control decreased from 1010 mg/L to 463 mg/L.

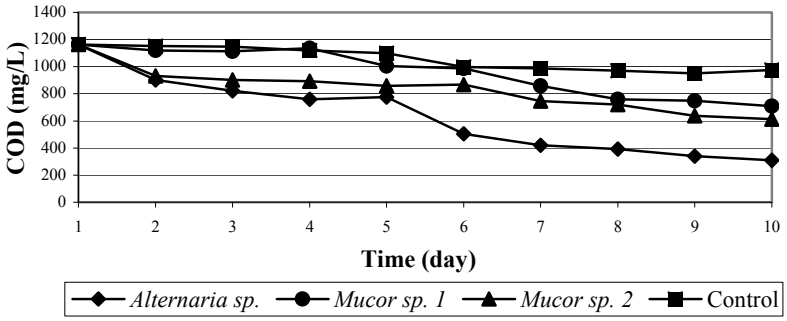

Figure 7.19: Total change in COD concentration over time by the fungal isolates at a temperature of 31°C and pH of 7

Figures 7.19 and 7.20. pH 7, 31°C

It was observed that the overall COD concentrations decreased at varying rates by the fungal isolates. The *Alternaria* sp. showed a rapid decrease in COD concentrations from 1163 mg/L to eventually 310 mg/L. A slower rate of COD utilisation was observed by the *Mucor* spp., whereby *Mucor* spp. 1 reduced the COD from 1163 mg/L to 709 mg/L and *Mucor* sp. 2 decreased the COD to 613 mg/L from the initial concentration. Also observed was a slow, yet insignificant decrease in the COD concentration of the control from 1163 mg/L to a mere 976 mg/L.

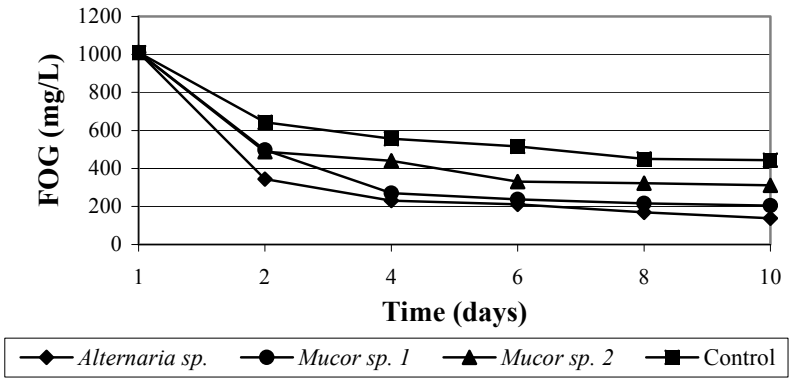

Figure 7.20: Total change in FOG concentration over time by the fungal isolates at a temperature of 31°C and pH of 7

It was observed that the *Alternaria* sp. and the *Mucor* sp. 1 reduced the FOG components of the edible oil effluent rapidly. This was shown by the initial FOG concentration of 1010 mg/L decreasing to 138 mg/L and 205 mg/L respectively. Also, *Mucor* sp. 2 and control showed a moderate decrease in FOG concentrations from 1010 mg/L to 311 mg/L and 443 mg/L respectively.

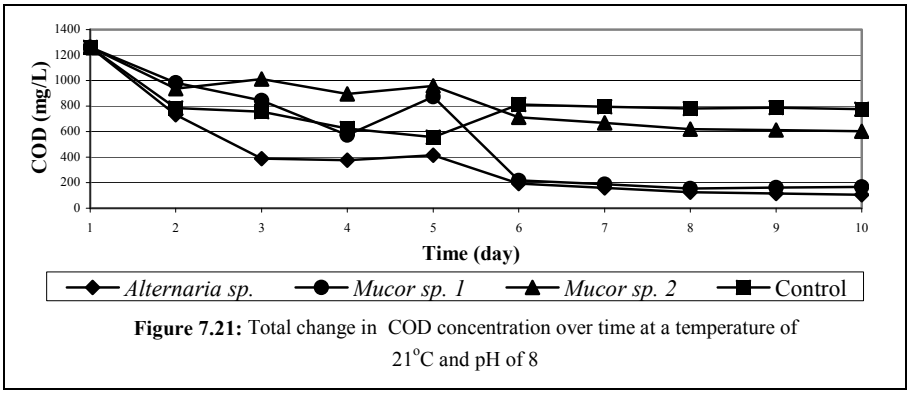

Figure 7.21: Total change in COD concentration over time at a temperature of 21°C and pH of 8

Figures 7.21 and 7.22. pH 8, 21°C

Slight fluctuations in the COD concentrations were observed by both *Mucor* spp. but an overall decrease of the total COD concentration from an initial concentration of 1262 mg/L to 166 mg/L by *Mucor* sp. 1 and 603 mg/L by *Mucor* sp. 2 was shown. The *Alternaria* sp. showed an efficient decline in COD concentrations from 1262 mg/L to 106 mg/L and the control also decreased to 776 mg/L from the initial COD concentration.

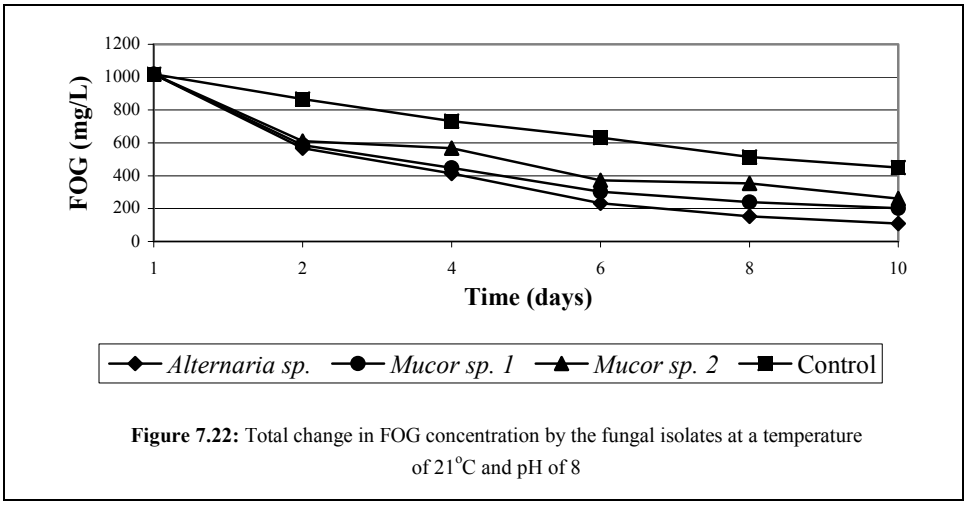

Figure 7.22: Total change in FOG concentration by the fungal isolates at a temperature of 21°C and pH of 8

An overall decrease of FOG concentrations during the 10 day experimental period by all the fungal isolates was observed. Initial FOG concentrations decreased from 1018 mg/L to 110 mg/L by the *Alternaria* sp., to 202 mg/L by the *Mucor* sp. 1, to 261 mg/L by the *Mucor* sp. 2 and, to 450 mg/L by the control.

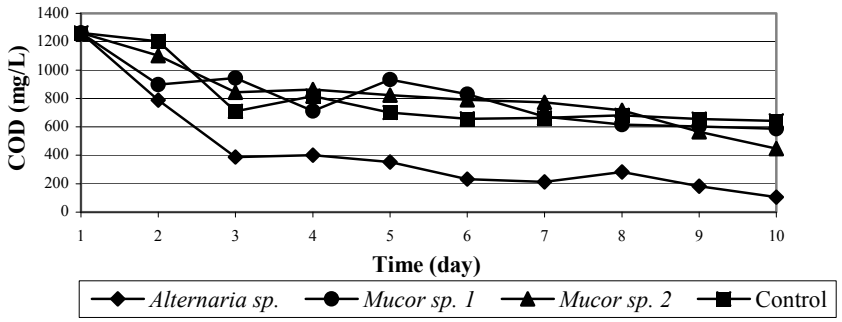

Figure 7.23: Total change in COD concentration by the fungal isolates at a temperature of 31^{o}C and pH of 8

Figures 7.23 and 7.24. pH 8, 31°C

It is observed that a reduction in the COD concentrations by all the fungal isolates and the control had occurred. The initial COD concentration of 1262 mg/L was decreased to 106 mg/L by the *Alternaria* sp., 588 mg/L by *Mucor* sp. 1, 446 mg/L by *Mucor* sp. 2 and 641 mg/L during the control experiments.

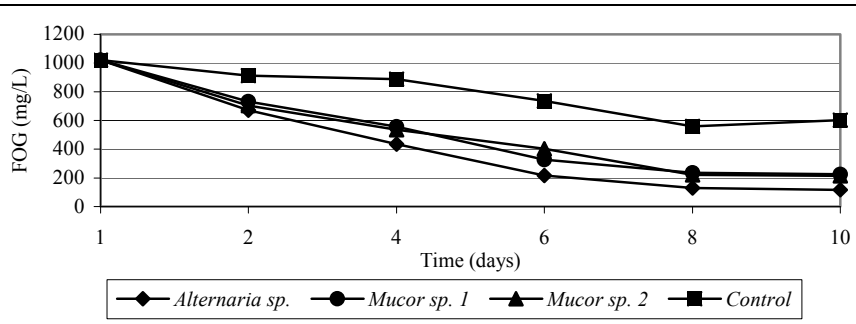

Figure 7.24: Total change in FOG concentration by the fungal isolates at a temperature of 31oC and pH of 8

The initial FOG concentration of 1020 mg/L was observed to decrease to 116 mg/L by the *Alternaria* sp., 225 mg/L and 214 mg/L by *Mucor* sp.1 and *Mucor* sp. 2 respectively. The control FOG concentration decreased to 602 mg/L from the initial concentration.