BIOLOGICAL PHOSPHORUS REMOVAL FROM EDIBLE OIL EFFLUENT BY ANAEROBIC-AEROBIC SEQUENCING BATCH REACTOR

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2003

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Dissertation submitted in compliance with the requirements of the Master' s Degree in Technology in the Department of Biotechnology, Durban Institute of Technology.

2008

DECLARATION

I hereby declare that the dissertation represents my own work, unless stated to the contrary in the text, and that it has not been submitted in part, or in whole to any other Technikon/University.

I hereby approve the final submission of the following dissertation.

10-2-2004

Supervisor

Date

Dr FAIZAL BUX
(D. TECH: BIOTECHNOLOGY, TECHNIKON NATAL)

DEDICATION

THIS DISSERTATION IS DEDICATED TO MY LATE FATHER

ABSTRACT

The objective of this study was to evaluate the characteristics and treatability of process wastewater from an edible oil refining industry, which discharge its effluent into a sewer system. The main objective was to assess a laboratory scale treatment process that would produce effluent having a regulatory acceptable phosphate concentration (below 20 mg/L) prior to discharge into municipal sewer system. A single stage laboratory-scale anaerobic-aerobic sequencing batch reactor (BPR-SBR) with a total volume adjustable up to 10L was designed for biological phosphorus removal. The BPR-SBR was run at 10 days sludge age, 8 hours hydraulic retention time and organic load of ~ 0.38 kg COD/kg MLSS.d for 158 days to evaluate its performance for bio-P removal efficiency. The BPR-SBR system showed a consistent P removal efficiency of up to 78.40 %, 80.15 % COD and 72.43 % FOG reduction. The laboratory scale study has demonstrated that the SBR technology is suitable for treating wastewater from edible oil producing industry.

ACKNOWLEDGEMENTS

Many people have provided invaluable support during this project. I wish to express my sincere gratitude to the following people and institutions for their assistance in the completion of this dissertation.

I. Dr Faizal Bux, my supervisor for keeping me focussed throughout the project and for always offering sound advice and encouragement.

II. Mr F. Swalaha, for his valuable input and guidance.

III. Blaise Atkinson, Sandile Mkhize, Delon Mudaly, Peter Osifo, Pumla Mathibela, Swastika Surujlal, Kureshnee Reddy and my colleagues at the Centre for Water and Wastewater Research for their various assistance and helpful comments.

IV. Durban Institute of Technology staff at the Department of Biotechnology for your support and assistance.

V. National Research Foundation and Centre for Water and Wastewater Research for the financial assistance.

VI. Paul from Company A, Darvill Wastewater Works and Durban Northern Wastewater Works for providing samples for the research project.

VII. My friends, Esrom, Patty, Phillip, Waren, Nomathembu, John, Thabang and all you people around me who made my study years in Durban so fantastic (you know who you are!).

VIII. My wife and children, Palesa and Nkateko for the patience and tolerance they showed through the years I took to complete this project.

IX. My Mom, two sisters, Violet and Alphinah, uncle Nick and his family for their encouragement and support.

TABLE OF CONTENTS

TITLE PAGE..... i

DECLARATION..... ii

DEDICATION..... iii

ABSTRACT..... iv

ACKNOWLEDGEMENTS..... v

TABLE OF CONTENTS..... vi

LIST OF TABLES..... xii

LIST OF FIGURES..... xiii

LIST OF ABBREVIATIONS..... xvi

CHAPTER 1

GENERAL INTRODUCTION

1.1 PHOSPHORUS..... 1

1.1.1 Characteristics of Phosphorus..... 1

1.1.2 Phosphorus Cycle..... 2

1.1.3 Importance of Phosphorus..... 4

1.2 SOURCES OF PHOSPHORUS..... 6

1.2.1 Point Sources..... 6

1.2.2 Non-point sources..... 8

1.3 EUTROPHICATION..... 9

1.3.1 Definition and Understanding..... 9

1.3.2 International Perspective..... 11

1.3.3 South African Perspective..... 12

1.3.4 Environmental and Health Impacts..... 13

1.4 POLICY APPROACH AND LEGISLATION..... 16

1.4.1 Policy Approach..... 16

1.4.2 Current Legislation..... 18

1.4.2.1 National Water Act..... 19

1.4.2.2 National Environmental Management Act..... 20

1.4.2.3 Water Services Act..... 21

1.5 THE SOUTH AFRICAN EDIBLE OIL INDUSTRY..... 22

1.5.1 Water Intake..... 23

1.5.2 Oil Processing..... 23

1.5.2.1 Degumming..... 24

1.5.2.2 Refining..... 24

(A) Chemical refining..... 25

(i) Neutralisation..... 25

(ii) Bleaching..... 27

(iii) Hydrogenation..... 27

(iv) Winterising..... 28

(v) Deodorising..... 28

(B) Physical refining..... 29

1.5.3 Effluent Quality..... 29

1.6 TREATMENT METHODS..... 30

1.6.1 Chemical Phosphorus Removal Method..... 32

1.6.2 Biological Phosphorus Removal Method..... 32

1.7 SEQUENCING BATCH REACTOR (SBR)..... 35

1.8 RESEARCH OBJECTIVES..... 37

CHAPTER 2

LITERATURE REVIEW

2.1 WASTEWATER TREATMENT..... 39

2.2 ACTIVATED SLUDGE..... 40

2.3 DEVELOPMENT OF BIOLOGICAL PHOSPHORUS REMOVAL..... 43

2.4 MECHANISMS OF BIOLOGICAL PHOSPHORUS REMOVAL..... 49

2.4.1 Anaerobic zone..... 50

2.4.1.1 Metabolic model of the anaerobic metabolism..... 53

2.4.2 Aerobic zone..... 57

2.4.2.1 Metabolic model of the aerobic metabolism..... 59

2.4.3 System phosphorus removal..... 61

2.5 FERMENTATION..... 62

2.5.1 First phase: VFA production..... 64

2.5.2 Second phase: VFA consumption..... 66

2.5.3 Prefermenter technology..... 67

2.6 PROCESS PARAMETERS FOR BPR..... 68

2.6.1 Wastewater characterisation..... 69

2.6.1.1 Carbonaceous material..... 69

2.6.1.2 Phosphorus fraction in wastewater..... 72

2.6.1.3 Nitrogenous constituents..... 74

2.6.2 Process control and design parameters..... 76

2.6.2.1 Loading rate..... 76

(a) Volumetric loading..... 76

(b) Organic loading..... 76

(c) Sludge loading..... 77

2.6.2.2 Solids retention time (SRT)..... 79

2.6.2.3 Mixed liquor suspended solids (MLSS)..... 80

2.6.2.4 Sludge settleability..... 81

2.6.2.5 Sludge bulking..... 83

2.6.3 Environmental factors..... 84

2.6.3.1 Dissolved Oxygen (DO)..... 84

2.6.3.2 Temperature..... 85

2.6.3.3 pH..... 87

2.7 MICROBIOLOGY OF BIOLOGICAL PHOSPHORUS REMOVAL... 88

2.8 SUMMARY..... 92

CHAPTER 3

EDIBLE OIL EFFLUENT CHARACTERISTION

3.1 INTRODUCTION..... 94

3.2 MATERIALS AND METHODS..... 95

3.3 RESULTS..... 97

3.4 DISCUSSION..... 101

CHAPTER 4

LABORATORY SCALE SEQUENCING BATCH REACTOR DESIGN FOR BIOLOGICAL PHOSPHORUS REMOVAL

4.1 INTRODUCTION..... 105

4.2 MATERIALS AND METHODS..... 106

4.2.1 Unit set-up..... 106

4.2.2 Sequencing Batch Reactor design for Biological Phosphorus

Removal..... 108

4.2.2.1 Effect of HRT..... 108

4.2.2.2 Effect of SRT..... 109

4.2.2.3 Effect of Organic loading..... 111

4.3 RESULTS..... 112

4.4 DISCUSSION..... 115

CHAPTER 5

OPTIMISATION AND PERFORMANCE EVALUATION OF SEQUENCING BATCH REACTOR FOR BIOLOGICAL PHOSPHORUS REMOVAL

5.1 INTRODUCTION..... 122

5.2 MATERIALS AND METHODS..... 124

5.3 RESULTS..... 127

5.4 DISCUSSION..... 132

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS 138

REFERENCES..... 142

APPENDICES

APPENDIX 1.....A

APPENDIX 2..... B

APPENDIX 3.....E

APPENDIX 4.....G

APPENDIX 5..... H

APPENDIX 6.....I

APPENDIX 7..... ...J

APPENDIX 8.....L

APPENDIX 9.....N

APPENDIX 10.....P

APPENDIX 11.....R

APPENDIX 12A.....S

APPENDIX 12B.....T

APPENDIX 13A.....U

APPENDIX 13B.....W

APPENDIX 13C.....Z

APPENDIX 14A.....DD

APPENDIX 14B.....FF

LIST OF TABLES

TABLE 1.1 Breakdown of water usage of a typical edible oil processing plant in South Africa..... 23

TABLE 2.1 Typical process loading ranges..... 79

TABLE 2.2 Biological phosphorus removal steps..... 93

TABLE 3.1 Wastewater characterisation of the untreated oil effluent from Company A refinery plant..... 97

TABLE 4.1 SBR unit operation.....110

TABLE 4.2 Relationship between sludge volume index and organic load.....115

TABLE 5.1 Operational conditions and characteristics of BPR-SBR.....124

TABLE 5.2 Parameters design for the lab-scale pilot plant for bio-P removal.....126

TABLE 5.3 Linear relationship between phosphorus uptake and phosphorus release.....135

LIST OF FIGURES

FIGURE 1.1 Biogeochemical phosphorus cycle..... 3

FIGURE 1.2 Schematic representation of the flow of P from various sources into the aquatic environment..... 7

FIGURE 1.3 Schematic representation of physical and chemical refining..... 26

FIGURE 1.4 Schematic representation of EBPR process..... 34

FIGURE 1.5 Schematic representation of SBR operation..... 36

FIGURE 2.1 Schematic diagram of the activated sludge process..... 42

FIGURE 2.2 Schematic diagram of Phostrip process..... 44

FIGURE 2.3: Four-stage Bardenpho activated sludge process for biological nitrogen removal..... 45

FIGURE 2.4 Five-stage Phoredox activated sludge process for biological nitrogen and phosphorus removal..... 48

FIGURE 2.5 Biochemical model proposed for anaerobic acetate uptake and PHA synthesis and aerobic PHA metabolism..... 56

FIGURE 2.6 Phenomena involved in biological phosphorus removal.....62

FIGURE 2.7 Schematic representation of the anaerobic degradation of wastewater.63

FUGURE 2.8 Division of COD into its various constituent fractions..... 70

FIGURE 2.9 Fractions of phosphorus in domestic wastewater.....73

FIGURE 3.1 Total and soluble COD profiles of untreated effluent over a period of twelve months fourteen months of experimental work..... 98

FIGURE 3.2 FOG profile of untreated effluent over a period of twelve months.... 98

FIGURE 3.3 TP profile of untreated effluent over the first three months period 99

FIGURE 3.4 Nitrate and ammonia profiles of untreated effluent over a period of twelve months..... 99

FIGURE 3.5 pH profile of untreated effluent over a period of twelve months..... 100

FIGURE 3.6 Sulphate profile of untreated effluent over the first twelve months period..... 100

FIGURE 3.7 Total suspended solids profile of untreated effluent over a period of twelve months..... 101

FIGURE 4.1 Schematic diagram of the experimental lab-scale SBR set-up.....107

FIGURE 4.2 COD removal at various HRT during the first 40 days of experimental period.....112

FIGURE 4.3 % TP removal at various HRT during the first 40 days of experimental period 113

FIGURE 4.4 Relationship between SRT and % P removal efficiency.....114

FIGURE 4.5 Relationship between Organic Loading and % P removal efficiency.....115

FIGURE 5.1 Total influent and effluent COD profiles for the BPR-SBR over the 158 days of operation.....127

FIGURE 5.2 COD_{util} and soluble COD profiles for the BPR-SBR during the 158 days of operation.....128

FIGURE 5.3 Calibration curve for P standards.....128

FIGURE 5.4 Influent and effluent TP profiles for the BPR-SBR over the 158 days of operation.....129

FIGURE 5.5 Relationship between influent COD and P over the 158 days of operation.....130

FIGURE 5.6 Linear relationship between P uptake and P release over the 158 days of operation 130

FIGURE 5.7 % FOG and % COD removal profiles for the BPR-SBR over the 158 days of operation.....131

FIGURE 5.8 SVI and effluent TSS profiles for the BPR-SBR unit over the 158 days of operation 131

FIGURE 5.9 Relationship between VSS and P over 158 days of experimental period...132

TABLE OF ABBREVIATIONS

ADP	adenosine diphosphate
AN	anaerobic reactor
ASP	Activated Sludge Process
bio-P	biological phosphorus
BNR	biological nutrient removal
BOD_5	Five day biochemical oxygen demand
BPR	biological phosphorus removal
CM	Continuous mixing
$Ca_5F(PO_4)_3$	calcium fluoride phosphate
CO_2	carbon dioxide
COD	chemical oxygen demand
COD_{SOL}	soluble COD
CMASS	completely mixed activated sludge system
CWWR	Centre for Water and Wastewater Research
DAF	Dissolved air floatation
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DSVI	dilute sludge volume index
DW A F	Department of Water Affairs and Forestry
Ø	diameter
EBPR	enhanced biological phosphorus removal
EFF	effluent
EM	Emblem-Meyerhof
Θ_X	effective sludge age

f	endogenous residue fraction of active mass
fbs	reactor biomass fraction
fb,i	biodegradable, soluble influent COD fraction
fbpi	biodegradable, particulate influent COD fraction
f_{cv}	COD to VSS ratio
f_{usi}	unbiodegradable soluble influent COD fraction
f_{upi}	unbiodegradable particulate influent COD fraction
FF A	Free fatty acids
FOG	Fats, oils and grease or separable fatty matter (SFM)
F-RBCOD	Fermentable RBCOD
FISH	fluorescent in situ hybridization
F /M	food:microorganism ratio
GAO	glycogen accumulating organism
H_2	hydrogen gas
HAB	Heterotrophic active biomass
HAc	acetate
HCl	Hydrochloric acid
HRT	hydraulic retention time
INF	influent
IAWQ	International Association on Water Quality
IAWPRC	International Association on Water Pollution Research and Control
K_{hm}	maximum substrate utilization rate
K_S	The half saturation constant or shape factor of the Monod equation
K_2HPO_4	Hydrogen potassium phosphate
MCRT	mean cell retention time

ML	mixed liquor
MLSS	mixed liquor suspended solids
MLTP	mixed liquor total phosphorus
MLVSS	mixed liquor volatile suspended solids
N	nitrogen
N_{ti}	total influent nitrogen
NDBEPR	nitrification denitrification biological excess phosphorus removal
NEMA	National Environmental Management Act
NWA	National Water Act
Ni	Nickel
NO_3	nitrate
NaOH	sodium hydroxide
ORP	oxidation-reduction potential
OUR	oxygen utilization rate
P	phosphorus
P_{ti}	total influent phosphorus
P_{INF}	Influent phosphorus
P_{EFF}	Effluent phosphorus
PAOs	phosphorus accumulating organism
PF	Plug Flow
PHA	polyhydroxyalkonoates
PHB	poly-p-hydroxybutyrate
PHV	poly 3-hydroxyvalerate
Pmf	proton motive force
PO_4	phosphate

poly-P	polyphosphate
PST	primary settling tank
Q	flow rate
Q_d	The specific denitrification rate
Qi	influent flow rate
Q_n	The specification nitrification rate
R_s	sludge age
RAS	return activated sludge
RBCOD	readily biodegradable COD
r	reaction
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
S_{bpi}	biodegradable particulate influent COD
S_{bsi}	biodegradable soluble influent COD
S_i	unbiodegradable soluble COD
S_{te}	total effluent COD
S_{ti}	total influent COD
SBCOD	slowly biodegradable COD
SCF	short chain fatty acid
Θ_c	sludge age
S_e	substrate concentration at the end of the react period
S_f	substrate concentration at the end of the fill period
SO_4	sulphate
SRP	soluble reactive phosphorus
SRT	solids retention time
S_{ui}	unbiodegradable COD

S_{usi}	soluble unbiodegradable COD
SSVI	stirred specific volume index
SV_{30min}	sludge volume after 30 min settling
SVI	sludge volume index
SWI	Specific water intake
t_c	cycle time
TCA	tricarboxylic acid
t_d	draw time
t_f	fill time
t_i	idle time
TKN	Total Kjeldahl Nitrogen
TP	total phosphorus
t_r	react time
t_s	settle time
T_bOD	total biological demand
TSS	total suspended solids
UCT	University of Cape Town
UES	uniform effluent standards
V	volume
VFA	volatile fatty acid
VM	vanadate-molybdate
VSS	volatile suspended solids
WAS	waste activated sludge
WRC	Water Research Commission
WSA	Water Services Act

WWW	wastewater works
WWTP	wastewater treatment plant
Xa	active organism concentration
X_e	endogenous residue concentration
Y_h	The cell yield coefficient defined as the mass of activated sludge or biomass produced per unit of substrate removed

CHAPTER 1

GENERAL INTRODUCTION

1.1 PHOSPHORUS

1.1.1 Characteristics of Phosphorus

Phosphorus (P) was discovered by Brand in 1669 (as cited from CST, 1997) by preparing it from urine. It is the eleventh- most abundant mineral in the earth's crust and does not exist in a gaseous state. Phosphorus exists in four or more allotropic forms: white (or yellow), red, and black (or violet). Ordinary P is a waxy white solid; which is colourless and transparent when pure. White phosphorus has two modifications: *alpha and beta* with a transition temperature at –3.8°C (CST, 1997; NCSU, 1998).

P may be grouped into two physical fractions i.e. dissolved and particulate P. Dissolved inorganic P (mainly $H_2PO_4^-$ or HPO_4^{2-}) is the directly bio-available form. Other forms are dissolved condensed P (P-O-P bonds) and dissolved organic P (P-O-C bonds) which, are bio-available only through conversion to inorganic P. Dissolved condensed P includes both natural compounds and the P compounds found in P detergents, and usually have a small lifetime in natural waters due to hydrolysis to dissolved inorganic P (Weddepohl and Meyer, 1992).

Particulate P often comprises a high proportion of the total P input to water-bodies. Particulate P fraction consists of inorganic, organic and condensed forms. Inorganic P is the

most significant as a source of bio-available P in most natural waters (NCSU, 1998). The condensed particulate P compounds generally comprises a small portion of the total particulate P. Organic P eroded from soil particles (the major source of particulate P in streams) is relatively stable and the fraction converted to dissolved inorganic P in natural waters is small (Weddepohl and Meyer, 1992).

P in soils occurs, as organic or inorganic P. Organic P is generally high in surface soils where organic matter tends to accumulate. Inorganic forms are more prevalent in sub-soils. Soil P is readily immobilised due to its affinity to certain minerals. P is rapidly immobilised as iron and aluminium phosphates in strong acid soils, and calcium as tricalcium phosphate in alkaline soils, which reduces the availability of soil P. Once it is lost to the aquatic environment, the nature of P existing in sediment or in solutions becomes significant in the nutrition of aquatic micro-organisms (Weddepohl and Meyer, 1992; Muyima, *et al.*, 1997).

Phosphorus has a major role in the structure of nucleic acids (e.g. DNA) and in molecules (e.g. ATP) involved in the storage and use of energy in cells. Soluble Reactive Phosphorus (SRP), i.e. immediately available phosphorus and P that can be transformed into an available form by naturally occurring processes, is seldom found in high quantities (it is usually < 0.01 mg/L P) in non-polluted water as it is utilised by plants and converted into cell structures (Dallas and Day, 1993).

1.1.2 Phosphorus Cycle

Phosphorus cycle (figure 1.1) occurs when phosphorus moves from land to sediments in the seas and then back to land again. Phosphorus is a metallic element which, occurs naturally in rocks and other mineral deposits. During the natural process of weathering, the rocks gradually release the phosphorus as phosphate ions (PO_4^{3-}), which, enters rivers and streams that transport them to the ocean. Once in the ocean the phosphorus accumulates on

continental shelves in the form of insoluble deposits. Over time, the crystal plates rise from the sea floor and expose the phosphates on land. After more time, weathering will release P from rocks and the cycle's geo-chemical phase begins again (Corbin, 2001; Wilkes, 2001).

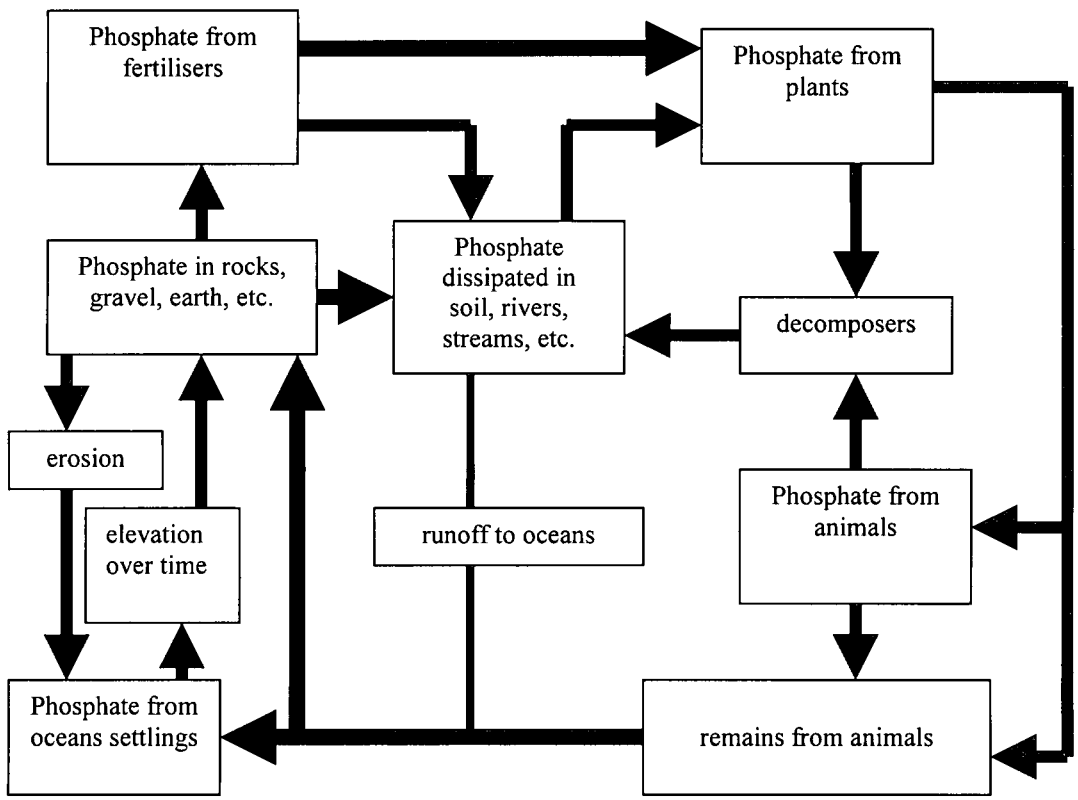


Figure 1.1: Biogeochemical phosphorus cycle (adapted from Corbin, 2001).

Knowledge of the P cycle is important as it influences the availability of P. During P transport from the primary sources to the receiving aquatic system, P undergoes transformations that are associated with its cycle, which changes their concentrations, chemical forms and availability. The main storage of phosphorus is on land and is usually found in the form of phosphates (Walmsley, 2000; Corbin, 2001). Phosphates exist in three forms: orthophosphate, metaphosphate (or polyphosphate) and organically bound phosphate each compound contains phosphorus in a different chemical arrangement (Wiechers, 1987; Wilkes, 2001).

Orthophosphate, forms are produced by natural processes but major man influenced sources include: partially treated and untreated sewage, runoff from agricultural sites, and application of some lawn fertilisers. Orthophosphates are readily available to the biological community, i.e. plant uptake (Weddepohl and Meyer, 1992).

All organisms, both plants and animals require phosphorus for synthesizing phospholipids, NADPH, ATP, nucleic acids, and other compounds. Polyphosphates in water are transformed into orthophosphate and available for plant uptake (Manson *et al.,* 2000). Plants dissolve ionised forms of phosphate for animals to feed on. Herbivores obtain phosphorus by eating plants, and carnivores by eating herbivores. Eventually both these organisms will excrete phosphorus as waste in the form of urine and faeces. Decomposition of waste will release phosphorus into the soil, which will eventually be absorbed by plants and then recycled within the ecosystem (Corbin, 2001).

The organic phosphate is the phosphate that is bound or tied up in the plant tissue, waste solids, or other organic material (Manson *et al.,* 2000). Organic P forms include relatively labile phospholipids, inositols and fulvic acids, while more resistant forms are comprised of humic acids (Sharpley, 1995). Between 25% and 90% of the total P in the soil is bound in the organic matter. This organic P is not plant-available, but it can be an important storehouse of P as it can be released by the process of mineralisation, i.e. when organic matter is decomposed by microorganisms to form orthophosphates (Manson *et al.,* 2000).

1.1.3 Importance of Phosphorus

There are 17 nutrients that are essential for plant growth and production. Insufficient supplies of one or more have an adverse effect on plant growth, maturity and yield (Sawyer and Mallarino, 2000). Phosphorus is one of the key elements necessary for growth of both plants and animals. Phosphorus is second only to calcium, in volume, in the human body. Like

calcium, it is found mainly in bones and teeth, with the remainder in body tissues and fluids. Phosphorus has a major role in the structure of nucleic acids (DNA; RNA) and in molecules (ATP; ADP) involved in the storage and use of energy in cells and a key stage in the Kreb's Cycle (Addiscott *et al.*, 1991). RNA and DNA are the backbones of life via genetics. Thus the availability of phosphorus is a key factor in controlling photosynthesis in plants (Lory, 1999). The average daily phosphorus requirement for adults human being is 0.8 to 1.5 grams per day (NIWR, 1985).

Phosphorus is an essential plant nutrient for terrestrial and aquatic plants (Voss and Griffith, 1999). In aquatic environment, it tends to be the growth-limiting nutrient. The presence of phosphorus is often scarce in the well-oxygenated lake waters and importantly the low levels of phosphorus limit the production of freshwater systems (van Loosdrecht, 2001; Wilkes, 2001).

Polyphosphates are used in some public water supplies as a means of controlling corrosion and residual aluminium in treated water, treating boiler waters and in most heavy-duty synthetic detergent formulations designed for the household and industrial markets as laundering and commercial cleaning fluids. Many of these detergents contain over 50% by weight of polyphosphates. They are also used in softened waters for stabilisation of calcium carbonate to eliminate the need for recarbonation (Sawyer *et al.*, 1994; Srinivasan *et al.*, 1999; Walmsley, 2000).

Phosphate rock is commercially available in the form called apatite and the phosphate is also present in fossilised bone or bird droppings called guano (Wilkes, 2001). Apatite is defined as a natural, variously coloured calcium fluoride phosphate ($Ca_5F(PO_4)_3$) with chlorine, hydroxyl, and carbonate sometimes replacing the fluoride. Apatite is found in igneous and metamorphic rocks, and sedimentary rocks (NCSU, 1998). Huge quantities of sulphuric acid

are used in the conversion of the phosphate rock into a fertiliser product called super-phosphate, which is applied to agricultural and residential lands as fertilisers (Wilkes, 2001).

1.2 SOURCES OF PHOSPHORUS

Various elements, including carbon, oxygen, hydrogen, sulphur, potassium, nitrogen and phosphorus are required for normal growth and reproduction in plants. Nitrogen and phosphorus are the most common limiting nutrients in that they are implicated in excessive plant growth resulting from nutrient enrichment (Davies and Day, 1998). Nutrients are exported to the receiving water body, in which concentrations change according to the nutrient loads it receives and in response to the biological and chemical processes taking place in it (Weddepohl and Meyer, 1992).

Population growth, increased economic activity and changes in land use all lead to new trends in water demand and use (Schreiner, 1999). Human activities make use of numerous products and resources (containing P compounds), which are ultimately converted into available P that is released into the environment through various pathways (Lilley *et al.*, 1997; Walmsley, 2000). P is not a conservative element and it undoubtedly incurs several changes once it enters an aquatic environment. On entering an aquatic system, phosphorus is dissolved in water environment or adsorbed onto soil and other particles. It can be taken up physically or assimilated biologically (Bath, 1989). There are two main ways (figure 1.2) in which P loads are introduced to the aquatic environment, namely point and non-point sources (Dallas and Day, 1993; Hohls *et al.*, 1998; Atkinson, 1999; MPCA, 2001).

1.2.1 Point Sources

Point sources are those inputs that are considered to have a well-defined point of discharge, which under most circumstances, is usually continuous. Point sources are easier to describe,

quantify and control since that they originate from a fixed point (Hohls *et al.*, 1998). Domestic sewage and industrial effluents are the major point source contributors of the P load on the water environment in South Africa. The major sources of P in domestic wastewater are human excreta and animals waste, which contributes 50 – 65% of P load. A normal adult excretes about 1.3 – 1.5 g of phosphorus per day (Wiechers and Heynike, 1986; NCSU, 1998).

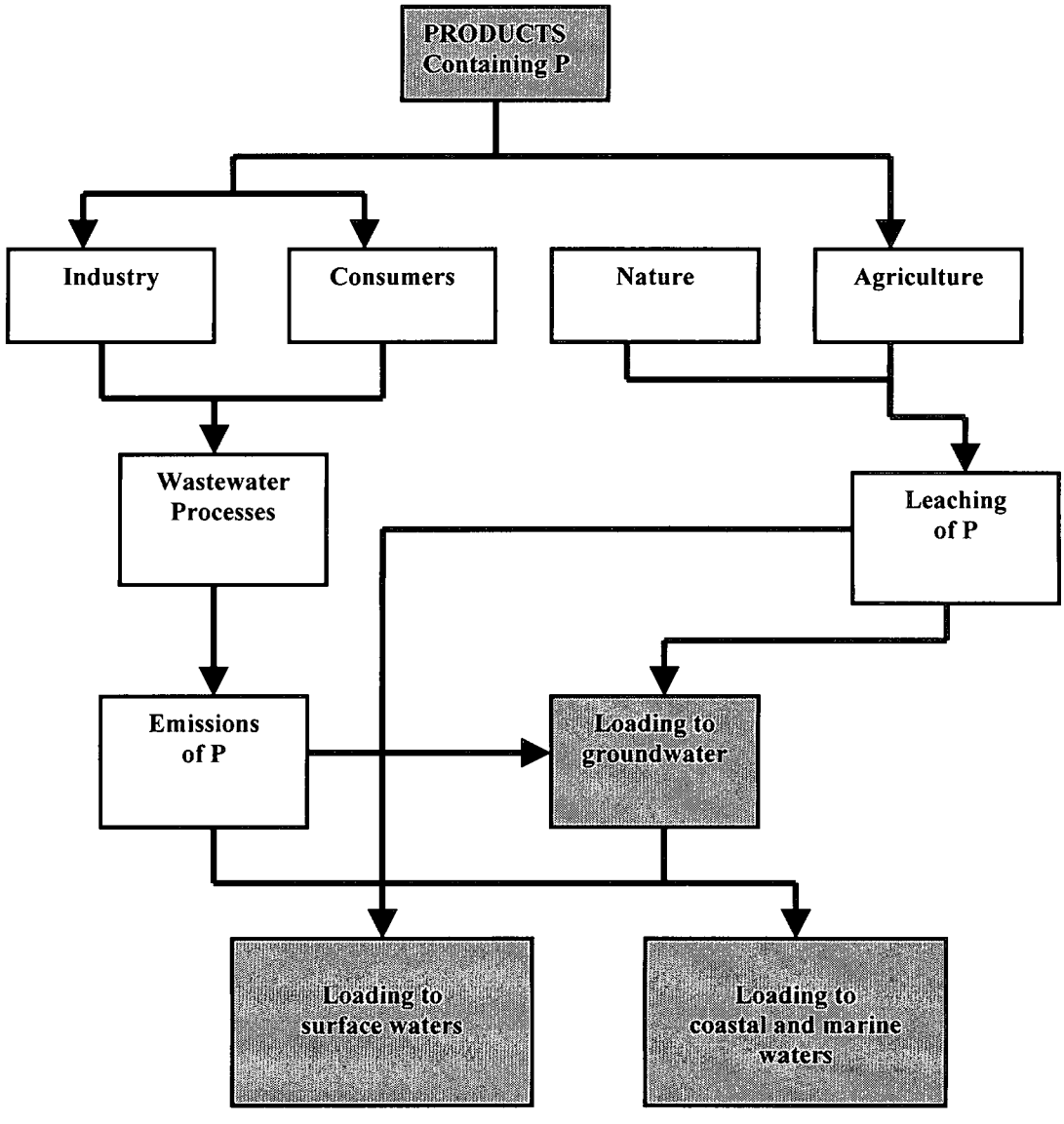


Figure 1.2: Schematic representation of the flow of P from various sources into the aquatic environment (adapted from Wiechers and Heynike, 1986)

About 35 - 50% of the point source P load originates from the use of industrial products, such as fertilisers, toothpaste, synthetic detergents, pharmaceuticals and food-treating compounds (Wiechers and Heynike, 1986; Wiechers, 1987). The effluent that is discharged from edible oil industries contains high concentrations of phosphorus in aqueous form (Steffen, *et al*, 1989; Hui, 1996).

Raw sewage contains a number of different P forms, *inter alia* organically bound and inorganic P. The P containing compounds may be in a particulate, colloidal or dissolved form. The concentrations and loads of these various P forms entering municipal sewage works may vary diurnally, daily and seasonally. Typically orthophosphate comprises 50% or more of the total P in raw sewage (Wiechers and Heynike, 1986). Conventional primary and secondary treatment systems do not reduce the P concentrations by much, but increase the soluble orthophosphate content from 50% to about 90% by transforming the organic and polyphosphates to orthophosphate (Weddepohl and Meyer, 1992). Primary treatment removes only about 10% of the P in the waste stream and secondary treatment removes about 30%. Orthophosphate form is the easiest to remove by tertiary treatment and is also most readily available form for assimilation by algae and aquatic plants. Tertiary treatment technologies include biological removal and chemical precipitation (Wiechers and Heynike, 1986; NCSU, 1998; AWWA, 2000).

1.2.2 Non-point Sources

Non point sources are those for which the origin of discharge is diffuse and are also referred to as diffuse sources (Hohls *et al.*, 1998). A diffuse source is a location with multiple nutrient sources that are spread over a much wider area and nutrients enter the aquatic environment through leaching and atmospheric deposition processes (e.g. fertiliser application, atmospheric precipitation, livestock waste, urban runoff, failing septic systems, contaminated groundwater and natural sources). For diffuse sources the nutrients are transported over the

landscape and come into contact with soils and vegetation before entering the aquatic environment (Weddepohl and Meyer, 1992; NCSU, 1998; Walmsley, 2000; Wilkes, 2001).

Surface drainage is the major non-point source of pollution in waterways (Bath, 1989). P loading from surface runoff is dependent on several factors which include P content of the soil, soil characteristics, topography, geology, vegetative cover, land use, manipulative practices, animal populations, pollution, precipitation and quantity and duration of runoff (Grobler and Silberbauer, 1985; Weddepohl and Meyer, 1992).

Non-point source pollution is typically significantly higher than the point sources of pollution Wilkes, 2001). P management and control is much more easily implemented at point sources than non-point sources (Little and Zander, 1996; Walmsley, 2000).

1.3 EUTROPHICATION

1.3.1 Definition and Understanding

Eutrophication is a traditional ecological term used to describe the process by which a water body becomes enriched with plant nutrients. During this process the water body accumulates organic matter (both living and decaying) and progressively changes its character from that of a deep- water body (lake) to that of a wetland and, ultimately to that of a terrestrial system (du Plessis *et al.*, 1990; Walmsley, 2000). This is a natural process in lake evolution, but may be accelerated by the activities of man. Under natural conditions the process takes place over tens of thousands of years. Over the last 100 years, human influences have greatly accelerated the rate of enrichment, thereby shortening the lifespan of water bodies (Hohls *et al.*, 1998; Walmsley, 2000).

The linkage between aquatic plant production, nutrients and human activities was first noted in the early part of the century. However, a clear scientific understanding was only developed in the 1960s. Limnological research conducted between 1960s and 1980s was directed at identifying and quantifying the key nutrients in the eutrophication process (Walmsley, 2000). There are two types of eutrophication (Walmsley, 2000):

- The natural process that is dependent on the geology and natural features of the catchment. It is not reversible and continues *ad infinitum*, albeit at a slow rate; and
- The human-induced process that is related to anthropogenic activities. This process is referred to as cultural eutrophication as it is associated with human activities. Cultural eutrophication accelerates the rate of ageing of a water body and it is reversible.

Classification of water impoundments can be described according to their trophic status. The trophic status refers to the state of nutrient enrichment of aquatic ecosystems. The trophic status of water impoundments can be classified as (Walmsley, 1980; Toerien *et al.*, 1978):

- Oligotrophic means the presence of low levels (5-10 μg P/L) of nutrients and no water quality problems;
- Mesotrophic means intermediate levels (10-30 μg P/L) of nutrients, with emerging signs of water quality problems;
- Eutrophic means high levels (30-100 μg P/L) of nutrients and an increased frequency of water quality problems; and
- Hypertrophic means excessive levels (>100 μg P/L) of nutrients, where plant production is governed by physical factors. Water quality problems are almost continuous.

1.3.2 International Perspective

Eutrophication has increased markedly throughout the world during the last four decades (Grobler, 1985; Bath, 1989). Most of the world's developed countries such as USA, Canada, European Union Countries, and Australia have regarded eutrophication as a priority water quality issue, since 1960s (as cited in Wiechers and Heynike, 1986; Toerien *et al.*, 1975). The eutrophication problem has also received attention in less industrialised countries of South America and Asia, but less so in Africa (Walmsley, 2000).

Worldwide, eutrophication management programmes have concentrated on reducing the total phosphorus load to water bodies (EPA, 1984). P load control has been demonstrated internationally as one of the most effective ways of dealing with man-made eutrophication, since phosphorus is generally considered the most manageable of the nutrients (Weddepohl and Meyer, 1992; Hohls *et al.*, 1998). This strategy has been applied successfully in countries like the USA, Europe, Scandinavia and Japan and is regarded to be the most desirable since it eliminates the cause of eutrophication (EWPCA, 1985). The rationale for this approach stems from the fact that, there are proven and cost effective technologies available for removal of phosphorus from wastewaters. In addition, a significant portion (35 - 50%) of the phosphorus in domestic wastewater, and the majority of the phosphorus in some industrial wastes, is contributed by controllable synthetic detergents (Pillay, 1994).

1.3.3 South African Perspective

The Department of Water Affairs and Forestry (DWAF) has long (about 30 years ago) recognised eutrophication as one of the major factors affecting the quality of South African water resources (DWAF, 1986). This was experienced in the more developed and industrialised areas of the country as it was greatly accelerated by human activities. South Africa has some of the most highly enriched surface waters in the world. Hartebeespoort, Rietvlei and Roodeplaat dams are ranked as some of the most eutrophied reservoirs in the world according to the eutrophication surveys that were conducted in the 1970s. The surveys further indicated that eutrophication of aquatic ecosystems is widespread in South Africa (Toerien *et al.*, 1975; NIWR, 1985).

The recent study that was conducted by DWAF provided evidence that eutrophication in South Africa is now as widespread as it was 25 years ago. The study also indicates that there have been some positive trends in the reduction of nutrient loads, and eutrophication status, of several reservoirs. At the same time, some reservoirs have shown little improvement, whilst others have shown deterioration (Walmsley, 2000).

South Africa has pursued a source directed approach to eutrophication management since the early 1970's (Weddepohl and Meyer, 1992; Walmsley, 2000). In 1977 Toerien reviewed eutrophication in South Africa and provided tentative guidelines for its control. After several years of research using data from selected South African reservoirs, Walmsley and Butty (1980) reported updated guidelines for the control of eutrophication in South Africa. On the basis of these reports DWAF decided to implement measures to control the causes of eutrophication and not the consequences (Weddepohl and Meyer, 1992). It was then in 1988 when DWAF introduced a receiving water quality objective for reservoirs in sensitive catchments (DWAF, 1997).

South Africa was regarded as one of the leading countries in the world in eutrophication management and research in the period between 1975 and 1985 (Moss, 1998). Since then, eutrophication management has been incapacitated as a result of an inability to transform policy into practice by government (Quibell *et al.*, 1997). However, DWAF is currently conducting situation-specific surveillance to manage the situation in South Africa (DWAF, 2000).

1.3.4 Environmental and Health Impacts

Gross eutrophication is marked when the inorganic soluble nitrogen and phosphorus in water reaches concentrations in excess of 0.3 mg N/L and 0.015 mg P/L respectively (Lilley *et al.*, 1997). Eutrophication can lead to a rapid numerical increase in fast-growing plant and animal species, which subsequently become pests and may affect water quality (Dallas and Day, 1993). Under certain conditions, P in water can act as a trigger for an outbreak of blue-green algae, also known as cyanobacteria (AWWA, 2000).

Blue-green algae (or cyanobacteria) are small single celled prokaryotic (having no nucleus or organelles) microorganisms, only a few microns long. When present in large groups or blooms, these algae appear as a blue-green discoloration in the water. This type of algae is mostly found in freshwaters with high levels of phosphates and warm, sunny, and calm conditions (Rae *et al.*, 1999; Wilkes, 2001). Some species of blue-green algae such as *Microcystis* can produce a range of toxins that are potentially harmful to both animals and human if consumed (Hohls *et al.*, 1998; Rae *et al.*, 1999). The most prevalent group is the hepatotoxic microcystin toxins and nodularin that have caused numerous animal deaths throughout the world, including South Africa. The first known case of livestock poisoning in South Africa dates back to the 1920s (DWAF, 2000). In humans, these toxins are suspected tumour promoters and have linked the death of 47 kidney dialysis patients in Brazil to microcystin toxin poisoning (Rae *et al.*, 1999).

Eutrophication of major water storage systems is regarded as one of the most serious threats to water quality for recreational water users (Grobler, 1985; Bath, 1989). Toxins produced by microcystin may be linked to health problems ranging from skin irritation to liver damage and gastroenteritis, depending on the type and duration of exposure for recreational water users. The livelihood of many fish, shellfish, and livestock has also been endangered through contact with this toxin (Wilkes, 2001). In 1998 a Toxic Algae Forum was established in South Africa to promote awareness, the causes and consequences of algal toxins (DWAF, 2000).

Phosphorus (as orthophosphate) has been widely implicated as the limiting nutrient in freshwater aquatic systems. That is, if all phosphorus is used, plant growth will cease, no matter how much nitrogen is available (Wilkes, 2001). Although P is necessary, excessive levels are detrimental to the aquatic ecosystem (Lory, 1999). When large numbers of algae die at the same time due to overstimulation of the aquatic environment, a large pool of nutrients will be released into the water body, resulting in extensive deoxygenation of lower layers of water bodies during stratification, which may cause disturbance in biological activity and water chemistry, due to the effects of hydrogen sulphide and elevated levels of heavy metals (Hohls *et al.*, 1998; Walmsley, 2000). This may lead to changes in ecological community structures and loss of biodiversity, and ultimately cause hypoxic or anoxic conditions, which may result in fish kills (Lilley *et al.*, 1997; Hohls *et al.*, 1998; Rae *et al.*, 1999; Wilkes, 2001).

Cyanobacterial blooms are common to many South African freshwater impoundments that supply bulk water to water works and may interfere with water treatment processes (Rae *et al.*, 1999; DEAT, 2000). This may lead to increased water purification costs due to filter clogging, increased chemical dosing, advanced water treatment processes to remove tastes and odours associated with some algal species such geosmin (trans-1,10-dimethyl-trans-9-decalol); MIB (2-methylisoborneol); ß-cyclocitral, IPMP (2-isopropylmethoxypyrazine) and IBMP (2-isobutylmethoxypyrazine) and high levels of control and operating expertise (Hohls

et al., 1998; Rae *et al.*, 1999). Phosphate itself at low concentrations does not have notable adverse health effects, but levels greater than 1.0 mg/L may interfere with water treatment processes (NCSU, 1998). Potentially carcinogenic trihalomethanes may be formed when water from eutrophied sources is chlorinated during water treatment process for domestic use (Hohls *et al.*, 1998).

Aesthetic degradation of water surfaces may occur as a result of algal scums and overabundant floating and rooted aquatic macrophyte growth. This may lead to adverse economic value of land (loss of property value) adjacent to such water bodies, because of the detrimental effect that the algae and aquatic macrophytes have on the aesthetic value of the water bodies. Concerns regarding the water and aesthetic quality of Roodeplaat Dam have led to a request to the Ombudsman in South Africa to investigate the situation (Silberbauer, 1982; Hohls *et al.*, 1998). Increased interference in recreational activities (boating, fishing, swimming) may occur (Walmsley, 2000).

Controlling P levels in inland and lakes has become a priority in water quality management (AWWA, 2000). A number of countries have introduced effluent phosphate standards or receiving water standards or guidelines, with the objective of controlling eutrophication (Wiechers, 1987). Countries have implemented regulations on phosphate emissions, which push for the development of technologies for phosphorus removal and recovery in wastewaters (Van Loosdrecht, 2001). The imposition of such laws and regulations has greatly stimulated research and development work for finding more cost effective methods for P removal processes (Wiechers, 1987). Research on phosphorus removal and determination has grown rapidly in recent years in environmental engineering and wastewater treatment practices. One of the major problems in wastewater treatment practice is the disposal of sludges containing phosphates from biological treatment processes (Sawyer *et al.*, 1994).

1.4 POLICY APPROACH AND LEGISLATION

1.4.1 Policy Approach

Management of natural resources and the environment has progressed rapidly in South Africa over the years. This progress has followed a global trend of increasing knowledge about the environment and the need to protect it (Harris *et al.*, 1997). There are numerous natural resources regulations in South Africa, which relate to the management of the environment and water resources (as cited in Gray, 1999).

Water quality management in South Africa started with the Union Health Act 36 of 1919, which gave the Chief Health Officer of the Public Health Department, the responsibility to control pollution by ensuring that "*the best known or the only or the most practicable methods*" for sewage disposal were used. The Act gave the Chief Health Officer the powers to prevent the disposal of effluent from sewage treatment plants into watercourses (van der Merwe and Grobler, 1990).

Water quality policy development has been an ongoing process, with notable milestones in the promulgation of the Water Act (Act 54 of 1956) in 1956. Subsequent to the Act in 1962, the General and Special Effluent Standards were introduced (Gray, 1999). Due to excessive phosphorus concentrations produced by municipal and industrial wastewaters, the Act was amended on the 1st August 1980 by the then Minister of Water Affairs, Forestry and Environmental Conservation when he announced the amendment of the standards for the discharge of industrial wastewater into a water resource in terms of Section 21(1)(a) of the Water Act. This led to the promulgation of the effluent standard of 1mg P/L as dissolved orthophosphate (Wiechers, 1987; Weddepohl and Meyer, 1992; Lilley *et al.*, 1997; Walmsley, 2000).

The Effluent Phosphate Standard was introduced in seven sensitive catchments throughout South Africa. The sensitive catchment areas included the following rivers or their tributaries (Wiechers, 1987; Lilley *et al.*, 1997; Walmsley, 2000):

- Vaal River upstream and inclusive of the Bloemhof Dam
- Pienaars and Crocodile Rivers upstream of their confluence
- Great Olifants River upstream and inclusive of the Loskop Dam
- Umgeni River upstream of the influence of tidal water
- Umlaas River upstream of its point of discharge into the sea
- Buffalo River upstream and inclusive of Bridle Drift Dam
- Berg River upstream of the influence of tidal water.

Local authorities were given five years to upgrade effluent treatment works in order to comply with the Standard. Local authorities were granted exemption from the Standard by DWAF for a further three years. The Standard was only implemented in 1988 (Hohls *et al.*, 1998). There has been a widespread non-compliance of the 1mg P/L standard by many wastewater treatment plants in the sensitive areas (van Niekerk, 2000). The extent of compliance is not well monitored or documented and there, have been few prosecutions for this non-compliance (Walmsley, 2000). However, many local authorities responsible for sensitive catchments in South Africa are legally obliged to discharge effluents containing low phosphorus concentrations, of less than 1.0 mg P/L (Pitman and Boyd, 1999).

Prior to 1990, DWAF controlled water quality and water pollution from point sources by requiring effluent to meet either uniform effluent standards (UES) or special effluent standards, which were set at technologically and economically feasible levels (van der Merwe and Grobler, 1990; Moore *et al.*, 1991). In 1994 the Minister of DWAF initiated a review programme of the South African water law. Through a detailed process of consultation and

negotiation, principles that would form the basis of the South African Water Act were defined in 1996 (Harris *et al.*, 1997). In 1998 the new National Water Act (Act 36 of 1998) was promulgated (Gray, 1999; Wepener *et al.*, 2000).

1.4.2 Current Legislation

South Africa has had the opportunity to completely reform its Constitution since the first democratic elections were held in 1994. This resulted in the enactment of the new Constitution of the Republic of South Africa (Act 108 of 1996), which created the right to the environment as a fundamental right (DEAT, 1999; Abrams, 2000). The Constitution is the supreme law of South Africa and it forms the cornerstone of the environmental law (Sampson, 2001). Section 24 of the Constitution states that *"the people of South Africa have a right to an environment that is not detrimental to human health"*. It also imposes a duty on the state to promulgate legislation and to implement policies to ensure that this right is upheld (DEAT, 1999).

There are currently in excess of one hundred laws in South Africa that are directly or indirectly relevant to the environment (Sampson, 2001). Since the enactment of the new Constitution there were many other laws that were promulgated such as the Water Services Act (WSA - Act 108 of 1997), the National Water Act (NWA – Act 36 of 1998) and the National Environmental Management Act (NEMA – Act 107 of 1998). Policies such as the National Waste Management Strategy and the Waste Discharge Charge System were developed as an integral part of the NWA and NEMA (DEAT, 1999; DWAF, 2000; Abrams, 2000).

There is a multitude of older legislation in South Africa, which either directly or indirectly manages pollution control, such as the Health Act 63 of 1977; Environmental Conservation Act 73 of 1989; Minerals Act 50 of 1991; Occupational Health and Safety Act 85 of 1993,

Local Government Transition Act 209 of 1993 and many others (DEAT, 1999; Osifo, 2000; Sampson, 2001).

1.4.2.1 National Water Act, 1998

This Act promotes sustainability and equity as the central guiding principles in the protection, use, development, conservation, management and control of the South African water resources (Steyl, 1999; DWAF, 2000). Several regulations have been promulgated in terms of the Act since its implementation in 1998 (Sampson, 2001). The Act makes provision for the Minister to make regulations limiting or restricting the purpose, manner or extent of water use (DWAF, 2000).

The NWA follows the precautionary principle in dealing with pollution management and in particular the situation where pollution of a water resource occurs or might occur as a result of activities on land. For example the Act requires that industrial water users treat their effluent before discharge into a water resource to prevent pollution (Walmsley, 2000; DWAF, 2000). Furthermore, it requires any person discharging wastewater into a water resource to register with DWAF prior to the commencement of discharge (Sampson, 2001).

Although no mention is made of nutrient enrichment in the Act, there are several features that merit the inclusion of eutrophication considerations. These include (Walmsley, 200):

- A National Classification System. The Act calls for the development of a system to classify the nation's water resources and thus requires guidelines and procedures for determining different classes of water resources. The NWA makes provision for the classification system to determine nutrient enrichment and trophic status of specified water body type (e.g. rivers, lakes, wetlands, reservoirs, estuaries and coastal waters).

- Establishment of Information Management Systems. The Act makes provision for DWAF to establish a national information systems on the quantity and quality of all water resources, i.e. including eutrophied water resources.

In terms of NWA any regulations made under the Water Act 54 of 1956 remain in force despite the repeal in 1999 and are considered to have been made under the NWA. The retention of this regulation is important as the national water standard (e.g. General Effluent Standard for Phosphate) were prescribed in terms of regulations promulgated under the Water Act of 1956 in 1984 for the treatment of wastewater or industrial effluent. These water quality standards remain in effect so far as they are not inconsistent with the NWA, and until such time as new water quality standards are prescribed (Sampson, 2001).

1.4.2.2 National Environmental Management Act, 1998

NEMA advocates that the principle of sustainable development is paramount and that all development must be socially, environmentally and economically sustainable (Walmsley, 2000). As part of sustainable development the Act imposes a duty on everyone who causes, has caused or may cause significant pollution or degradation of the environment to take reasonable measures to prevent it from occurring, continuing or recurring (Section 28(1)). Although everyone has a duty in terms of section 28(1), the Act singles out an owner of land or a person in control or a person who has the right to use land or premises on which any activity or process is or was performed or undertaken, or any other situation exists, which causes, has caused or is likely to cause significant pollution or degradation of the environment, to take reasonable measures (Section 28(2)).

Various environmental principles such as "the polluter pays", "cradle-to-grave" and "waste prevention and minimisation" have been given legal effect in this Act (Sampson, 2001). NEMA also makes provision for all spheres of government (central, provincial and local) to

participate co-operatively in the governance of the environment (Walmsley, 2000). Furthermore the Act sets out various instances in which information must be made available to government authorities. This entitles the state to have access to information relating to the state of environment and the actual or future threats to the environment, including any emissions to water, and the production, handling, transportation, treatment and disposal of waste (Sampson, 2001).

1.4.2.3 Water Services Act, 1997

Provision is made in this Act for the Minister of DWAF to prescribe compulsory national standards from time to time relating to the quality of water discharged into any water resources system. This stems from major economic, political, social and demographic changes taking place in South Africa, which have a significant impact on water quality. A steady decline in water quality is being experienced in South Africa. Future scenarios indicate a continuing trend due to the greater demands on the water resources to satisfy the needs of industrialisation, urbanisation, agriculture, recreation and nature conservation (WRC, 1996b; DWAF, 1997). Water quality criteria, standards and the related legislation are used as the main administrative means to manage water quality in order to achieve user requirements (Chapman, 1992).

The Act also makes provision for local authorities to enforce stringent regulations in the form of bylaws in improving the quality of wastewater that is discharged into sewerage systems by industries (Pitman and Boyd, 1999). The discharge of poor quality effluent by edible oil industries is posing a threat to water sources and wastewater treatment installations (Atkinson, 1999; Adam and Marjanovic, 1996).

1.5 THE SOUTH AFRICAN EDIBLE OIL INDUSTRY

The South African edible oil industry is dominated by a few very large diversified oil producers (ITC, 1997). There are 16 edible oil plants in South Africa, run by 10 separate groups. The most commonly grown oil bearing crops in South Africa are sunflower, groundnut and maize, but other seeds such as cotton and soyabean are also processed (Steffen, *et al*, 1989). The market for edible soya products has continued to increase over the last few years and local producers of edible soya products are unable to meet total requirements due to limited crop sizes (ITC, 1997). South Africa is the world's tenth-largest producer of sunflower seed, with an annual harvest of between 186 000 and 780 000 tons (GCIS, 2000).

The edible oil industry in South Africa utilises only about 65% of its total capacity. The amount of oil that is produced in the country depends on the climatic conditions (Steffen, *et al*, 1989). South Africa is a dry country with only 8.6% of the rainfall available as surface water. The country has a very low average rainfall of 475 mm a^{-1} compared to the rest of the world, estimated just over half of the world average of 860 mm a^{-1} (Davies and Day, 1998). Droughts are a constant threat to the country's economic growth and social development (Bosch and Cloete, 1993; Steyl, 1999). Good rains lead to good maize, groundnut and sunflower crops, which will result in good oil seed production (Steffen, *et al*, 1989).

The total quantity of oil refined in South Africa has remained relatively constant over the years, at approximately 250 000 t/a (Steffen, *et al*, 1989). 253 315 and 245 222 t/a were produced in 1998 and 1999 respectively (CSS, 2000). Shortages of edible oil are supplemented in the form of unrefined oil imports, which are refined and packaged by local oil producers. Crude sunflower oil accounts for the bulk of imports. During 1995, imports of edible oils and fats totalled R1 billion and exports totalled R188 billion (ITC, 1997).

1.5.1 Water Intake

The edible oil industry as a whole uses vast quantities of water and produces a wide range of pollutants. The edible oil industry consumes about 1.75 $x10^6$ m^3 a^{-1} of water, which is about 0.11% of the total industrial water use (Steffen, *et al*, 1989; DWAF, 2000). The water is used as a solvent, a coolant, a dust-settler, a cleanser and even as a means of transport. A breakdown of water usage in a typical edible oil plant is shown in table 1.1. Much of this water is put to drain as wastewater. This wastewater or industrial effluent is loaded with pollutants of different kinds, depending on the production process type used (Sutton, 1994).

Table 1.1: Breakdown of water usage of a typical edible oil processing plant in South Africa.

	Mill (%)	Refinery (including hydrogenation) %	Total (%)
Boilers	10	30	40
Cooling	25	10	35
Domestic	1	1	2
Process	2	13	15
Washdown	1	7	8
TOTAL	39	61	100

1.5.2 Oil Processing

The vegetable oil processing industry involves the extraction and processing of oils and fats from vegetable sources. Vegetable oils and fats are principally used for human consumption but are also used in animal feed, for medical purposes, and for certain technical applications (Khan and Akhtar, 1998; WBG, 1998; ISEO, 1999). Vegetable oil may be produced from a wide variety of seeds, including cotton seed, soyabean, peanuts, sunflower seed, maize, rice bran, palm kernels, linseed, olives and coconut (Steffen *et al.*, 1989; WBG, 1998; ISEO, 1999).

Fats and oils obtained from the extraction of the oilseeds are termed "crude" fats and oils. Crude fats and oils contain varying but relatively small amounts of naturally occurring non-glyceride materials that are removed through a series of processing steps. For, example, crude soybean oil may contain small amounts of protein, free fatty acids and phosphatides which must be removed through subsequent processing to produce the desired oil products (ISEO, 1999). Crude oil refining includes degumming, neutralisation, bleaching, deodorisation, and further refining (Ozturk *et al.*, 1990; Eroglu *et al.*, 1990; WBG, 1998).

1.5.2.1 Degumming

Crude oils having relatively high levels of phosphatides such as soybean, corn and sunflower oil may be degummed prior to refining to remove the majority of phospholipid compounds (Steffen *et al.*, 1989; ISEO, 1999). The process generally involves treating the crude oil with a limited amount of water to hydrate the phosphatides and make them separable by centrifugation (ISEO, 1999). 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 (Steffen *et al.*, 1989). Soybean oil is the most common oil to be degummed; the phospholipids are often recovered and further processed to yield a variety of lecithin products (ISEO, 1999).

1.5.2.2 Refining

The principal product of an edible oil refinery is liquid oil which may be produced as cooking or salad oil or may be further processed to increase the market value of the product as margarine, cooking and bakers'fat, peanut butter and mayonnaise (Steffen *et al.*, 1989). There are basically two types of edible oil refining processes, i.e. physical and chemical refining.

(A) Chemical refining

Chemical refining process is sometimes referred to as alkali refining. It is a classic method for refining crude or degummed oil, generally performed on vegetable oils to reduce the free fatty acid content and to remove other gross impurities such as phosphatides, proteinaceous and mucilaginous substances (ISEO, 1999). Chemical refining consists of five inter-related processes as shown in figure 1.3, viz. neutralisation, bleaching, winterisation, hydrogenation and deodorisation (Mkhize, 2001).

(i) Neutralisation

Crude oil may sometimes contain extremely high quantities (as high as 40 %) of free fatty acids but usually range between 0.5 % and 8 %, depending on the oil type (Steffen *et al.*, 1989). By far the most important and widespread method of neutralising oil is with the alkali solution by the addition of caustic soda (ISEO, 1999). Caustic soda solution of up to 4N in concentration is usually used to neutralise oils at a predetermined optimum temperature in either batch, semi-continuous or processes for edible oil processing plant continuous systems (Steffen *et al.*, 1989). This results in a large reduction of free fatty acids through their conversion into water-soluble soaps. Most phosphatides and mucilaginous substances are soluble in the oil only in an anhydrous form and upon hydration with caustic soda solutions (ISEO, 1999). The soap or soapstock that is produced upon neutralisation is separated from the neutralised oil using centrifuges or gravitational settling (Hui, 1996). After alkali refining, the oil is water-washed to remove residual soap (ISEO, 1999). The soap or soapstock that is produced upon neutralisation is separated from the neutralised oil using centrifuges or gravitational settling (Hui, 1996; ISEO, 1999). Phosphoric or nitric 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 the aqueous phase (Steffen *et al.*, 1989; Hui, 1996). Neutralised oil is then subjected to further refining steps, as shown in figure 1.3.

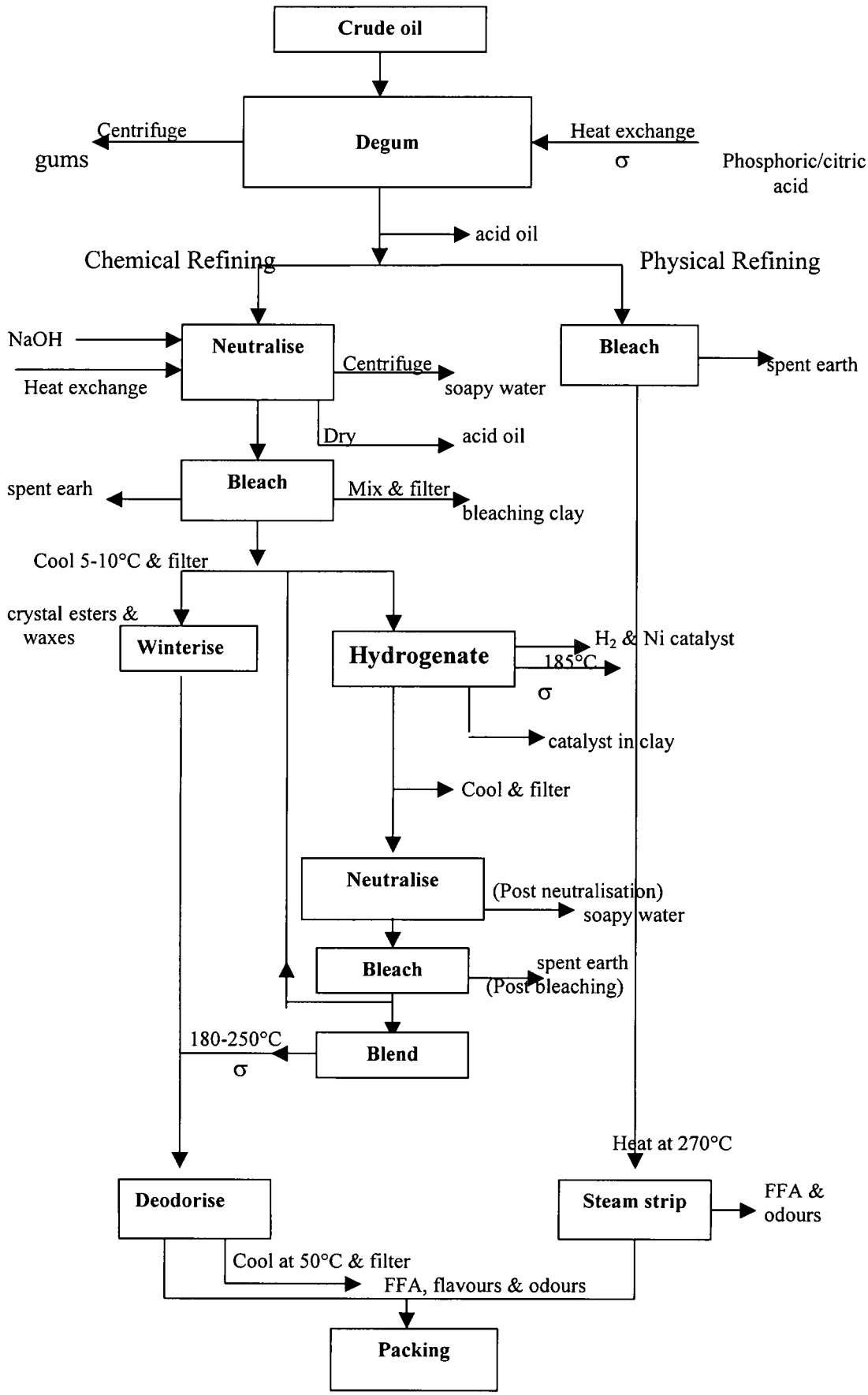

Crude oil
Degum
Centrifuge
gums
Heat exchange
σ
Phosphoric/citric acid
acid oil
Chemical Refining
Physical Refining
NaOH
Heat exchange
Neutralise
Centrifuge
soapy water
Bleach
spent earth
Dry
acid oil
spent earh
Bleach
Mix & filter
bleaching clay
Cool 5-10°C & filter
crystal esters & waxes
Winterise
Hydrogenate
H2 & Ni catalyst
185°C
σ
catalyst in clay
Cool & filter
Neutralise
(Post neutralisation)
soapy water
Bleach
spent earth
(Post bleaching)
180-250°C
σ
Blend
Heat at 270°C
Deodorise
Steam strip
FFA & odours
Cool at 50°C & filter
FFA, flavours & odours
Packing

Figure 1.3: Schematic representation of physical and chemical refining (Steffen *et al.*, 1989)

(ii) Bleaching

Bleaching refers to the process for removing colour producing substances and acts as a further purification step in the refining process. This step is conducted under vacuum at raised temperatures and may be performed in batch, semi-continuous or continuous systems like in the neutralisation process (Steffen *et al.*, 1989).

The usual method of bleaching is by adsorption of the colour producing substances on an adsorbent material. Acid-activated bleaching earth or clay, sometimes called bentonite, is the adsorbent material that has been used most extensively. This substance consists primarily of hydrated aluminium silicate. Anhydrous silica gel and activated carbon are also used as bleaching adsorbents to a limited extent (ISEO, 1999).

(iii) Hydrogenation

Oil, which is intended for use in margarine and other similar products must be hydrogenated, this produces fats with superior keeping qualities as well as higher melting points (Steffen *et al.*, 1989). In the process of hydrogenation, hydrogen gas is reacted with oil at elevated temperature (~ 185°C) and pressure in the presence of a catalyst. The catalyst most widely used is nickel supported on an inert carrier (diatomaceous earth), which is removed from the fat after the hydrogenation processing is completed (ISEO, 1999).

Hydrogenation is an exothermic reaction that is mostly carried out in batch systems (Steffen *et al.*, 1989). The process is easily controlled and can be stopped at any desired point, i.e. the hydrogenation conditions can be varied by the manufacturer to meet certain physical and chemical characteristics desired in the finished product. This is achieved through selection of the proper temperature, pressure, time, catalyst and starting oil (ISEO, 1999).

(iv) Winterising

Winterisation is a process whereby undesirable wax material is crystallised and removed from the oil by filtration to avoid clouding of the liquid fraction at cooler temperatures. The term winterisation was originally applied decades ago when cottonseed oil was subjected to winter temperatures to accomplish this process (ISEO, 1999). Winterisation processes using temperature to control crystallisation are performed on certain vegetable oils, such as sunflower, maize, corn and canola (Steffen *et al.*, 1989; Hui, 1996).

A process similar to the winterisation process, called dewaxing is sometimes utilised to clarify oils containing trace amounts of clouding constituents (ISEO, 1999). The winterisation process is usually performed on oil that is to be marketed as such without further processing. Winterisation process is not necessary for oil, which is to be hydrogenated. Oil is usually deodorised after winterisation (Steffen *et al.*, 1989).

(v) Deodorising

Deodorising is the last processing step applied in the production of edible oil (Hui, 1996). It is normally accomplished after refining and bleaching processes (Steffen *et al.*, 1989; ISEO, 1999). The process is defined as a vacuum steam distillation process with the purpose of removing trace constituents that give rise to undesirable flavours, colours and odours (Steffen *et al.*, 1989, Hui, 1996; ISEO, 1999).

Deodorisation of oil is simply a removal of residual free fatty acids, aldehydes and ketones that are responsible for undesirable odours and flavours in the final refined oil using steam (Steffen *et al.*, 1989). This process is carried out under vacuum to facilitate the removal of volatile substances, to avoid undue hydrolysis of the oil, and to make the most efficient use of

the steam (ISEO, 1999). After deodorising process has been completed, the refined oil is cooled and then stored for packaging (Steffen *et al.*, 1989).

(B) Physical refining

Physical refining is an alternative to alkali refining, in which free fatty acids (FFA) in the crude or degummed oils are removed by steam stripping rather than by neutralisation and subsequently removed as soap stock as in the alkali refining process (Steffen *et al.*, 1989). Oils low in phosphatide content (palm and coconut) may be physically refined (i.e. steam stripped) to remove FFA (ISEO, 1999).

Physical refining process has a very important requirement, i.e. the feedstock or crude oil should be rigorously pre-treated to ensure that it is free from phosphatides, trace metals and earth removable pigment (Steffen *et al.*, 1989). The extent of pre-treatment necessary depends on the particular oil type and its quality (Hui, 1996). Pre-treatment of high FFA oils such as sunflower and maize prior to physical refining, typically comprises the addition of phosphoric or citric acid at temperatures of approximately 70°C followed by high speed centrifugation to remove the hydrated gums (WBG, 1998). The centrifuged oil is then dried, bleached and winterised before physical refining (Steffen *et al.*, 1989).

1.5.3 Effluent Quality

A typical, oil processing plant discharges approximately 35% of the incoming water to sewer, which varies considerably by quality over a 24-h period. The largest volume and load of the effluent discharged by an edible oil plant arises from the refining operations. The type of method applied for oil processing has a strong bearing on the quantity and quality of effluent produced. Typically about 80% of the effluent volume is attributed to the refinery (Steffen, *et al*, 1989).

Pollution of water by the edible oil industry occurs on every level of production, as shown in figure 1.3 (Sutton, 1994). The main types of pollutants in edible oil plant effluents are fats, oil and grease (FOG), sodium, sulphates and phosphates (Steffen *et al.*, 1989; Adam and Marjanovic, 1996; Hwu, 1998; Khan and Akhtar, 1998; WBG, 1998). Gums (phosphatides, sugars, resins and proteinaceous materials) produced during the degumming process may be separated into hydrated and non-hydrated types (Hui, 1996). Pre-treatment of high FFA oils such as maize and sunflower prior to physical refining, comprises of the addition of phosphoric or citric acid. This is done during the degumming process to remove non-hydrated gums (Steffen *et al.*, 1989).

The first three stages of refining is carried out in the same reactor as a batch process that produce a soapstock from which fatty acids are recovered by means of acids splitting. Acids splitting process is carried out by addition of sulphuric acid to the soapstock, which causes the FFA to be separated from the medium. The resulting effluent is highly acidic and contains considerable quantities of fats and oils and very high levels of total suspended solids (TSS), chemical oxygen demand (COD), sodium and sulphate (Steffen *et al.*, 1989; Eroglu *et al.*, 1990; Ozturk *et al.*, 1990; Abou-Elela and Zher, 1998). During neutralisation stages, citric or phosphoric acids 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 the aqueous phase. If phosphoric acid is used, the effluent produced will contain high concentrations of phosphorus in aqueous form (Hui, 1996).

1.6 TREATMENT METHODS

Due to the water shortage problems in the foreseeable future in South Africa, more research is conducted on possible new treatment methods for industrial wastewater treatment (Sutton, 1994). Wastewater treatment has been a challenge throughout the years due to varying

influent chemical and physical characteristics and stringent effluent regulations (Abreu and Estrada, 1998).

Edible oil effluents can be treated by either chemical or biological means, or by combination of both methods (Seng, 1980). The problems with chemical treatment are the increased chemical handling costs and the production of chemical sludge, which often results in sludge with poor settling and dewatering characteristics. It is also difficult to treat and dispose the sludge produced from chemical precipitation. Precipitation with metal salts can depress the pH. Biological treatment methods offer an easy and cost effective alternative to chemical methods in treating edible oil effluents (Seng, 1980; Novotny, 1998).

Effective treatment of refinery effluent from the edible oil industry may be achieved by a combination of treatment methods, such as screening, acid splitting of oil emulsions, skimming of fats and oils and neutralisation (Steffen *et al.*, 1989). The removal of emulsified oils and other biodegradable organics present in the refinery effluent can be accomplished by screening, dissolved air floatation (DAF) and biological treatment methods (Seng, 1980; Khan and Akhtar, 1998; WBG, 1998). More stringent environmental standards have promoted the development of new intensive biological processes for treating edible oil effluents (Bekir, 2001).

The use of phosphoric acid results in effluent that contains high concentrations of phosphorus in aqueous form (Hui, 1996). The removal of phosphate from wastewater may be achieved by chemical or biological means (Momberg, 1991; Bond *et al.*, 1999). Sometimes a chemical method is used in conjunction with a biological method (Lilley *et al.*, 1997).

1.6.1 Chemical Phosphorus Removal Method

Chemical methods involve precipitation by the addition of metal oxides (e.g. calcium, magnesium or sodium aluminate), or by addition of chlorides or sulphates of metal salts (e.g. iron or aluminium). These chemicals can be applied at various stages of the treatment process, viz. during the primary sedimentation phase, within the secondary treatment system or after the secondary sedimentation (Momberg, 1991; Lilley *et al.*, 1997). The chemistry of phosphate removal by precipitation with metallic ions is complex (Wierchers, 1987).

The disadvantages of the chemical methods have been well documented in South Africa (van Dijk and Braakensiek, 1985). This may include pH control, personnel, equipment, chemical costs and finished water quality. Metal oxides are strongly alkaline resulting in an increase in pH, which must be controlled so as not to interfere with the ensuing stages of the treatment process. Metallic salts are strongly acidic and may result in similar problems. Efficient control of chemical dosage or continuous operator attention is therefore a prerequisite for success. Efficient phosphate removal may require additional flocculation for which equipment such as clarifiers are needed to dispose voluminous sludge produced. Ions derived from the metal oxides or salts may not be utilised in the reaction and remain in solution as contaminant thereby increasing the water conductivity levels and reducing the value of the treated water for subsequent use (Momberg and Oellermann, 1993).

1.6.2 Biological Phosphorus Removal Method

Worldwide, biological phosphorus removal has been studied for several decades and this has led to the proposal of many different processes (Kuba *et at,* 1993; Bond *et al.*, 1999). One such system is the activated sludge process, used mainly for the treatment of both municipal and industrial effluents (Barnard, 1974, 1976; Davelaar *et al.*, 1978; Gray, 1989; Toerien *et al.*, 1990). Much attention has been devoted to understand the metabolism and operational

parameters involved, but certain aspects are still unclear (Serafim *et al.*, 2000). Treatment systems using activated sludge have been able to handle many of these difficulties (Abreu and Estrada, 1998). There are however, numerous problems associated with the operation of any activated sludge treatment plant and these vary from site to site and process to process (Watts, 1996).

With the introduction of legislation in 1980 limiting P concentrations in effluents discharged from municipal wastewater treatment plants, intensive efforts were directed by the South African research community towards biological excess phosphorus removal (BEPR) in activated sludge system. Millions of rands have been invested in the development of activated sludge plants for carbon, nitrogen and phosphorus removal from wastewater (Bosch and Cloete, 1993). Considerable new information on the nutrient (N & P) removal in activated sludge system has become available since 1984, particularly on denitrification and BEPR (Wentzel *et al.*, 1997).

Biological phosphorus removal (BPR) process is based on the cycling of activated sludge through anaerobic and aerobic zones, resulting in accumulation of phosphorus-accumulating organisms (PAO). PAOs are able to accumulate phosphorus in excess of requirements for cell synthesis (Ydstebø *et al.*, 2000). PAOs normally are present in activated sludge systems but cannot achieve phosphorus removal in the absence of readily biodegradable chemical oxygen demand (RBCOD) and anaerobic-aerobic cycling (Jeyanayagam *et al.*, 2000).

PAOs take up volatile fatty acids (VFAs) under anaerobic conditions and store them as polyhydroxybutyrate (PHB). Energy for uptake of VFAs is provided through hydrolysis of stored polyphosphate, which results in release of orthophosphate. In the following aerobic zone, the PHB is oxidised, and the polyphosphate pool is recovered through uptake of orthophosphate (see figure 1.4). The net result is a reduction of phosphorus in wastewater,

and withdrawal of phosphorus rich-sludge completes the phosphorus removal process (Ydstebø *et al.*, 2000).

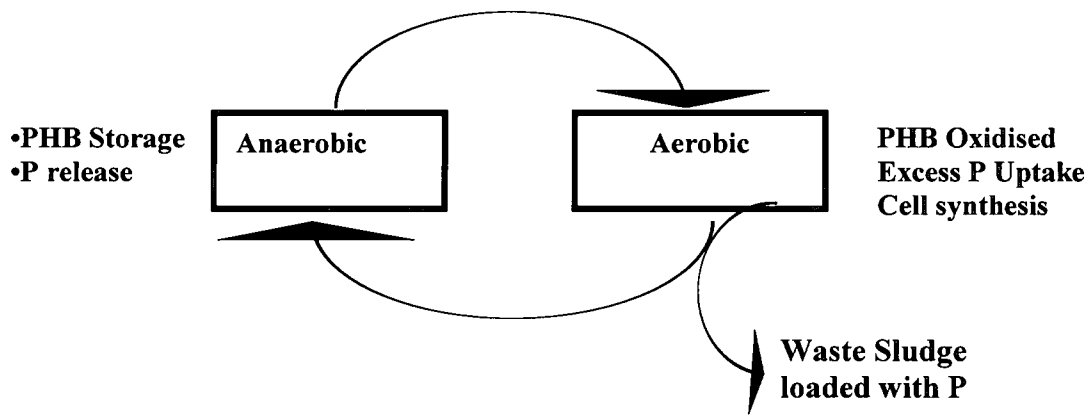


Figure 1.4: Schematic representation of EBPR process (Jeyanayagam *et al.*, 2000)

Biological methods have been used successfully at municipal and industrial levels to remove bio-P from wastewater (Garzón-Zúñinga and González-Martínez, 1996). BPR operations are increasingly gaining support over chemical precipitation methods (Wentzel, 1992; Henze, 1996; Lilley *et al.*, 1997). BEPR process is one of the most economical options available for the removal of phosphorus from wastewater (Satoh *et al.*, 1994). BEPR processes typically involve higher capital investment than chemical phosphorus precipitation, however, BEPR processes have the potential to offer lower operation and maintenance costs than conventional processes with chemical dosing (de Haas *et al.*, 2000).

Technical and economic presentations were made on chemical and biological phosphorus removal by Ir. P. de Jong in 1999 and he considered the operating costs of biological phosphorus removal to be somewhat lower and indicated that approximately 40% of the operators in Holland prefer biological systems than chemical systems. Dr. Ir. Klapwijk, 1999 considered biological phosphorus removal to be more sustainable than chemical treatment (Bommele, 1999). A number of biological phosphorus removal processes using the sequencing batch reactors (SBRs) principle have been developed in recent years (Keller *et al.*, 2000).

1.7 SEQUENCING BATCH REACTOR (SBR)

Sequencing batch reactors have been around for a long time. They were in existence and working well, long before continuous flow activated sludge system were designed (Walden, 1997). The operation of an activated sludge process using a "batch process" was identified around the turn of the century when activated sludge treatment was first discovered (Shamskhorzani and Norcross, 2000). By the early 1920s, however, virtually all of these plants were converted to continuous flow operation even though the volume needed for continuous flow systems was about twice that needed for the corresponding fill-and-draw system (Wilderer *et al.*, 1997). However, SBRs were forgotten for over half a century and were only revived in the early 1970s by the work of Irvine and Davis (Sanchez *et al.*, 1990).

SBR technology is an inherently non-steady state, activated sludge process (de Silva and Rittmann, 2000). An SBR is a fill-and-draw system that operates in a true batch mode with aeration and sludge settlement both occurring in the same tank, i.e. SBRs operate by a cycle of periods consisting of fill, react, settle, decant and idle (Abreu and Estrada, 1998). The operation of sequencing batch reactor (SBR) systems is described in figure 1.5. SBR systems are hybrid systems with some characteristics of continuous flow PF and CM systems but they have other characteristics that are truly unique. During the fill phase the reactor contents are mixed with the continuously incoming wastewater. Aeration may be delayed or not be applied at all during the fill period to improve sludge settleability by favouring the growth of microorganisms that form flocs. During the react phase, although the reactor contents are mixed, the whole reactor contents remain in the tank for the specified duration of this phase. This is an ideal PF condition for this phase. At the end of the react phase, reaction and mixing are terminated. Ideal quiescent settling, not subject to flow currents or other irregularities, occurs.

SBRs bare no similarities to the extended aeration process, as is often assumed (Norcross, 1992). SBR system differs from conventional activated sludge processes in that it performs all unit processes in one basin (Abreu and Estrada, 1998). A typical SBR plant produces about 25% reduction in capital cost and about 10% reduction in running cost over conventional activated sludge process (West, 1998). The many advantages offered by the SBR system justify the recent increase in the implementation of this technology in industrial and municipal wastewater treatment (Abreu and Estrada, 1998).

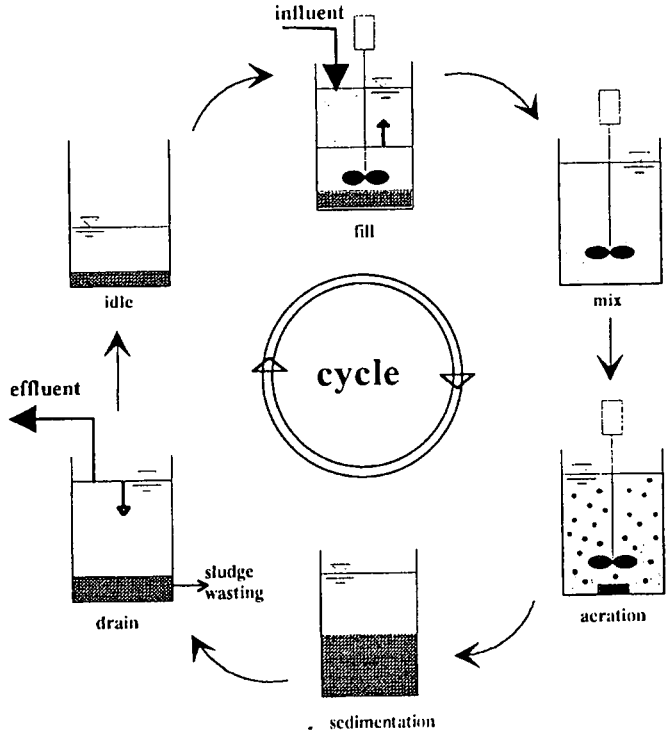


Figure 1.5: Schematic representation of SBR operation (Irvine *et al.*, 1997)

SBRs are versatile systems that can be used for a wide range of different wastewater types and for different treatment purposes (Cuevas-Rodrígues *et al.*, 1998). Because SBR technology is sequential, it is possible to tailor the treatment sequence to meet special requirements. The phases can be modified at any point in time to achieve the highest quality effluent (Walden, 1997). The primary application areas of SBR systems are in small communities that require treatment facilities which have low capital and operating costs; in

industries and municipalities that have unique applications, such as the removal of priority pollutants, nitrogen, phosphorus, and specific components in pre-treatment, and finally in research (Irvine and Busch, 1979).

1.8 RESEARCH OBJECTIVES

In order to limit phosphate discharge to the water environment, technology for phosphate removal at municipal and industrial wastewater treatment works is to be made available (Wiechers, 1987). A great need for technologies and strategies to reduce the discharge of phosphorus to the water environment has been established. It is, therefore, necessary for South Africa to use more advanced water treatment processes to produce water of an acceptable quality (WRC, 1996b).

Research on industrial water use and effluents in South Africa is oriented towards developing and promoting integrated water conservation and effluent management strategies through the application of sustainable water pollution prevention practices, and cost effective water abatement technologies. The implementation of these strategies is expected to result in significant savings in freshwater intake, reduced pollution load discharge to the environment per unit of production, minimisation and elimination of toxic chemical discharges, optimisation of energy use and the utilisation of environmentally friendly technologies for not only the purification of effluents and the implementation of closed-loop systems, but also for the production of high value by-products (WRC, 1996a).

The main objectives of this research study was:

- To characterise untreated edible oil effluent from the refinery processing plant.
- To design a laboratory-scale SBR system for simultaneous COD and phosphorus removal.
- To optimise the operational conditions for the BPR process using SBR.
- To evaluate the performance of the SBR system for BPR from edible oil effluent.

CHAPTER 2

LITERATURE REVIEW

2.1 WASTEWATER TREATMENT

Wastewater treatment is the largest biotechnology industry in the world, handling and disposing of domestic and industrial wastes. The basic function of wastewater treatment is to speed up the natural processes by which water is purified. There are three basic stages in the treatment of wastes, primary (physical), secondary (biological) and tertiary treatment (physical-chemical). In the primary stage, solids are allowed to settle and removed from wastewater. The secondary stage uses biological processes to further, treat wastewater (EPA, 1998). Tertiary treatment processes are used for handling increasingly complex wastewaters that contain high quantities of nonbiodegradable materials by means of advance treatment such as ion exchange, reverse osmosis, chemical reaction, electrodialysis, filtration and adsorption by activated carbon (Schroeder, 1977; Cavaseno, 1980).

Wastewaters are normally treated by a combination of physical-chemical and biological operations (Droste, 1997; EPA, 1998). Several countries in developed world of Western Europe and North America use secondary treatment in combination with physical-chemical processes (Schroeder, 1977). However, it is possible to treat wastewaters solely with either physical-chemical or biological methods. Physical-chemical processes can logically be applied as a form of pretreatment and then followed at a later stage by secondary treatment in treating industrial wastewaters (Droste, 1997).

The secondary stage of treatment removes about 85% of the organic matter in sewage by making use of bacteria in it. The principal secondary treatment techniques used in secondary treatment are trickling filter and activated sludge processes (EPA, 1998). Activated sludge is the most commonly specified biological treatment process for removing soluble and colloidal organic contaminants from process wastewaters (Cavaseno, 1980).

2.2 ACTIVATED SLUDGE

Activated sludge process is the cornerstone of sewage treatment systems and has become the most widely used secondary treatment unit process for treatment of both domestic and industrial wastewaters (Muyzer and Ramsing, 1995; Muyima *et al.*, 1997). It is used throughout the world and remains the most popular treatment process available, with the majority of new treatment plants of the activated sludge type (Gray, 1990). The trend today is towards the use of activated sludge process instead of other biological treatment methods such as trickling filters, rotating biodisc contractors, oxidation ponds (EPA, 1998). However, unlike the other processes it requires a high degree of operational control and management (Gray, 1990).

The activated sludge process refers to a continuous or semi-continuous (fill and draw) aerobic method for biological wastewater treatment. The process relies on a dense microbial population being mixed in suspension with the wastewater under aerobic conditions (Tchobanoglous and Burton, 1991). With unlimited food and oxygen, extremely high rates of microbial growth and respiration can be achieved, resulting in the utilisation of the organic matter present either as oxidised end products (CO_2, NO_3, SO_4, and PO_4) or the biosynthesis of new micro-organisms (Gray, 1990; Muyima *et al.*, 1997).

The main components of all activated sludge systems are (Gray, 1990; Toprak, 2001):

- The reactor. This can be a tank, lagoon, or ditch. The main criteria of a reactor are that the contents should be adequately mixed and aerated. It is also known as the aeration tank or basin.
- Activated sludge. This is the microbial biomass within the reactor, which is comprised mainly of about 95% bacteria and 5% of other microfauna and flora. The sludge is a flocculant suspension of these organisms and is often referred to as mixed liquor suspended solids (MLSS).
- Aeration/mixing system. Aeration and mixing of activated sludge and incoming wastewater are essential. The contents of the aeration tank are referred to as mixed liquor. Either surface aeration or diffused air is used for mixing to bring organisms, oxygen, and nutrients together, and to remove metabolic waste products.
- Waste activated sludge (WAS). The excess sludge that is disposed of when the activated sludge exceeds the amount that is needed to break down the wastes.
- Sedimentation tank. Also referred to as final settling tank or secondary clarifier. The tank receives the overflow of the aeration basin. This separates the microbial biomass from the treated effluent.
- Returned activated sludge (RAS). The settled activated sludge in the sedimentation tank is recycled back to the reactor to maintain the microbial population at a required concentration in order to ensure continuation of treatment.

The activated sludge process is essentially a two-stage process as shown in figure 2.1. It involves an aeration phase in which the wastewater to be treated is brought into contact with a bacterial floc in the presence of oxygen to degrade polluting matter. The second stage is a

settlement phase where the flocs are allowed to settle to produce a well-clarified effluent (Gray, 1989). Most of the activated sludge is returned (RAS) to the aeration tank to act as an inoculum of microorganisms ensuring that there is an adequate microbial population to fully oxidise the wastewater during its retention within the aeration tank. The excess sludge (WAS) is wasted from the settling tank (Gray, 1990).

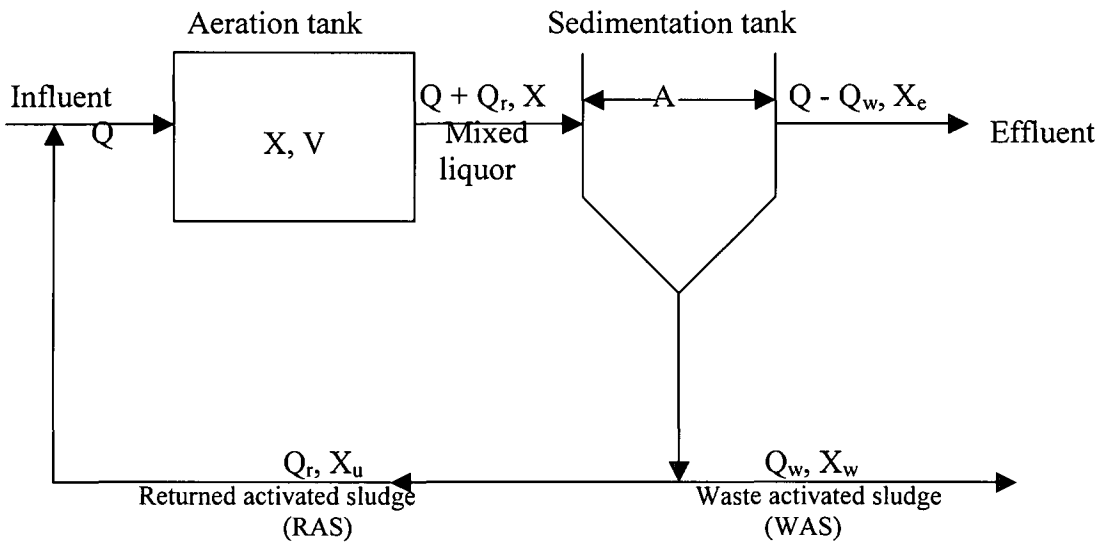


Figure 2.1: Schematic diagram of the activated sludge process (Sedlak, 1991).

Where A is the surface area of the sedimentation tank, V the aeration tank volume, Q the influent flow rate, Q_r the RAS flow rate, Q_w the WAS flow rate, X the aeration tank MLSS concentration, X_u the RAS suspended solids concentration, X_e the effluent suspended solids concentration, and X_w the WAS suspended solids concentration, which is normally equal to X_u.

The activated sludge process can be modified to achieve a high degree of phosphorus removal from wastewater (Jenkins, 1991; Bond, 1999). The evolution of the design and working of activated sludge process for biological phosphate removal has been reviewed in depth and will only receive a brief explanation here (Barnard, 1976; Toerien *et al.*, 1990).

2.3 DEVELOPMENT OF BIOLOGICAL PHOSPHORUS REMOAL

The first major drive towards the development of technology for phosphate removal from wastewater started in Switzerland in the early sixties. Elevated phosphorus concentrations in Swiss lakes and adverse eutrophication effects had prompted the Swiss researchers and engineers to develop chemical precipitation techniques for the removal of phosphate from effluent discharge. This resulted in a considerable degree of success in controlling eutrophication in Switzerland. In the late sixties USA and Sweden followed, and then in the early seventies Denmark, Norway, Germany, Finland and South Africa followed the Swiss lead and also initiated investigations for the development of technologies for phosphorus removal (Wiechers, 1987).

Jenkins and Lockett first investigated P removal by an activated sludge process in 1943. They noted that only 54% of the P entering a treatment works was discharged in the effluent. Harris, 1957 (as cited in Toerien *et al.*, 1990) reported that certain microbial strains can accumulate P in excess of metabolic requirements, and Srinath *et al.* (1959) carried out the first detailed laboratory experiments on enhanced P removal in an activated sludge system.

The evolution of the design and application of biological phosphorus removal systems is unique in the field of Sanitary Engineering. The phenomenon was unknowingly observed in full-scale plants in the early 1960s, but only after research in the early 1970s identified the necessary operating conditions. Srinath and Alarcon (as cited from Sedlak, 1991) were the first researchers to report the occurrence of biological phosphorus removal from wastewater treatment plant sludges. Both observed rapid phosphorus uptake when sludge samples taken from a local plug flow activated sludge plant were mixed and aerated with raw wastewater. However, they could not explain this phenomenon (Sedlak, 1991). Levin and Shapiro in 1965 introduced the term "luxury uptake" to describe the ability of activated sludge to remove more P than that required for growth, after they have observed enhanced biological

phosphorus removal using activated sludge from the District of Columbia activated sludge plant (Toerien *et al.*, 1990). The first commercial biological phosphorus removal process was developed from this work, known as the Phostrip process (Sedlak, 1991).

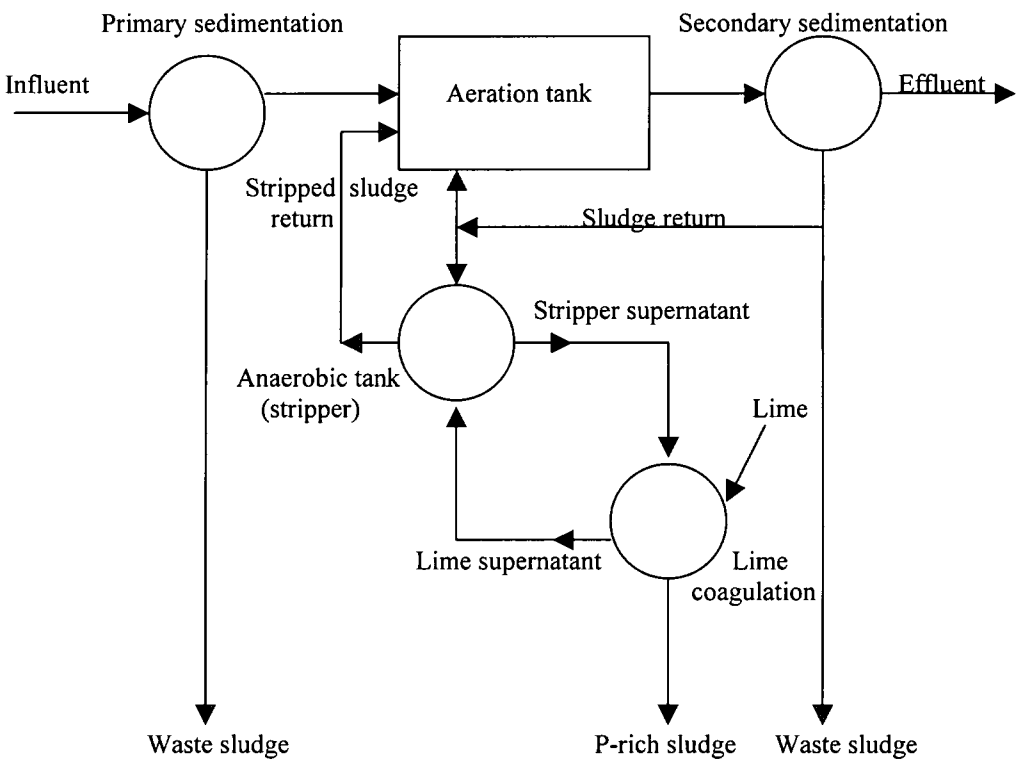


Figure 2.2: Schematic diagram of Phostrip process (Lilley *et al.*, 1997).

The Phostrip process (fig. 2.2) is a combined biological and chemical system. P is accumulated biologically and then released back into solution under anaerobic conditions in a special reactor. The concentrated phosphorus solution is then removed by chemical coagulation. Nitrogen removal is not included in this process. P is taken up in the aeration tank of a conventional activated sludge system. A proportion of the separated mixed liquor is diverted to an anoxic or anaerobic 'stripper tank' and excess P is released from storage in bacterial cells into solution, while the solids settle to form sludge. The stripped sludge is returned to the aeration tank where it carries out carbonaceous oxidation as well as absorbing more P from the wastewater. The supernatant from the stripper is treated by lime coagulation to remove P in solution, producing P rich sludge. The process is a continuous one with about

15-20 % of the total wastewater flow passing through the stripper (Toerien *et al.*, 1990; Gray, 1990; Lilley *et al.*, 1997).

Bacteria present in the mixed liquor of activated sludge for excess biological phosphate removal in full-scale plant was first observed by Vacker *et al.*, 1967. The plant was not specifically designed to achieve enhanced P remove. Barnard in 1973 reviewed the available technology for biological nitrification and denitrification and proposed a four-stage process, the Bardenpho activated sludge process (fig. 2.3), for which the South African Council for Scientific and Industrial Research was granted patent rights (Wentzel *et al.*, 1992). The Bardenpho process consists of four-stages, primary anoxic, primary aerated, secondary anoxic and secondary aerated followed by a clarifier (Ekama *et al.*, 1984; Gray, 1990; Lilley *et al.*, 1997; Muyima *et al.*, 1997).

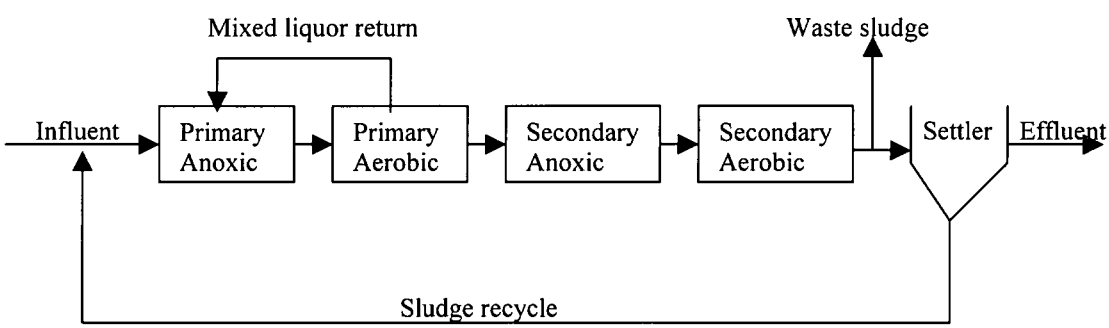


Figure 2.3: Four-stage Bardenpho activated sludge process for biological nitrogen removal (Lilley *et al.*, 1997).

Carbon removal and nitrification take place in the main aeration basin. Nitrified mixed liquor is recycled from this basin to the primary anoxic basin where, in the absence of free dissolved oxygen (DO), denitrification occurs, using the organic compounds in the influent wastewater as the carbon source. A proportion of mixed liquor from the primary aerobic basin is passed through to the secondary anoxic basin, where additional denitrification takes place at a slow rate under conditions of endogeneous respiration. Before entering the secondary clarifier,

mixed liquor from the secondary anoxic basin passes through a small reaeration basin, the function of which is to ensure that (Toerien *et al.*, 1990):

- NH_3-N formed during endogeneous respiration in the secondary anoxic basin is converted to NO_3-N,
- Aerobic conditions exist, as any denitrification that occurs there under anoxic conditions would produce nitrogen gas that could cause rising sludge, and
- Aerobic conditions exist in the secondary clarifier to prevent P release from the sludge into the effluent.

After pilot plant trials with settled domestic wastewater, Barnard in1974 reported N and P removals of up to 95 and 97 %, respectively, without the addition of chemicals. He also postulated that the key requirements for enhanced P removal are exposure of sludge microorganisms to anaerobic conditions, under which P release could occur (Sedlak, 1991). When subsequently exposed to an aerated or aerobic environment, the P that was previously released into solution and the P entering in the influent wastewater could either be taken up by the microorganisms in quantities greater than their normal metabolic requirements or precipitated from solution as a result of changes in redox potential caused by exposure to different oxygen regimes (Barnard *et al.*, 1997).

This led to the modification of the Bardenpho process to include five stages, i.e. the Bardenpho sequence with an additional anaerobic stage placed at the head of the process. The five-stage process is known as the Phoredox process in South Africa and Modified Bardenpho process in the United States (fig. 2.4), which is designed mainly to remove both nitrogen and phosphorus (Ekama *et al.*, 1984; Gray, 1990; Lilley *et al.*, 1997; Muyima *et al.*, 1997).

Phoredox process was derived from phosphorus and redox potential to signify the lower reduced conditions required in the anaerobic zone (Sedlak, 1991). In the Phoredox activated sludge process, the incorporation of an anaerobic zone at the head of the process allows the release of phosphate, after being released from the biomass in the anaerobic zone, phosphate is reincorporated into the biomass during aerobiosis, together with part or all of the influent phosphate (Gerber *et al.*, 1986; 1987). Bernard noted that the release of phosphate is a basic requirement for successful uptake of phosphate. He further reported that the presence of nitrates in the anaerobic zone had an adverse effect on the biological phosphorus removal efficiency. Experiments in pilot plant and full-scale facilities confirmed the negative impact of nitrates on biological phosphorus removal (Bernard, 1976; Marais *et al.*, 1983).

The anaerobic reactor receives the influent sewage feed and the sludge recycled from the clarifier underflow. The outflow from the anaerobic reactor is passed through to the primary anoxic reactor, which also receive the mixed liquor recycle. The mixed liquor recycle is drawn from the primary aeration basin, which is the third stage of the process. The out flow from this reactor is passed through to the secondary anoxic reactor, and followed by the secondary aeration basin and finally through the clarifier, from which the supernatant liquor is discharged and the settled solids returned to the head of the process.

In some applications, the last two basins of the five-stage Phoredox process have been excluded, because the inclusion of the secondary anoxic basin can result in only slightly greater N removal due to the low rate of denitrificaton under endogenous respiration. Such systems are known as three-stage Phoredox process (Lilley *et al.*, 1997). The application of the Phoredox process in South Africa has led to the removal of phosphate in the final effluent of activated sludge plants to levels between 0.2 and 0.8 mg/L, together with the removal of 80-90 % of nitrogen (Barnard, 1976). Since then the phenomenon has gained worldwide support and is utilised in both new and existing wastewater treatment plants which are either

constructed or upgraded to accommodate biological nutrient removal (Henze, 1996; de Haas *et al.*, 2000).

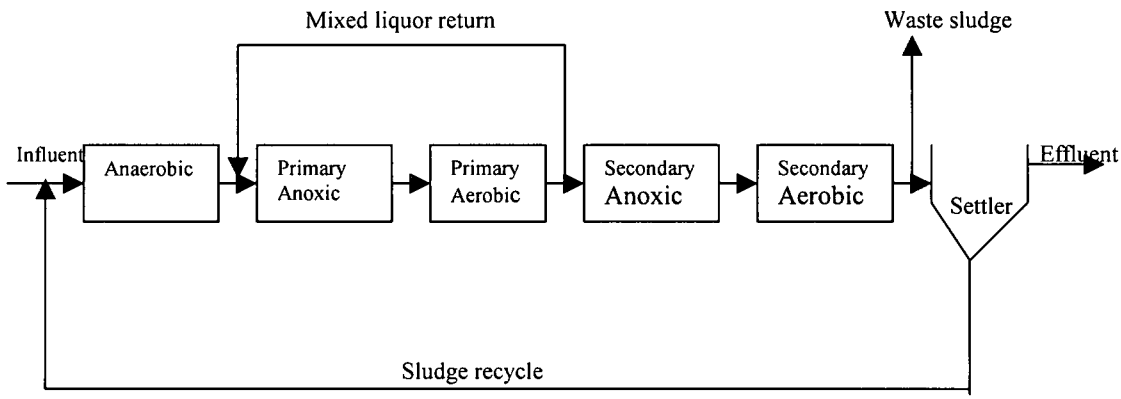


Figure 2.4: Five-stage Phoredox activated sludge process for biological nitrogen and phosphorus removal (Lilley *et al.*, 1997).

There are several other activated sludge processes that are currently being applied worldwide for biological phosphorus removal. Some process configurations incorporate nitrogen removal by nitrification and denitrification along with biological phosphorus removal such as University of Cape Town (UCT) process, A/O process, DEPHANOX process, Biodenipho process, Modified UCT process, Johannesburg process (Lilley *et al.*, 1997; Novotny, 1998).

Nicholls *et al.*, 1986 provided direction for further understanding and modification of the anaerobic-aerobic biological phosphorus removal system. They proposed a biochemical model involving carbon storage products, such as polyhydroxybutyrate (PHB), and polyphosphates to explain biological phosphorus removal. Recent development in the use of biological treatment processes for the removal of phosphorus in wastewater can be found in the work of Ekama *et al.*, 1999; Orhon *et al.*, 1994; Wentzel *et al.*, 1992.

2.4 MECHANISMS OF BIOLOGICAL PHOSPHORUS REMOVAL

The general tendency in recent years has been a decline in pre- and postprecipitation, and an increase in biological phosphorus (bio-P) removal. Bio-P removal is receiving increased interest because of its low sludge production and the fertiliser value of the sludge. Biological phosphorus removal (BPR) is presently carried out using activated sludge technology exclusively in both domestic and industrial wastewater treatment (Henze, 1996; Keller *et al.*, 2000). BPR process using activated sludge systems is considered one of the most economical, efficient and environmentally friendly methods for bio-P removal (Novotny, 1999; Jeon *et al.*, 2001). Substantial savings can be achieved through biological process rather than chemical P removal process. For instance, savings estimated at US$ 5-6 million per annum for some 32 nutrient removal treatment plants in South Africa were achieved (Toerien *et al.*, 1987).

Biological phosphorus removal has been studied for several decades and this has led to the proposal of many different processes (Wentzel *et al.*, 1991; Satoh *et al.*, 1992; Kuba *et al.*, 1993). Although considerable research has been directed towards improving understanding of enhanced biological phosphorus removal (EBPR) phenomenon, the real features of the process still remain ambiguous (Satoh *et al.*, 1996; Jeon *et al.*, 2001). Its biological mechanisms are not deeply understood at present, and operational guidelines based on scientific knowledge still need to be developed. Knowledge of the biochemical reactions of the EBPR process is largely derived from indirect observations and theoretical considerations. Because the biochemical details are lacking, engineers use a "black box" –type approach for design and optimisation of EBPR activated sludge systems (Siebritz *et al.*, 1983; Wentzel *et al.*, 1985; Satoh *et al.*, 1994; Momba and Cloete, 1996; Scheer and Seyfried, 1996; Carucci *et al.*, 1997; Brdjanovic *et al.*, 1998; Bond *et al.*, 1999; Jeon *et al.*, 2001).

Phosphorus is removed in EBPR process by incorporating phosphorus into the cell tissue of microorganisms (poly-P organisms or PAOs, mainly *Acinetobacter*) in quantities greater than

needed for anabolic processes (Chang *et al.*, 1996; Muñoz-Colunga and Gonzalez-MartPnez, 1996). This is achieved by exposing the micoorganisms to alternating anaerobic and aerobic conditions in a continuous or batch activated sludge process (Rensink, 1991; Smolders *et al.*, 1994a; 1994b; Rodrigo *et al.*, 1996; Ydstebρ *et al.*, 2000; Randall *et al.*, 1997; Jeon *et al.*, 2001). The amount of phosphorus removed depends on the net sludge production since waste in the cell tissue is the only means of removing phosphorus in EBPR process (Barnes *et al.*, 1984; Chang *et al.*, 1996; Scheer and Seyfried, 1996; Liu *et al.*, 1997; Bond *et al.*, 1999). The biological phosphorus removal mechanism is based on the following key facts (Wentzel *et al.*, 1989; Sedlak, 1991; Rusník and Wanner, 2000):

- In anaerobic zone, phosphorus is released from the microorgainsms. Accompanying this release, the organic substrate (lower fatty acids, mainly acetate) is taken up and stored into the cell by P-removing bacteria as polyhydroxyalcanoates (PHA).
- In the aerobic zone, storage products (PHA) are oxidised. Parallely, soluble orthophosphate provides for the resynthesis of the intracellular polyphosphates.

2.4.1 Anaerobic zone

In wastewater treatment plants phosphorus can be removed biologically by introducing an anaerobic phase at the first stage of the activated sludge process, in which recycled activated sludge microorganisms encounter a high concentration of food in the form of COD from the influent wastewater (Maurer and Gujer, 1997; 1998; Van Loosdrecht, 2001). Anaerobic zone is described as the zone in, which both dissolved oxygen and oxidised nitrogen (nitrites and nitrates) are absent (Barnard, 1976; Buchan, 1983; Muyima *et al.*, 1997). The presence of nitrate in an anaerobic zone has been reported as a handicap to the phosphate removing potential of activated sludge systems (Barnard, 1976; Marais *et al.*, 1983). High

concentrations of nitrate present in the anaerobic zone can result in poor phosphate removal efficiency (Toerien *et al.*, 1990).

The function of the anaerobic zone in EBPR process is two-fold: (i) the reduced redox potential induces conversion of the influent readily biodegradable COD (RBCOD) to short chain fatty acids (SCFA) via acidogenisis by non-poly-P heterotrophs, and (ii) it provides an ideal environment where polyphosphate accumulating organisims (PAOs) are able to take up the volatile fatty acids (VFAs) and accumulate them intracellularly as PHAs (Bosch and Cloete, 1993; Ekama and Wentzel, 1997).

During the anaerobic phase, the energy requirements of the poly-P organisms are supplied by the hydrolysis of the polyphosphate compounds in the cells, a process, which results in the release of phosphate to the liquid environment (Comeau *et al.*, 1986). Biological phosphate release is dependent on the presence of readily biodegradable substrate mainly volatile fatty acids such as acetate in the absence of nitrates. (Satoh *et al.*, 1992; Maurer and Gujer, 1997). VFAs are produced in the anaerobic zone as a result of fermentation reactions due to the presence of organic compounds in the influent wastewater (Nicholls *et al.*, 1985; Comeau *et al.*, 1986; Gerber *et al.*, 1986; Jones *et al.*, 1987; Appeldoorn *et al.*, 1992; Satoh *et al.*, 1992; Van Loosdrecht, 2001). The phosphorus release, which occurs in the presence of VFAs is called the primary release, and the release in the absence of VFAs is defined as the secondary release (Barnard, 1993; 1994).

Secondary release of phosphorus is not however associated with the storage of organic compounds and phosphorus thus released will not be removed by the poly-P organisms in the subsequent aerobic stage. Hence any secondary release of phosphorus is detrimental to the performance efficiency of bio-P removal and should be prevented. Long hydraulic retention time in the anaerobic zone, inadequate supply of organic compounds in the wastewater and

insufficient activity of the fermentation bacteria in the anaerobic zone could all lead to the secondary phosphorus release and inefficient operation (Danesh and Oleszkiewicz, 1997).

The ability of PAOs to store polyphosphate is dependent on the availability of fatty acids, under anaerobic conditions (Henze, 1996). Hence, poly-P organisms compete with facultative bacteria (particularly denitrifiers) for the substrate by obtaining energy through the utilisation of stored polyphosphates. The addition of substrate during this phase will favour poly-P organisms over the facultative bacteria by capturing organic material first before the facultative bacteria can make use of it (Muñoz-Colunga and Gonzalez-Martinez, 1996). The amount of phosphorus that can be removed by bio-P activity is directly coupled to the amount of VFAs that the PAOs can take up in the anaerobic zone (Henze, 1996;Van Loosdrecht *et al.*, 1997). Hence, the ratio between the phosphate release and VFAs, mainly acetate uptake should be constant (Smolders *et al.*, 1994a). Data from available literature show that a range of values (0.25 to 0.75 P-mol/C-mol) for phosphate/acetate ratio has been observed (Arvin, 1985; Wentzel *et al.*, 1986, 1988; Arun *et al.*, 1988).

Under anaerobic conditions, most of the aerobes cannot perform metabolism. However; when readily biodegradable COD, particularly, VFAs, are available, PAOs can take up the readily biodegradable substrate and store as poly-β-hydroxyalkanoates (PHAs), using stored polyphosphate as an energy source. Phosphate in the form of orthophosphate (ortho-P) is released from the cells to the bulk liquid in the anaerobic zone as a by-product of this type of metabolism (Danesh and Oleszkiewicz, 1997; Zhao, 2000).

Theoretically, COD requirements for P release can be calculated from the stoichiometric relationship (Gujer *et al.*, 1995; Tasli *et al.*, 1999):

$$\Delta COD_P = \frac{\Delta P_{released}}{Y_{PO4}}$$

where,

ΔCOD_P is the amount COD removed from solution for PHB storage.

$\Delta P_{released}$ is the amount of P released into solution.

Y_{PO4} is a coefficient reflecting the stoichiometric relationship between P released and PHB storage under anaerobic conditions. The suggested value for Y_{PO4} is 0.4 g P/g COD.

2.4.1.1 Metabolic model of the anaerobic metabolism

It is widely recognised that PHAs, an important intracellular carbon and energy storage material of bacteria, are an essential component of PAOs in EBPR process. Moreover, various bio-P models have illustrated the relationship between PHAs accumulation/degradation and phosphorus release/uptake (Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Smolders *et al.*, 1995). Previous studies on PHAs have verified that the poly 3-hydroxybutyrate (PHB) and poly 3-hydroxyvalerate (PHV) are the major PHAs components involved in biological phosphorus removal (Pereira *et al.*, 1996; Maurer *et al.*, 1997; Chuang *et al.*, 1998).

Two biochemical models have been proposed for the anaerobic metabolism of organisms that describe the synthesis of PHAs and the source of the reducing power required for the anabolic reaction. These are the Comeau-Wentzel (TCA cycle) and Mino (glycolysis) models (Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Mino *et al.*, 1987; Smolders *et al.*, 1994a). In the Comeau-

Wentzel model, the tricarboxylic acid (TCA) cycle operates under anaerobic conditions to partially oxidise acetate (HAc) to carbon dioxide (CO_2) and to generate reducing power in the form of NADH (Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Randall *et al.*, 1997). The Comeau-Wentzel model is specific for *Acinetobacter* spp., i.e. for organisms not possessing Embden-Meyerhof (EM) pathway (Wentzel *et al.*, 1992).

In contrast Mino *et al.* (1987) proposed a biochemical model for poly-P organisms that possess the Embden-Meyerhof pathway, but did not identify the specific poly-P organisms. This model hypothesis suggests that degradation of intracellularly stored glycogen in the Embden-Meyerhof pathway is the source for reducing power needed for NADH production. In the degradation of glycogen, ATP is also produced, which lowers the required energy contribution of the hydrolysis of poly-P, resulting in a decreased ratio for phosphate released per acetate taken up (Mino *et al.*, 1987; Wentzel *et al.*, 1992; Smolders *et al.*, 1994a).

For both models, the acetate uptake is divided into a transport and storage process in which the energy necessary for the transport of acetate is highly dependent on the pH (Smolders *et al.*, 1994a, 1994b; Liu *et al.*, 1994; Satoh *et al.*, 1994; Jeon *et al.*, 2001). At low pH, no energy is involved in the transport of acetic acid and the observed P release originates from the ATP used in the conversion of acetate to acetyl CoA. The P release at low pH in the glycogen model is half the amount released in the TCA model, due to the contribution of the ATP generated in the conversion of glycogen into PHB (Smolders *et al.*, 1994a). The acetate uptake and phosphate release at high pH can be described with the use of three basic reactions: the transport and storage of acetate as PHB (reaction 1), polyphosphate degradation (reaction 2), and a reaction which supplies the reduction equivalents needed in reaction 1. This reaction is different for the TCA model and the glycogen model (reaction 3a and 3b respectively).

r_1: $CH_2O + (½ + \alpha_1)ATP + ¼\ NADH \rightarrow CH_{1.5}O_{0.5} + ½\ H_2O$

Acetic acid PHB

where ,

α_1 : is the amount of ATP requirement for the acetate transport (mol ATP/C mol)

The uptake of 1 C-mol acetic acid (which is equal to 0.5 mol HAc) in the above reaction, r_1 and conversion to PHB is described in three steps: (i) the uptake of acetate which requires α_1 mol ATP depending on the pH (α_1 = 0 – 0.5), (ii) the conversion to acetyl CoA which requires 0.5 mol ATP, and (iii) the subsequent conversion to PHB which requires 0.25 mol NADH per C-mol acetic acid.

r_2: $HPO_3 + H_2O \rightarrow \alpha_2 ATP + H_3PO_4$

polyphosphate

For the uptake and storage of acetate, ATP is produced by the degradation of polyphosphate. Polyphosphate is represented as HPO_3 in r_2 above. The amount of ATP, which is generated from the degradation of polyphosphate, is represented with α_2 in the r_2 reaction. The hydrolysis of 1 P-mol polyphosphate yields 1 mol ATP and 1 mol phosphate, and hence, α_2 = 1, when presumed that no energy is produced by the export of phosphate.

r_{3a}: $CH_2O + (½ + \alpha_1)ATP + H_2O \rightarrow 2NADH + CO_2$

NADH is required in reaction 1 and is produced from the conversion of some of the acetate in the TCA cycle in reaction 3a. It is assumed that the amount of ATP produced in the TCA cycle is used for the conversion of FADH to NADH (Comeau *et al.*, 1987; Wentzel *et al.*, 1991; Smolders *et al.*, 1994a).

$$r_{3b}: \quad CH_{10/6}O_{5.6} + 1/6\ H_2O \rightarrow 2/3\ CH_{1.5}O + 1/3\ CO_2 + \tfrac{1}{2}\ NADH + \tfrac{1}{2}ATP$$

glycogen PHB

In reaction 3b, NADH is produced from the conversion of a 0.5 C-mol glycogen through the EM pathway to acetyl CoA that is subsequently converted to PHB, which also yields 0.25 mol ATP.

The reactions r_1 to r_3 are termed the "internal reactions" and they are based on biochemical knowledge and stoichiomestry. These reactions occur in the cell and cannot be observed directly. However, the internal reaction rates can be related to the observable conversion rates outside the cell (Smolders *et al.*, 1994a).

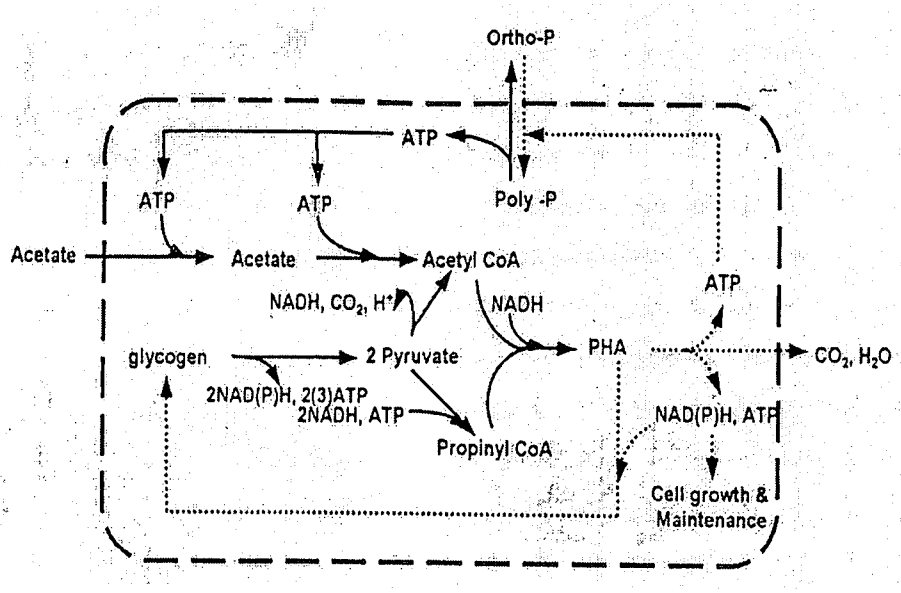


Figure 2.5: Biochemical model proposed for anaerobic (∏) acetate uptake and PHA synthesis and aerobic (--->) PHA metabolism (schematised according to Smolders *et al.*, 1994b, and Satoh *et al.*, 1994)

The schematic representation for the mechanisms of phosphate release and acetate uptake is presented in figure 2.5 by Smolders *et al.*, 1994b and Satoh *et al.*, 1994. According to the model, there are two possible pathways for the synthesis of PHA from glycogen: via acetyl-

CoA or via propionyl-CoA (succinate-propionate pathway). When one molecule of glycogen is consumed, two molecules of pyruvate are produced. If pyruvate is metabolised to PHA through acetyl-CoA, more NADH will be produced per ATP production. In this case, polyphosphate will be the primary source of energy for acetate uptake and conversion of acetate to acetyl-CoA (Jeon *et al.*, 2001).

2.4.2 Aerobic zone

Oxygen is available as an external electron acceptor and the previously stored organic material (PHAs) are utilised as substrate source in the aerobic zone (Wentzel *et al.*, 1990; 1992). During the aerobic phase the PAOs metabolise the anaerobically produced PHA to generate energy for cell growth, polyphosphate synthesis and glycogen formation (Smolders *et al.*, 1994b; MuZoz-Colunga and Gonzalez-Martinez, 1996; Danesh and Oleszkiewicz, 1997; Zhao, 2000). This will result in an increase in the population of the PAOs due to substrate utilisation and a net uptake of the P released during the anaerobic phase into the cell (Sedlak, 1991; Randall *et al.*, 1997)

Various factors can affect the rate of uptake of phosphate by PAOs. Efficient uptake of phosphate requires high concentrations of fermentable substrates or volatile fatty acids in the influent to the anaerobic zone (Muyima *et al.*, 1997). The findings by Wentzel *et al.*, 1985 indicate that excessive phosphate uptake in the aerobic zone is associated directly with the degree of phosphate release during the previous anaerobic phase, and that more phosphate release leads to more phosphate uptake.

The role of polyphosphate as energy source during anaerobic conditions implies that, during aerobic conditions, energy has to be spent on the synthesis of polyphosphate and on the transport of phosphate into the cell. In addition cell growth and glycogen synthesis from PHB requires energy in the form of ATP. Because ATP results from oxidative phosphorylation, all

conversions (growth, polyphosphate and glycogen synthesis) are coupled to oxygen consumption (Smolders *et al.*, 1994). The efficiency of phosphate removal seems to be dependent on the intensity of aeration (Muyima *et al.*, 1997).

In aerobic conditions the TCA cycle and oxidative phosphorylation are active resulting in a decrease in the NADH/NAD ratio and an increase in the ATP/ADT ratio. The decreased NADH concentration will stimulate polyphosphate synthesis with phosphate uptake occurring via a hydroxyl mediated antiport and cation uptake via a proton mediated antiport and the proton motive force (pmf) being maintained by ATP (Bosch and Cloete, 1993). In completely aerobic environments, where oxidative phosphorylation is able to proceed the ATP/ADP ratio will be high, and thus stimulate polyphosphate accumulation (Wentzel *et al.*, 1986). If sufficient substrate is available PHB synthesis will also be stimulated with the protons and electrons for the reduction of acetate to PHB being supplied by the operation of the TCA cycle (Bosch and Cloete, 1993).

The principal mechanism of polyphosphate synthesis is via the phosphorylation of accumulated phosphate by ATP, the reaction of which is as follows:

$$ATP + (PO_4)_n \Leftrightarrow ADP + (PO_4)_{n+1}$$

It is evident from this reaction that the pathway controls both polyphosphate synthesis and degradation, the direction of which is regulated entirely by intracellular ATP/ADP ratios (Wentzel *et al.*, 1986).

2.4.2.1 Metabolic model of the aerobic metabolism

The metabolic reactions in the aerobic phase can be separated into two groups, i.e. firstly the energy generating reactions and secondly the energy consuming reactions (Smolders *et al.*, 1994b). The energy producing reactions can be described by equations for PHB catabolism, (reaction 5) and oxidative phosphorylation, (reaction 6). Whilst the energy consuming reactions can be described by the: production of biomass (reaction 6), polyphosphate synthesis (reactions 8a and 8b) and glycogen synthesis (reaction 9). These reactions are based on biochemical knowledge and stoichiometry.

$$r_5: \quad -CH_{1.5}O_{0.5} - 1.5H_2O + 2.25\ NADH_2 + 0.5ATP + CO_2 = 0$$

PHB

In the above reaction, r_5 PHB is degraded to acetylCoA and converted in the TCA cycle. The $FADH_2$ that is produced is assumed to be equivalent to $NADH_2$ in the above reaction.

$$r_6: \quad -NADH_2 - 0.5O_2 + H_2O + \delta\ ATP = 0$$

Oxidative phosphorylation process is stoichiometrically represented in reaction 6. In the oxidative phosphorylation reaction ATP is produced from $NADH_2$, and the amount of ATP produced per electron pair is represented by δ, the P/O ratio, which resembles the efficiency of the oxidative phosphorylation.

$$r_7: \quad -1.27CH_{1.5}O_{0.5} - 0.20NH_3 - 0.015H_3PO_4 - (K + m_{ATP}/\mu)ATP - 0.385H_2O +$$

PHB $\qquad CH_{2.09}O_{0.54}N_{0.20}P_{0.015} + 0.615NADH_2 + 0.27CO_2 = 0$

where,

m_{ATP}: is the specific ATP consumption due to the maintenance processes (mol/C-mol·h)

μ: is the growth rate (h^{-1})

Reaction 7 represents the synthesis of biomass from PHB, where 0.27 mol CO_2 is produced per C-mol biomass. The amount ATP needed for the formation of biomass precursors from acetyl CoA and polymerisation of these precursors to 1 C-mol biomass is represented by K in r_7. According to biochemical analysis, the value of K is estimated as $K = 1.5$ mol ATP per C-mol biomass (as cited in Smolders *et al.*, 1994a).

$$r_{8a}: \quad -H_3PO_4^{out} - 1/\epsilon\ NADH_2 - 1/2\epsilon\ O_2 + H_3PO_4^{in} + 1/\epsilon\ H_2O = 0$$

where,

ϵ: is the energy for transport of phosphate (P-mol/mol $NADH_2$)

Reaction 8a represents the phosphate transport. The transport of phosphate across the cell membrane is a process, which requires energy. Phosphate is a negatively charged ion and has to be taken up against the electric potential difference over the cell membrane. Cations required for the polyphosphate synthesis (Mg^{2+}, Ca^{2+} and K^+) are taken up by the cell without energy costs via the proton mediated antiport (Wentzel *et al.*, 1986; Bosch and Cloete, 1993). The energy used for the transportation of phosphate is generated by the import of protons that are subsequently exported over the cell membrane in the oxidation of $NADH_2$. Therefore, a certain amount of phosphate, ϵ, can be transported for each $NADH_2$ consumed (as cited in Smolders *et al.*, 1994b).

$$r_{8b}: \quad H_3PO_4^{in} - \alpha_3 ATP + H_3PO_4 + H_2O = 0$$

polyphosphate

The polyphosphate synthesis is stoichiometrically represented in reaction 8b. The composition of polyphosphate is based on phosphorus, magnesium and potassium. Magnesium and potassium elements were not considered in the above reaction, r_{8b}, hence polyphosphate is represented as H_3PO_4. The amount of ATP required for the synthesis of polyphosphate is represented by α_3 in r_{8b}. For the synthesis of polyphosphate, 1 ATP is required, hence a typical value of $\alpha_3 = 1$ is given (Stryer, 1981).

$$r_9: \quad -4/3CH_{1.5}O_{0.5} - 5/6ATP - 5/6H_2O + CH_{10/6}O_{5/6} + 1/3CO_2 + 1NADH_2 + = 0$$

PHB glycogen

Reaction 9 represents the glycogen production. This reaction is based on the production of glycogen from oxaloacetate in glyconeogenesis. Oxaloacetate is produced from PHB through the glyoxylate cycle (Stryer, 1981).

2.4.3 System phosphorus removal

The behaviour of P removal activated sludge systems is characterised by a P release in the anaerobic stage followed by excess P uptake in the aerobic stage as shown in figure 2.6. A number of factors have been postulated to play a role in P release (anaerobiosis, redox potential, nitrate, sulphide, substrate composition, and concurrent P and metal ion release and uptake) and uptake (aerobiosis, the presence of PAOs in a preconditioned sludge and certain metallic ions) phenomena (Toerien *et al.*, 1990).

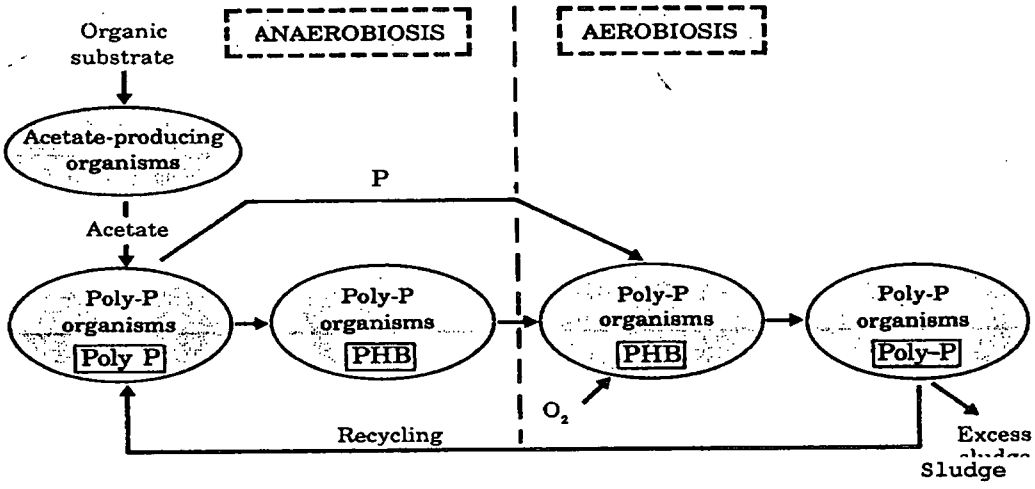


Figure 2.6: **Phenomena involved in biological phosphorus removal (Toerien *et al.*, 1990)**

The amount of phosphorus that can be removed by PAOs is dependent on the availability of fermentation products and soluble fermentable organics, which are very limited in wastewaters (Christensson, 1997; Artan *et al.*, 2000). The establishment of conditions to stimulate excess P removal becomes increasingly more difficult as the influent COD strength of the wastewater decreases. Special measures such as fermentation of part or all the influent wastewater may be implemented to increase RBCOD fraction (Toerien *et al.*, 1990). The BEPR mechanism discussed above indicates that the level of biological phosphorus removal achieved is directly related to the amount of substrate that can be fermented in the anaerobic phase (Fuhs and Chen, 1975; Nicholls *et al.*, 1985, 1986; Osborn *et al.*, 1986; Sedlak, 1991).

2.5 FERMENTATION

The anaerobic fermentation of organic substances in wastewater is well known in anaerobic digestion (Cuevas-Rodríguez *et al.*, 1998). This is because the fundamental biological processes involved are the same as those in anaerobic digestion, a field that has been researched extensively in the past 20-30 years (v. Münch and Koch, 1997a). Anaerobic digestion research over these years has included efforts to better understand fermentation of

primary sludge for the production of VFAs in wastewater treatment because of the beneficial effects attributed to VFAs in BEPR processes (Pitman *et al.*, 1992; Elefsiniotis and Oldham, 1991; 1994).

Anaerobic digestion is a complex process consisting of a series of microbial reactions catalysed by consortia of different bacteria (McInerney *et al.*, 1981; Ross *et al.*, 1992). Biological processes occurring during anaerobic digestion in wastewater treatment are well understood. Figure 2.7 summarises the current understanding of the main biological reactions occurring during the anaerobic degradation of wastewater, a process, which occurs in two distinguishable phases. These two phases can either occur coupled in a syntrophic relationship or separate for process optimisation and control (Fox and Pohland, 1994).

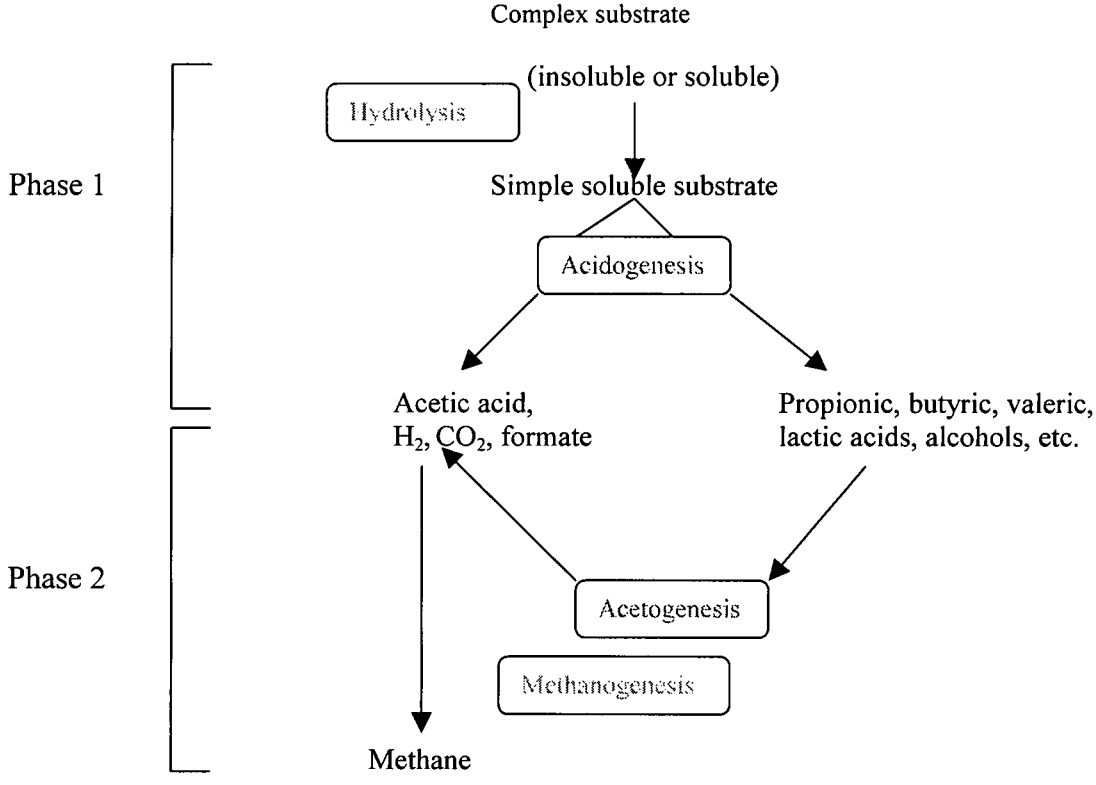


Figure 2.7: Schematic representation of the anaerobic degradation of wastewater (adapted from Fox and Pohland, 1994)

2.5.1 First phase: VFA production

Fermentation is the first phase of the anaerobic degradation process, "acid-phase". It is described as the production of VFAs (mainly acetic and propionic acid) from domestic or industrial wastewater by subjecting the wastewater to anaerobic conditions for a period of time (Deinema *et al.*, 1985; Brodisch, 1985; v. Münch, and Pollard, 1997b). This phase consists of two processes, hydrolysis and acidogenesis (Rustrian *et al.*, 1997).

Hydrolysis is a process whereby particulate or high molecular weight soluble substrates are broken down to smaller molecules by incorporation of water molecules (v. Münch, 1998). Hydrolysis of particulate organic matter to soluble substrate has been identified as the rate-limiting step during the acid generation phase (Eastman, 1977). During the fermentation process, hydrolysis is catalysed by hydrolytic enzymes excreted by bacteria (v. Münch, 1998). Acidogenesis is a process whereby the smaller molecules produced during hydrolysis are converted anaerobically to VFAs and other lower molecular weight soluble carbon compounds (Gujer and Zehner, 1983). The principal VFAs formed during acidogenesis are acetic, propionic and butyric acids (Lilley *et al.*, 1997).

Fermentation can be expressed as a first order process with respect to particular fermentable components (X_{acid}) and is characterised by hydrolysis constant (k_{acid}). Thus the temperature dependent acid production rate can be written as (Moser-Engeler *et al.*, 1998):

$$r_{acid} = k_{acid} \cdot X_{acid}$$

The pH dependence in the above reaction has been neglected. During fermentation period, the pH decreases and may decline to below 6.0. This however, does not adversely affect the acid forming bacteria, but does retard the growth of methane producing bacteria (Lilley *et al.*, 1997). Lilley *et al.*, 1990 also reported that VFAs formation is a first-order reaction with a maximum potential conversion of influent COD to VFA of 17%, at 20°C, at retention times

of less than 10 days. Research, undertaken over the years has indicated that VFAs production are greatly influenced by parameters such as hydraulic retention time (HRT), solid retention time (SRT), pH, temperature, wastewater characteristics, reactor configuration, trace minerals and oxidation-reduction potential (ORP) (Andrews and Pearson, 1965; Ghosh *et al.*, 1975; Elefsiniotis and Oldham, 1991; 1994; Banerjee *et al.*, 1998).

Until about 1970's, when BPR process was pioneered in South Africa it was observed that fermentation was beneficial to BPR systems. Since then fermentation has increasingly gained popularity in BPR wastewater treatment systems (v. Münch, 1998).

VFAs are generally regarded as the best carbon sources for bio-P removal (Comeau *et al.*, 1987; Aruna *et al.*, 1988; 1989; Abu-ghararah and Randall, 1991; Satoh *et al.*, 1992; Lilley *et al.*, 1997). The net amount of phosphorus that can be removed per unit VFA generated in the anaerobic zone is a function of the cell yield and net amount of phosphorus stored in the wasted biological mass (Sedlak, 1991). About 6-9 mg of VFA is required to remove 1 mg of phosphorus (Barnard, 1993).

The VFAs production (activity of fermentation bacteria) is a slow process when compared to the uptake of VFAs by PAOs (Danesh and Oleszkiewics, 1997). The relative slowness of fermentation activity (VFAs production) compared to the VFAs uptake was indicated by Wentzel *et al.*, 1988 and Barnard, 1994. A large number of microorganisms have been found to be capable of producing VFAs. Some predominant microorganisms responsible for acidogenesis are the species belonging o the family of Streptococcaceae and Enterobacteriaceae and to the genera of *Bacteroids, Clostridium, Butyrivibrio, Eubacterium,* and *Lactobacillus*. Aeromonas species have also been identified as the important microorganisms for accomplishing fermentation and VFAs production in BEPR wastewater treatment (Sedlak, 1991).

2.5.2 Second phase: VFA consumption

The second phase of anaerobic digestion consists of two processes, acetogenesis and methagenosis. Acetogenesis process is also known as the anaerobic oxidation of short-chain fatty acids and the main products of this process are acetate and hydrogen gas. Acetogenesis cannot be encouraged without encouraging methanogenesis. This is unfortunate as acetogenesis by itself would lead to "higher-quality" VFAs for the BNR process, namely acetic acid (Degrémont, 1991; v. Münch, 1998).

In order to maximise the mass of VFAs produced during acidogenesis, the fermentation period must be terminated prior to the onset of methanogenesis. Methanogenesis is aglobal microbiological process in which organic matter is metabolised to methane (Pauss and Nyns, 2000). In this stage, VFAs produced are utilised by methane producing organisms to form methane, carbon dioxide and water from the decarboxylation of acetate and from the reduction of carbon dioxide and hydrogen gas (Lilley *et al.*, 1997; Münch, 1998). Acetate is the prime precursor for methane production, contributing about 70% of the total methane produced (Kaspar and Wuhrmann, 1978; Smith *et al.*, 1980; Degrémont, 1991).

Methane production is more likely to occur at longer retention times (6 to 10 days) and reduce yields of VFAs (Osborn *et al.*, 1986; Lilley *et al.*, 1990). Methanogens are the slowest growing population in anaerobic digestion and their presence or absence can be controlled by maintaining the sludge age or fermentation period to less than 6 days. In some cases, it may be possible to exceed the 6-day fermentation period without observing any methane production (Lilley *et al.*, 1997). The separation of the acidification phase (fermentation) from methanogenesis is a normal practice in anaerobic digestion of wastewaters (Cuevas-Rodríguez *et al.*, 1998).

2.5.3 Prefermenter technology

The amount of phosphorus that can be removed by PAOs is dependent on the availability of fermentation products and soluble fermentable organics, which are very limited in wastewaters (Artan *et al.*, 2000). To enhance biological nutrient removal and to improve the process operation, acid fermentation of primary sludge has been employed in some full-scale plants practising BNR in conventional flow-through treatment (Danesh and Oleszkiewicz, 1997). The use of primary settled sludge to improve biological phosphorus removal efficiency is increasingly most notable in South Africa and other parts of the world such as Canada, to generate VFAs, so as to boost the fraction of readily biodegradable carbon in the feed wastewater (Sedlak, 1991; Pitman, 1992, 1995; Muench *et al.*, 1996).

Prefermenters are an increasingly popular unit operation in wastewater treatment for BNR. They are used to significantly improve the performance and reliability of BNR in wastewater treatment plants (v. Münch and Pollard, 1997b; Graja and Wilderer, 2000). Prefermenters provide a suitable carbon source for the BNR process by producing VFAs from the influent wastewater (v. Münch, 1998).

Prefermenters can either be side-stream or in-line (Barnard, 1993; Frese *et al.*, 1993; Pitman, 1995; Lilley *et al.*, 1997). In-line prefermenters are fed with raw sewage rather than with primary sludge. An example of an in-line prefermenter is the primary sedimentation tank (PST), where fermentation takes place within PST, with the sludge being continuously recycled to elutriate the fermentation products. Most full-scale prefermenters are fed with primary sludge or raw sludge from the underflow of a primary clarifier (Rabinowitz and Oldham, 1985a and b; Comeau *et al.*, 1987). These types of prefermenters are called side-stream prefermenters (v. Münch, 1998). Side-stream prefermenters can be operated as a batch fermentation system, or as a continuous system (Lilley *et al.*, 1997).

Single-stage or multiple-stage prefermenters can be used as batch fermentation systems. Single-stage prefermenters can be either mixed or non-mixed. Non-mixed single-stage prefermenters are often referred to as static prefermenters, and are basically an extension of the fermenting potential in the primary clarifier (v. Münch, 1998). Where multiple batch fermenters are used, it is usual to have the same number of units as the fermentation period in days (Lilley *et al.*, 1997).

Continuous fermentation takes place in either a single unit or multiple units operated in parallel. In continuous fermentation system sludge age is controlled by wasting a measured volume of sludge from the prefermenter unit each day. Sludge is continuously recycled during fermentation period and is stopped only when sludge wasting is being done to allow the thickening of the sludge prior to wasting (Lilley *et al.*, 1997).

2.6 PROCESS PARAMETERS FOR BPR

This section deals with the major design and operational principles, which almost inevitably have an ecological impact on activated sludge plants or are affected by microbial considerations. No attempt has been made to present detailed mathematical modelling or engineering design procedures for bio-P removal in this section.

When designing and operating activated sludge systems for biological phosphorus removal, cognisance must be taken of a number of key parameters to ensure maximum bio-P removal efficiency (Atkinson, 1999). These key parameters can be divided into the following categories (Toerien *et al.*, 1990; Sedlak, 1991):

- wastewater characterisation
- process control and design parameters such as SRT, HRT, flow regime, reactor design

- environmental factors such as DO, temperature and pH

2.6.1 Wastewater characterisation

Wastewater can be characterised chemically and physically. Chemical characteristics comprise of carbonaceous and nitrogenous constituents in their various fractions, soluble and particulate, biodegradable and non-biodegradable; and inorganic constituents principally total alkalinity, total acidity, pH and phosphorus. Inorganic constituents such as calcium, sulphate, sodium, magnesium and chloride are of minor importance. Physical characterisation comprises of dissolved, suspended and settleable constituents (Ekama and Marais, 1984).

Wastewater is a source of substrate and nutrients for the microbial consortium of activated sludge (Atkinson, 1999). Carbon, nitrogen and phosphorus are required by microorganisms in balanced amounts to satisfy the metabolic requirements. A reasonable estimate for C:N:P ratio can be expressed as 100:5:1 on a mass basis (Pipes, 1979; Droste, 1997). Nitrogen can only be considered available for biological activity when present in the form of ammonia and phosphorus as soluble phosphate (Atkinson, 1999).

Traditionally parameters used in evaluating the feasibility of BPR include ratios of biochemical oxygen demand to phosphorus (BOD/P), COD/P and total Kjeldahl nitrogen to COD (TKN/COD) (Randall *et al.*, 1992; Ekama *et al.*, 1984). The BOD/P and COD/P ratios indicate the importance of the strength of substrate in BPR process (Park *et al.*, 2001).

2.6.1.1 Carbonaceous material

Characterisation of the carbonaceous material in the influent wastewater is done through the COD test. For BPR activated sludge process design and operation it is necessary to identify and know the magnitudes of the various fractions of the influent COD as these significantly

affect the response of the process (Ekama and Marais, 1984; Ekama *et al.*, 1986). These fractions are needed to accurately describe the behaviour of the biological phosphorus removal process (Novotny, 1998). The efficiency of the BEPR process is influenced greatly by the carbon source and concentration (Lemos *et al.*, 1998). Figure 2.8 shows the COD subdivisions as presented by Dold *et al.*, 1991.

The influent COD (S_{ti}) of municipal wastewaters is divided into three main fractions, biodegradable (S_{bi}), unbiodegradable (S_{ui}) and heterotrophic active biomass (HAB) fractions. The biodegradable (S_{bi}) consists of two fractions: readily biodegradable soluble COD (RBCOD) and slowly biodegradable particulate COD (SBCOD). The RBCOD is further subdivided into two sub-fractions, SCFA (also called volatile fatty acids) and fermentable RBCOD (F-RBCOD) (Dold *et al.*, 1980; 1991; Nicholls *et al.*, 1985; Pitman, 1991; Wentzel *et al.*, 1994, 1999).

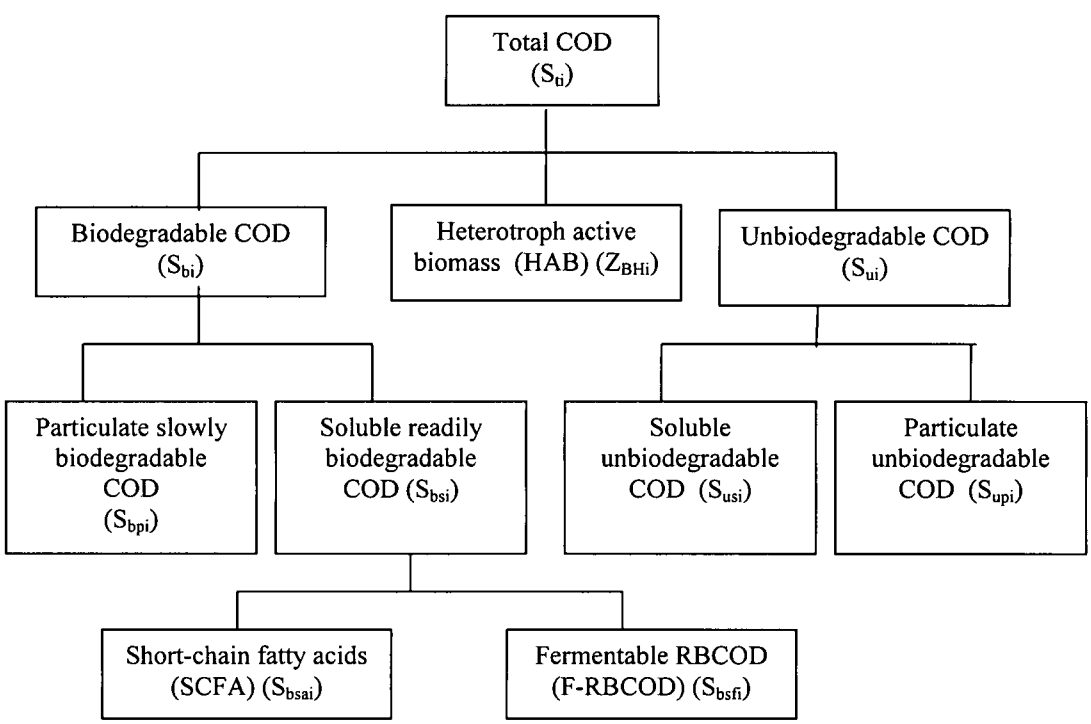


Figure 2.8: Division of influent COD into its various constituent fractions (Dold *et al.*, 1991).

RBCOD sub-fractions (SCFA and F-RBCOD) induce different responses during anaerobisis in BEPR system (Wentzel *et al.*, 1990). The SCFA component is directly available to PAOs for uptake in the anaerobic phase, whereas the F-RBCOD first requires conversion in the anaerobic reactor to SCFA to become available to PAOs. This conversion is an acid fermentation mediated (Lilley *et al.*, 1997; Mark *et al.*, 1997; Wentzel *et al.*, 1985, 1990, 1991).

The mathematical modelling of biological wastewater treatment processes and the design and operation of selector systems and nutrient removal plants require a reliable and accurate estimate of the readily biodegradable portion of the influent wastewater COD (Mamais *et al.*, 1993). One of the most significant achievements in the conceptual understanding and modelling of activated sludge systems is the recognition of substrate fractions with different biodegradation rates (Orhon *et al.*, 1999). The influent wastewater COD may be determined using the total biological demand (T_bOD) concept of Mullis and Schroeder (1971). RBCOD and SBCOD can be quantified either biological by the bioassay method or the rapid physical-chemical method, or a combination of both (Mamais *et al.*, 1993; Wentzel *et al.*, 1995; 1999; Novotny, 1998).

Studies indicate that the slowly biodegradable COD accounts for the bulk of organic content of domestic sewage and industrial wastewaters (Henze, 1992; Orhon *et al.*, 1994; Orhon *et al.*, 1998; Orhon and Ubay Çokgör, 1997). The particulate slowly biodegradable COD must first be sorbed onto the microorganisms and hydrolysed by extracellular enzymes before it can be incorporated into the cell mass (Ekama *et al.*, 1992; Orhon *et al.*, 1999). S_{bpi} can be determined from the influent biodegradable COD (determined by the T_bOD method) and the influent readily biodegradable soluble COD by:

$$S_{bpi} = S_{bi} - S_{bsi}$$

The unbiodegradable COD fraction (S_{ui}) is divided into unbiodegradable soluble COD (S_{usi}) and unbiodegradable particulate COD (S_{upi}). The soluble component will pass through the system unaltered and be discharged with the effluent, whilst the particulate portion will be enmeshed to the sludge floc surface and removed from the system via sludge wastage (Novotny, 1998).

HAB arises from the synthesis of living hetrotrophic organisms on biodegradable organic substrates and is "lost" via endogenous respiration/death processes (Ubisi *et al.*, 1997). HAB exists only as a hypothetical parameter within the structure of the design procedures and kinetic models of activated sludge system. Indirect evidence provides support for this parameter. A simple batch test procedure detailed by Wentzel *et al.*, 1995 and Mbewe *et al.*, 1995, has been developed to quantify heterotrophic active biomass (Ntshudisane *et al.*, 2000).

2.6.1.2 Phosphorus fraction in wastewater

Phosphorus is found in wastewater as phosphates. These can be categorised by physical (dissolved and particulate fractions) and chemical (orthophosphate, condensed phosphate and organic phosphate fractions) characteristics. The fraction of phosphorus in domestic wastewater is shown in figure 2.9 (Novotny, 1998). In both settled and raw municipal wastewaters, the orthophosphate fraction predominates, ranging between 70 to 90 per cent of the total phosphorus as PO_4^{3+}, HPO_4^{2-}, $H_2PO_4^-$, and H_3PO_4, which are available for immediate biological metabolism (Ekama and Marais, 1984; Gray, 1990).

Total phosphorus removal consists of adsorption and precipitation of colloidal and particulate P, uptake of P for cell synthesis, and EBPR for removal of the remaining P fraction not removed by other mechanisms (Ydstebø *et al.*, 2000). In activated sludge process, condensed and organically bound phosphorus in the influent is converted to orthophosphate by biological activity (Novotny, 1998). After secondary treatment, about 80% of the total phosphorus in the

final effluent is in the orthophosphate form (Sedlak, 1991). Average phosphorus concentrations in sewage range from 5-20 mg P/L as total phosphorus, of which 1-5 mg P/L is the organic fraction and the rest is inorganic (Gray, 1990).

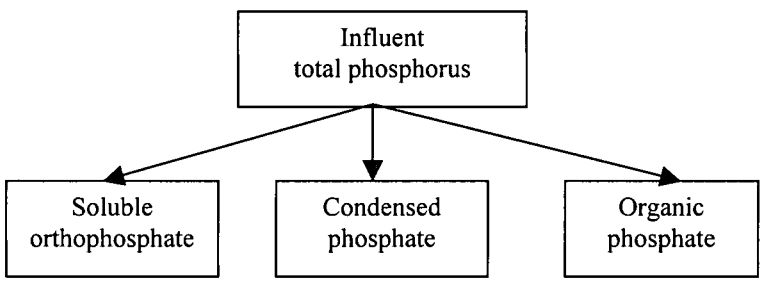


Figure 2.9: Fractions of phosphorus in domestic wastewater (Novotny, 1998).

Effluent phosphorus has been related to influent BOD/P and COD/P ratios (Sedlak, 1991). The COD/P ratio resulting in P-limitation depends on the fractions of dissolved and particulate COD and P (Randall *et al.*, 1992). It has been known that BOD/P ratio in the range 20-30 would provide effluent, soluble phosphorus concentration < 1mg/L for systems with relatively low sludge age (Sedlak, 1991). Whilst on the other hand influent total COD/P ratio > 35 would result in an effluent total phosphorus concentration < 1 mg/L (Randall *et al.*, 1992). A lower COD/P ratio enhances the fraction of poly-P organisms in the sludge and thus increases the P content. To ensure high EBPR, at reduced COD/P ratios sludge yield should be increased by reducing SRT (Ydstebø *et al.*, 2000).

By using the measured amount of phosphorus released under the anaerobic condition, a conservative P uptake-P release ratio of 1.15 and a ratio for metabolic phosphorus requirement of BOD/P, which is a function of mean cell residence time (MCRT), the feasibility of EBPR system can be evaluated (as cited by Park *et al.*, 2001).

The soluble effluent phosphorus concentration can be predicted as follows (Park *et al.*, 2001):

$$P_{eff} = P_{inf} - P \text{ release} \times 1.15 - \left[\frac{BOD}{5 \times (MRCT) + 90} \right]$$

where, P_{eff} and P_{inf} are influent and predicted effluent phosphorus concentrations, mg/L, and BOD and MCRT are measured in mg/L and days, respectively.

Total phosphorus and orthophosphate analysis involves two procedural steps, i.e. conversion of the phosphorus form into the dissolved orthophosphate by a digestion method (perchloric acid, nitric acid-sulphuric acid, persulphate oxidation) and colorimetric evaluation of the dissolved orthophosphate concentration (APHA, 1989). Colorimetric test methods include ascorbic acid method, automated ascorbic acid reduction method, vanadomolybdophosphoric acid method and stannous chloride method (NCSU, 1998).

2.6.1.3 Nitrogenous constituents

Nitrogen (N) exists in many forms because of the high oxidation states it can assume. The most prevalent forms of nitrogen in wastewaters are organic, ammonium and nitrate nitrogen (Sedlak, 1991). In untreated domestic wastewater, nitrogen (N) will be found primarily in the form of organic and ammonium nitrogen (NH_4^+-N). Little (<1 %) ammonia nitrogen (NH_3-N) exists in a normal domestic wastewater with a pH of 7. Typically total nitrogen in domestic wastewater consists of about 60% ammonium nitrogen and 40% organic nitrogen with less than 1% in the form of nitrate and/or nitrite (Novotny, 1998).

As with COD, nitrogenous material can be subdivided into fractions, i.e. biodegradable and unbiodegradable fractions (Ekama and Marais, 1984). The biodegradable organic N is ammonified in the anaerobic zone of the biological system, i.e. converted to NH_3-N through

various hydrolytic reactions, where it is made available for immediate incorporation into the bacterial protoplasm (Wanner, 1997). Nitrogen entering a wastewater treatment plant (WWTP) in the organic or ammonia form can be transformed to another form. Transformation of ammonia and organic nitrogen to the oxidised form of nitrate is accomplished through biological nitrification (Sedlak, 1991).

The generally accepted theory for biological phosphorus removal is that sequential anaerobic-aerobic contacting processes result in the selection of phosphorus removing microorganisms (Novotny, 1998). For ideal phosphorus removal systems, electron donors (COD) and acceptors (oxygen or nitrate) should not be present simultaneously. Disturbances occur when nitrate (or oxygen) and electron donors (COD) are both present in the anaerobic phase of the process, because energy can be obtained from the denitrification processes (Kuba *et al.*, 1994). Ekama *et al.*, 1984 suggested that if nitrates are positively excluded from the anaerobic reactor, excess P removal can be achieved if RBCOD fraction is in excess of 50 mg COD/L.

Nitrate can be introduced into the anaerobic zone by returned activated sludge from final clarifiers (in the case of a conventional activated sludge treatment plant) or by direct circulation flow (in the case of an oxidation ditch). The introduction of nitrate and nitrite depletes the readily biodegradable substrate, which is necessary for the PAOs. Therefore, the presence of nitrates in the recycled stream significantly inhibits the biological phosphorus removal potential (Comeau *et al.*, 1990; Jenkins and Tandoi, 1991; Sedlak, 1991; Kuba *et al.*, 1994; Tasli *et al.*, 1999).

2.6.2 Process control and design parameters

2.6.2.1 Loading rate

Loading rate is defined as the rate at which sewage (raw or settled) is applied to an activated sludge reactor. The rate at which the plant is loaded with carbonaceous materials directly influences the effluent quality, settling properties of the sludge and overall plant performance (Horan, 1990). Three loading factors are used in the design and operation of activated sludge systems, i.e. volumetric loading, organic loading and sludge loading (Gray, 1990).

(a) Volumetric loading

The volumetric loading is described as the flow of wastewater in relation to the reactor capacity. The retention time of the wastewater in the reactor, the hydraulic retention time (HRT), is expressed as (Gray, 1990):

$$HRT = \frac{V \times 24}{Q}$$

where, V is the reactor volume (m^3) and Q the rate of flow of the influent wastewater to the reactor ($m^{-3}d^{-1}$), so that the hydraulic retention time is expressed in hours. The HRT must be sufficiently long to allow the required degree of adsorption, flocculation and mineralisation (Gray, 1989).

(b) Organic loading

Organic loading is the measure of the amount of biological oxygen demand (BOD), which is applied per unit volume of aeration tank capacity (Gray, 1990; Horan, 1990). The BOD and COD tests are employed to measure the carbonaceous energy content of wastewater via its

oxygen demand (Novotny, 1998). Wastewaters have different organic contents and so it is useful to express organic loading in terms of COD in relation to the reactor capacity. COD offers a more quantitative representation of the electron donor capacity than BOD (Marais and Ekama, 1984). Another great advantage of the COD parameter is that it provides a direct estimate of the oxygen or energy potential of the volatile solids (Novotny, 1998). Organic load can be expressed in terms of Kg COD per tank capacity per day as (Gray, 1990):

$$\text{Organic load} = \frac{Q \times COD}{V}$$

where, Q is the influent wastewater flow rate (m^3/d), COD of influent wastewater in (Kg/m^3) and V is the volume of the reactor (m^3). The organic load is expressed as Kg COD $m^{-3}d^{-1}$. The organic load has a significant effect on the biological phosphorus removal system, hence higher organic loading rates provides higher VFA amounts and higher capacity to the bacteria to accumulate phosphates. The amount of polyphosphates can contribute to higher relative densities contributing to the settling speed of the sludge (Cuevas-Rodríguez *et al.*, 1998).

(c) Sludge loading

Research has indicated the relationship between biomass and phosphorus removal in activated sludge (Bosch, 1990; Streichan *et al.*, 1990; Sidat *et al.*, 1999). MLSS and mixed liquor volatile suspended solids (MLVSS) are often used as indicators of biomass, and used as such in the mathematical modelling of biological phosphorus removal (Sidat *et al.*, 1999; Ntshudisane *et al.*, 2000). With the biomass actively removing the organic fractions of the wastewater it follows that the COD loading should be related the amount of activated sludge in the aeration tank.

The sludge loading is referred to as the food (*f*) to microorganism (*m*) ratio, U, and is expressed as (Droste, 1997):

$$U = \frac{f}{m} = \frac{\text{Organic loading rate}}{\text{Volume of sludge}} = \frac{Q \times COD}{V \times X}$$

where, X is the MLSS in the aeration tank. The *f/m* ratio is expressed as grams COD per day per gram MLSS ($gg^{-1}d^{-1}$ or alternatively $Kg\ Kg^{-1}d^{-1}$).

The *f/m* ratio describes the degree of starvation of the microorganisms (Droste, 1997). The rate of metabolism of the microorganisms in the activated sludge aeration tank is controlled by the *f/m* ratio (Viessman and Hammer, 1985). The type of activated sludge process can be defined by the *f/m* ratio as being high-rate, conventional, or extended, see table 2.1 (Gray, 1990). To ensure optimum performance under field conditions, it is recommended that the maximum organic loading, expressed in terms of *f/m* ratio, be in the range from 0.05 to 1.5 Kg COD/Kg MLVSS.day, see table 2.1 for typical process loading ranges (Degrémont, 1991; Chudoba *et al.*, 1992; Toprak, 2001).

When the *f/m* ratio is high the microorganisms are in the exponential growth phase, which is characterised by excess substrate and maximum rate metabolism. While at low *f/m* ratios the overall metabolic action in the aeration tank is endogenous, with the substrate limiting microbial growth so that cell lysis and resynthesis occurs. The lower the *f/m* ratio, the greater the COD removal efficiency and the overall oxygen demand for the system (Gray, 1989, 1990).

Table 2.1: Typical process loading ranges (Toprak, 2001).

Loading Range	SRT (day)	*F:M* (Kg COD/Kg MLVSS. day)
High	3 –5	0.4 –1.5
Medium	5 – 15	0.2 – 0.4
Low	15- 30	0.05 – 0.2

2.6.2.2 Solids retention time (SRT)

SRT is the most important control parameter for biological phosphorus removal in the performance of activated sludge system (Sedlak, 1991; Lilley *et al.*, 1997). SRT affects the character and condition of activated sludge flocs within the aeration tank (Gray, 1990). SRT can be referred to as either sludge residence time or sludge age (θ_c). It can also be referred to as the mean cell residence time (MCRT) (Gray, 1990; Toerien *et al.*, 1990; Sedlak, 1991).

SRT is describes the ratio between the mass of sludge present in the reactor and the daily mass of excess sludge extracted from the unit, i.e. the residence time of the sludge in the system (Degrémont, 1991). The sludge or biomass requires a certain amount of time to assimilate the substrate and reproduce (Droste, 1997). SRT can be expressed in a simplified equation as:

$$\text{SRT or } \theta_c = \frac{V \times X}{X_w}$$

Where, X_w is the daily MLSS in the waste sludge stream. SRT is an operational factor giving control over sludge activity because SRT is the reciprocal of the net specific growth rate of the sludge and so can be considered a measure of sludge activity (Gray, 1990). SRT is related to the *f/m* ratio describing the relative state of starvation of the microorganisms.

Selection of the sludge age is the most fundamental and important decision in the design of an activated sludge process. The choice depends on many factors, including consideration of the effluent quality required (Ekama and Marais, 1984). The SRT value selected for design will be a function of treatment requirements (Sedlak, 1991). Where P removal is required, the sludge age can be selected to prevent nitrification (Toerien *et al.*, 1990). Consequently, EBPR systems should be operated at lowest SRT compatible with the nitrification/denitrification needs of the plant (Sedlak, 1991).

The SRT can be controlled by either altering the sludge wastage rate from the bottom of the clarifier or by direct abstraction of the sludge from the reactor (Gray, 1990; Osifo, 2000). Abstraction of the sludge directly from the reactor is a simple and more preferable method of sludge wastage. The mixed liquor concentration within the reactor is not affected by the diurnal flow patterns as much as in the clarifier underflow. The wasted sludge MLSS and the reactor MLSS concentrations are the same; Lilley *et al.*, 1997). Volumetric control is the simplest way of ensuring adherence to prescribed SRTs (Toerien *et al.*, 1990). Using this type of control, if a sludge age of say 15 days is required, one-fifteenth of the reactor volume is wasted every day (Ekama and Marais, 1984; Lilley *et al.*, 1997).

2.6.2.3 Mixed liquor suspended solids (MLSS)

The concentration of suspended solids in the aeration tank is referred to as the mixed liquor suspended solids (Gray, 1990). It is a parameter, which is used in activated sludge to measure biomass within the aeration tank (Ntshudisane *et al.*, 2000). It is measured in the same manner as suspended solids in wastewater, by filtering a known sample of mixed liquor through Whatman GF/C filter paper and weighing it after drying in an oven at 105°C (APHA, 1989).

MLSS comprises of three components viz., heterotrophic active biomass, endogenous residue and inert material (Ubisi *et al.*, 1997; Ntshudisane *et al.*, 2000). The viable biomass fraction of the MLSS is the key to phosphate removal by activated sludge (Ntshudisane *et al.*, 2000). The successful operation of EBPR activated sludge plants is dependent on good operation, coupled with good design and maintenance of the correct biomass load (Ntshudisane *et al.*, 2000). Previous research has indicated that phosphate uptake was related to an increase in biomass concentration (Bosch, 1990; Streichan *et al.*, 1990). In theory the higher the MLSS concentration in the aeration tank the greater the efficiency of the process, as there is a greater biomass to utilise the available food (Gray, 1990). An increase in biomass concentrations in EBPR system increases phosphate uptake capacity, resulting in higher phosphate removal efficiency (Sidat *et al.*, 1999; Ntshudisane *et al.*, 2000).

MLSS is a basic parameter that is used in the calculation of a number of other operating parameters in activated sludge system. For daily operational control the MLSS is quite adequate with the MLVSS and other measures of sludge activity used mainly for research and development work. Normal MLSS concentrations range from 1500-3500 mg/L for conventional activated sludge units, rising to 8000 mg/L for high-rate systems. MLSS concentration is controlled by altering the sludge wastage rate (Gray, 1990).

2.6.2.4 Sludge settleability

Most problems associated with the activated sludge process involve poor settleability (Gray, 1990). The effectiveness of settling of activated sludge from the treated mixed liquor is mainly dependent on the ability of the activated sludge to form flocs. The settleability is mainly dependent on the structure, size and density of the activated sludge flocs (Wilén and Balmér, 1998). Therefore a rapid method of assessing settleabiity is vital if good separation of the sludge in the sedimentation tank is to be maintained and a final effluent with low suspended solids concentration (Gray, 1990). The ability of activated sludge to separate is

normally measured by an index of settleability such as the sludge volume index (SVI), the sludge density index (SDI), or the stirred specific volume index (SSVI) (Keerfer, 1963; Dick and Vesilind, 1969; Lee *et al.*, 1996). SVI is the most widely used test for sludge setlleability in wastewater treatment. It is a measure of sludge settleability and compactibility that is made from a laboratory column-settling test (Droste, 1997). The procedure is out lined in Standard Methods (APHA, 1989).

SVI is defined as the as the volume in milliliters occupied by 1 g of sludge after it has settled for a specific period of time (normally 30 minutes) (Droste, 1997). It is expressed as (Gray, 1990):

$$SVI = \frac{V \times 1000}{MLSS} \ (ml\ g^{-1})$$

Where, V is the volume of settled sewage and MLSS is the mixed liquor suspended solids in (mg/L). A low SVI is indicative of a sludge that settles well, i.e. a good sludge would have SVI < 80 ml g^{-1} and very good one around 50 ml g^{-1}. A high SVI indicates a poor settleability and in general a sludge with SVI > 120 ml g^{-1}. Whereas SVI > 150 ml g^{-1} indicates settling problems and possibly bulking (Gray, 1990).

The *f/m* ratio and SRT influence the settleability and compactibility of the sludge. When the biomass is in a state of endogenous decay, it tends to form polymers that results in natural flocculation under quiescent conditions. In a complete mixed reactor at very high SRT or highly starved conditions, the sludge forms pinpoint floc (like the head of a needle) and does not flocculate well as in the intermediate ranges. At the other extreme at low SRT the sludge tends to become populated with filamentous organisms that exhibit poor settleability and the sludge does not flocculate as well (Droste, 1997). This indicates that the sludge settleability is

dependent on the microbiological, biochemical and physico-chemical properties of sludge (Gray, 1990).

2.6.2.5 Sludge bulking

Another major problem in the operation of activated sludge process is the growth of filamentous organisms, which leads to sludge bulking (Dick and Vesilind, 1969). Bulking is a phenomenon where filamentous organisms extend from the flocs into the bulk solution, interfering with the settlement and subsequent compaction of the activated sludge, with SVI > 150 ml g^{-1} (Pipes, 1967).

The major problem associated with bulking is poor sludge compaction. This results in much thinner sludge being returned to the aeration tank with low MLSS, which leads to difficulty of maintaining the desired operational MLSS in the aeration tank with a subsequent fall in effluent quality (Gray, 1990). Many causes of bulking have been identified although in practice it is extremely difficult to accurately diagnose the particular operational factor or set of conditions, which has caused the onset of filamentous growth in the mixed liquor. The most frequently cited causes of bulking are low dissolved oxygen (DO) concentration in the aeration tank, low organic loading (*f/m*), septic influent, nutrient deficiency and low pH (< 6.5) (Jenkins *et al.*, 1984).

The problem of bulking sludge in enhanced phosphorus removal in activated sludge plants has been reported (Pitman, 1984). In bio-P removal systems, with sequential anaerobic-aerobic zones, the proliferation of PAOs leads to the rapid sequestration of low molecular mass compounds, generated by acidogenesis and acetogenesis, in the anaerobic phase. Provided that the available substrate is largely utilised in this way, filamentous microorganisms growth can be effectively suppressed in P-removal plants (Wanner *et al.*, 1987). However, if slowly hydrolysable compounds pass through this reactor into the aerobic

stage, then the relatively high rate of microbial utilisation of hydrolysed products in this zone may result in low concentrations of organic compounds in the bulk liquid, a condition favouring the growth of filamentous microorganisms over floc-forming species (Ekama and Marais, 1985).

There is currently no specific control method for sludge bulking. Many of the control methods available are contradictory while others are quite bizarre (Chambers and Tomlinson, 1982). However, controlling bulking can be categorised into three broad categories, viz., process modification, operational control and chemical addition. Chemical control with the use of disinfectants or coagulants is extremely popular as it provides an immediate solution to the problem (Jenkins *et al.*, 1984; Gray, 1990). Tomlinson and Chambers, 1984 have proposed the use of an "Action Flow Sheet" to not only help diagnose the cause of bulking but to remedy the problem. However, in practice this approach is an extremely lengthy process, which has various options are investigated, leads to the mixed liquor being constantly washed out of the system in the final effluent.

2.6.3 Environmental factors

2.6.3.1 Dissolved Oxygen (DO)

In many types of SBRs, operation depends heavily upon dissolved oxygen. Therefore, a reliable DO measuring probe is extremely important for measuring the DO levels in the SBR basins. A DO control system optimises batch reactor performance (Laughlin *et al.*, 1998; Shamskhorzani and Norcross, 2000). The availability of DO and organic loading are the two most significant factors influencing the size distribution of activated sludge flocs (Wilén and Balmér, 1998). DO is also a function of sludge loading, increases as the sludge loading increases (Palm *et al.*, 1980). Starkey and Karr, 1984 reported that low DO concentrations lead to a poorer flocculated activated sludge and a more turbid effluent (as cited in

Shamskhorzani and Norcross, 2000). Knudson *et al.*, 1982 found a trend towards larger flocs with increased DO concentration (as cited in Shamskhorzani and Norcross, 2000).

Practical experience has shown that an average DO in the aerobic zone should be about 2-3 mg/L and should not be allowed to decline below 1 mg/L at any stage. A DO profile starting at about 1 mg/L near the inlet and rising to about 3 mg/L at the outlet is suitable. Filamentous bacterial growth is enhanced and nitrification is suppressed by DO values below 0.5 mg/L (Adamse, 1968; Palm *et al.*, 1980; Toerien *et al.*, 1990; Wilén and Balmér, 1998). Excessively high DO levels (4-5 mg/L) should be avoided, because "pin-point" floc formation can be caused and power will be wasted (Toerien *et al.*, 1990; Shamskhorzani and Norcross, 2000).

Oxygen is the terminal electron acceptor in aerobic metabolic processes (Droste, 1997). The phosphorus removal efficiency has been found to be related to the efficiency of the DO control in the EBPR system. DO concentrations of 1.5 to 3.0 mg/L are generally considered adequate for phosphorus uptake in EBPR systems (Pipes, 1979; Sedlak, 1991). If the DO is less than this, phosphorus removal efficiency may be reduced (Sedlak, 1991). The mechanism of biological phosphorus uptake suggests that a higher DO level may increase the rate, but not the magnitude of phosphorus uptake (EPA, 1987).

2.6.3.2 Temperature

The effect of temperature on bio-P removal process has been studied during recent years (Mamais and Jenkins, 1992; McClintock *et al.*, 1993). Temperature has an important influence on bio-P processes, especially due to the storage processes involved. However, the temperature effect on the stoichiometric and kinetics of the anaerobic and aerobic metabolism of EBPR process is not a straightforward case because of the different influence of temperature on the sub-processes (Baetens *et al.*, 1999). For some sub-processes, such as P

release, acetate uptake, PHA and oxygen consumption, it is not clear where the optimal temperature lies, between 20 °C and 30 °C, or even above 30 °C (Brdjanovic *et al.*, 1998a; 1998b).

The bacterial population in EBPR activated sludge system shifts with temperature changes (Kavanaugh and Randall, 1994; Brdjanovic *et al.*, 1998a; Baetens *et al.*, 1999). This means that the different metabolic processes occurring in bio-P bacteria might have a different temperature optimum. According to the current models of EBPR the uptake of phosphorus in the aerobic zone is related directly to the quantity of phosphate released in the anaerobic zone at temperatures between 5 °C and 30 °C (Barnard, 1983; Hong *et al.*, 1984; Spatzierer *et al.*, 1985; Brdjanovic *et al.*, 1997).

Helmer and Kunst, 1998 found that at temperature of 10 °C aerobic phosphate uptake was increasingly independent of phosphate release in the anaerobic zone. At 5 °C absolutely no correlation between anaerobic P release and aerobic P uptake occurred. Shapiro *et al.*, 1967 reported that anaerobic P release rates decreased by 2.1 – 2.6 times for every 10 °C temperature decrease in the range 10 – 30 °C. Boughton *et al.*, 1971 found that 24 – 37 °C to be the optimum range for aerobic P uptake. The aerobic batch experiments carried out by Mamais and Jenkins, 1992 indicate that the optimum temperature for maximum EBPR is in the range 28 – 33 °C. Declining phosphate release and uptake rates were observed at temperatures of 35 °C and higher, with a significant inhibition at 42.5 °C while no phosphate release or uptake was observed at 45 °C, indicating that at this temperature the phosphate removing bacteria were probably dead (Jones and Stephenson, 1996).

Several studies have coupled SRT with wastewater temperature (Brdjanovic *et al.*, 1998; Ydstebø *et al.*, 2000). Performance of EBPR at low temperatures is related to reduced biological reaction rates. However, the potential reduced performance can be controlled by SRT. McClintock *et al.*, 1993 observed EBPR at a 5-day SRT and 10 °C. Mamais and

Jenkins, 1992 reported a limiting SRT of 2.9 days at 13 °C. Marklund and Morling, 1994 reported that the limiting low temperature for EBPR might be between 4 and 5 °C at a 15-day SRT.

EBPR was studied in laboratory batch units over a temperature range of 5 – 15 °C by Sell *et al.*, 1981. They found that the amount of phosphorus removed at 5 °C was 40% greater than that removed at 15 °C. In contrast Smolders *et al.*, 1995 reported that at 5 °C, it was not possible to operate the EBPR process at SRT of 16 days. But by increasing the SRT seems to be appropriate to achieve acceptable EBPR at these temperatures.

Within the temperature range 13.5 – 20 °C, (Baetens *et al.*, 1999) it was demonstrated that EBPR functions efficiently and independently of SRT for aerobic SRTs above 2.1 days. At lower SRT values EBPR capability may be lost at an aerobic SRT that depends on temperature. Higher temperatures allow efficient EBPR to be maintained at lower SRT values (Mamais and Jenkins, 1992). Smolders *et al.*, 1995 reported that at 20 °C the minimum SRT is around 2 days. This was also observed by Shao *et al.*, 1991; McClintock *et al.*, 1993 and Baetens *et al.*, 1999.

2.6.3.3 pH

The efficiency of biological phosphorus removal system is influenced by parameters such as pH (Liu *et al.*, 1996). Smolders *et al.*, 1994 found that the phosphate release was kinetically and thermodynamically influenced by pH in the range 5.5 – 8.2. Liu *et al.*, 1996 also found that pH values in this range have influence on both substrate uptake and phosphate release. Tracy and Flamino, 1987 studied the effect of pH on the specific phosphorus uptake rate in the aeration zone. There was little effect of pH within the range of 6.5 to 7.0. Below the pH value of 6.5 activity steadily declined and all activity was lost at pH of 5.2.

Many researchers reported that the success of EBPR system depends on the competition between glycogen-accumulating organisms (GAOs) and PAOs. It was found that pH could be used as a tool to control this energy based competition. Jeon *et al.*, 2001 found that at a relatively high pH (~ 8.0), acetate uptake requires more ATP compared with low pH conditions. EBPR can be achieved when PAOs dominate over GAOs in the competition of acetate uptake (Matsuo, 1994; Smolders *et al.*, 1994). Jeon *et al.*, 2001 also found that pH increase result in a shift of the dominant population to PAOs, which resulted in the success of EBPR. Pure culture studies by Groenestijn and Deineman, showed that the maximum specific growth rate of *Acinetobacter* was 42 percent higher at a pH of 8.5 compared to a pH of 7.0 (as cited by Sedlak, 1991). Results of studies on the effects of pH suggest that more efficient biological phosphorus removal occurs at pH value 7.3 ± 0.5 (Sedlak, 1991; Liu *et al.*, 1996). Operation of reactor without pH control was found to be more favourable in terms of stability and efficiency of EBPR system (Bond, *et al.*, 1999; Serafim, 2000; Jeon *et al.*, 2001).

2.7 MICROBIOLOGY OF BIOLOGICAL PHOSPHORUS REMOVAL

The microbiology of enhanced P-removal activated sludge systems has received some attention but is far from completely understood (Toerien *et al.*, 1990). In spite of the widespread use of EBPR systems there are still uncertainties about which groups of organisms participate in the process and about the underlying metabolism. As activated sludges consist of mixed populations with a changing combination of bacterial species, the determination of the microorganisms involved is exceedingly difficult (Helmer and Kunst, 1998). The microorganisms responsible for bio-P removal are partly unknown with respect to taxonomy, but we know that they are present in all biological wastewater treatment processes (Henze, 1996; Van Loosdrecht *et al.*, 1997; Crocetti, 1999).

Microorganisms, which are able to accumulate phosphate as polyphosphate inside the cell, are widely used in enhanced bio-P removal from wastewater (Van Loosdrecht *et al.*, 1997). The

success of bio-P removal process is based on the ecological selection of a group of bacteria (poly-P organisms) capable of accumulating phosphates in quantities greater than needed for anabolic processes. The conditions that favour the growth of these microorganisms are subjecting the biomass in the wastewater treatment system to alternating anaerobic aerobic conditions. Other conditions have to be provided too to guarantee the development of poly-P organisms (Muñoz-Colunga and González-Martínez, 1996; Ydstebø *et al.*, 2000).

Biological models have been developed to explain how the poly-P organisms achieve phosphate removal and how they are selected for in the EBPR system (Wentzel *et al.*, 1991; Satoh *et al.*, 1992; Smolders *et al.*, 1994). These models have been established primarily from the findings of investigations carried out on mixed culture activated sludge. Therefore, knowledge of the biochemical reactions of the EBPR process is largely derived from indirect observations and theoretical considerations (Bond *et al.*, 1999). Microbiological details pertinent to EBPR are lacking since it has not yet been established which bacteria are important to the process (Cloete and Steyn, 1987; Wagner *et al.*, 1993; Manz *et al.*, 1994).

One genus of bacterium frequently cultured and suspected to have a role in EBPR is *Acinetobacter*, in the γ subclass of the class *Proteobacteria* (Fuhs and Chen, 1975). Traditionally, bacteria of the genus *Acinetobacter* are more accepted to be model organisms responsible for the excess phosphate removal (Fuhs and Chen, 1975; Buchman, 1983; Helmer and Kunst, 1998). Their growth kinetics and physiology underlie current bio-chemical and mathematical process models (Wentzel *et al.*, 1991). The methods employed for the enumeration of polyphosphate bacteria has led to the assumption that *Acinetobacter* is the main polyphosphate bacteria in enhanced phosphate removing activated sludge systems (Toerien *et al.*, 1990; Christensson, 1997). The use of fluorescence *in situ* hybridization (FISH) probing and cloning of 16S ribosomal DNA to describe activated sludge bacterial

communities has shown that *Acinetobacter* and β - *Proteobacteria* are dominant in EBPR mixed communities (Wagner *et al.*, 1994; Bond *et al.*, 1995).

The genus *Acinetobacter* consists of aerobic cocco-bacilli, which occur in pairs and chains or clusters, and are found naturally in soil, water and sewage (Carberry and Tenney, 1973; Juni, 1984; Sedlak, 1991). These bacteria are Gram-negative with a size of 1 – 1.5 μm (Benefield *et al.*, 1990; Bosch, 1992). They are known to prefer simple substrate (acetate) as would be produced in fermentation reactions in anaerobic zones of biological phosphorus removal systems (Sedlak, 1991). *Acinetobacter* cells can accumulate polyphosphate to *ca* 60 % of their cell volume, which corresponds to 18 % (m/v) polyphosphate or 10 – 20 % polyphosphate on a dry weight basis (Deinema *et al.*, 1980; Buchan, 1983). By using various size and volume calculations, Momba and Cloete, 1996 found that *Acinetobacter* is capable of removing a maximum of 10^{-10} mgP/cell. However, Bosch and Cloete observed P removal capacities of *ca* 10^{-11} to 10^{-12} mgP/cell for *Acinetobacter* isolates from activated sludge mixed liquor. Drysdale *et al.*, 2000 observed removal capacities of 8.2×10^{-11} and 6.1×10^{-11} mgP/L respectively in their studies.

More recent research has shown that while *Acinetobacter* species can always be detected in the activated sludges of EBPR plants, their actual numbers evaluated by using chemotaxonomical or molecular biological methods are below 10% of the population (Hiraishi *et al.*, 1998; Cloete and Steyn, 1988a). The ability to store phosphate could not be detected in all *Acinetobacter* strains isolated from activated sludges (Bayly *et al.*, 1991; Beacham *et al.*, 1992). According to these recent studies other bacterial species may also be of importance in EBPR process (Appeldoorn *et al.*, 1992). The importance and function of *Acinetobacter* spp. in the phosphorus removal process is not clear, as too little is known about the phosphate storing capacities of other bacteria in activated sludge (Helmer and Kunst,

1998). Brodisch and Joyner, 1983 found that activated sludge plants had a variety of populations without any microorganism dominating.

Nakamura *et al.*, 1989 have isolated a number of Gram-positive bacteria from sludge, which are able to accumulate high amounts of phosphorus. Cloete and Steyn, 1988a, b could not detect enough *Acinetobacters* in their sludge to account for the observed phosphorus removal. Lötter, 1985 found that biological phosphorus removal is the result of cooperation of different groups of bacteria, primarily fermentation bacteria, PAOs, other heterotrophs, and autotrophs, in some systems. Drysdale *et al.*, 2000 observed that *Pseudomonas* spp. play a vital role in P removal in the BNR activated sludge system. Drysdale *et al.*, 2000 also isolated other genera that show the ability to accumulate P when studied in homogenous cultures, which include *Aeromonas*, *Bacillus*, *Micrococcus*, *Moraxella*, *Staphylococcus*, *Streptococcus*, *Alcaligenes* and *Enterobacter*. Brodisch and Joyner, 1983; Lötter, 1985; Suresh *et al.*, 1985; Venter *et al.*, 1989; Streichan *et al.*, 1990; Auling *et al.*, 1991 also found that *Aerobacter*, *Arthrobacter*, *Proteobacteria* and *Xanthobacter* spp.as well as the filamentous *Microthrix* and *Norcardia* spp. can accumulate polyphosphates.

There have been recent reports of bacteria inhibiting EBPR in laboratory-scale activated sludge system designed for P removal (Cech and Hartman, 1993; Satoh *et al.*, 1994). These organisms are called G-bacteria or GAOs, which grow as cocci arranged in tetrads are thought to be able to out compete the PAOs in anaerobic-oxic biological nutrients removal plants by accumulating polysaccharide and not polyphosphate (Cech and Hartman, 1993; Mino *et al.*, 1995; Maszenan *et al.*, 1998). As with PAOs, there is little known about the ecological details of GAOs and how they affect EBPR. GAOs are thought to be able to effectively compete with fermentative organisms for RBCOD and PAOs for VFAs in the anaerobic zone as they are able to obtain the necessary reducing power and energy required for uptake through glycolysis, i.e. the Embden-Meyerhof pathway. Their proliferation in an EBPR system will eventually lead to a decline in phosphorus removal efficiency (Atkinson, 1999).

The success of EBPR depends on the competition between PAOs and GAOs, and some operational factors, which are suspected to be the possible causes of GAO dominance, such as long SRT (Fukase *et al.*, 1985), high ratio of anaerobic-aerobic HRT (Matsuo, 1994), addition of glucose as a cosubstrate (Cech and Hartman, 1993), low COD/P ratio in the feed (Liu *et al.*, 1997), and excessive aeration (Brdjanovic *et al.*, 1998b). However, in most studies, no clear indication of GAOs dominance resulting in deterioration of EBPR has been obtained (Jeon *et al.*, 2001). The mechanism of enhanced P-removal in activated sludge systems must therefore depend on PAOs, which in nature are favoured by fluctuating conditions of anaerobiosis-aerobiosis and conditions that are favourable to PAOs over the GAOs (Fuhs and Chen, 1975; Brodisch and Joyner, 1983; Brodisch, 1985; Toerien *et al.*, 1990; Muñoz-Colunga and González-Martínez, 1996).

2.8 SUMMARY

A brief summary of EBPR system may be explained as follows: Under anaerobic poly-P organisms take up VFAs and store them as PHB. During anaerobic phase poly-P organisms compete with facultative bacteria for the substrate by obtaining energy through hydrolysis of stored polyphosphate, which results in release of orthophosphate. In the following aerobic zone poly-P organisms metabolise the previously stored organic material (PHB) to obtain energy to capture phosphate and replenish the energy rich polyphosphate. The net results are a reduction of P in the wastewater, and withdrawal of P-rich sludge completes the P removal.

A number of factors have been postulated or shown to play a role in P release and uptake phenomena as discussed in section 2.4 on mechanisms of biological phosphorus removal system. These mechanisms are summarised in table 2.2.

Table 2.2: Biological phosphorus removal steps

Phase/Process	End results
Anaerobic Zone 1. Fermentation 2. Bio-P storing obtains VFAs	• RBCOD converted to VFAs by facultative organisms • VFAs transferred into cell • Orthophosphate release provides energy • VFAs converted to PHB
Aerobic Zone 1. Phosphate uptake 2. New cells produced	• PHB oxidised • Energy captured in polyP bonds • Orthophosphate removed from solution
System Phosphorus removal 1. Excess sludge wasting	• Phosphorus removal via wasted sludge

CHAPTER 3

EDIBLE OIL EFFLUENT CHARACTERISATION

3.1 INTRODUCTION

Wastewater characterisation is an important first step when designing new plants or evaluating the operation and efficiency of existing plants. It also provides useful information for the construction and operation of future plants. It accumulates useful input data for modelling studies, which can then be used to simulate best and worst case, scenarios with regard to biological processes (Schroeder, 1977).

When designing and operating a BPR activated sludge process, the characteristics of the wastewater to be treated are the most significant factors to consider. These characteristics dictate the selection of the process to be employed as well as the degree of phosphorus removal, which will be attainable (Temmink *et al.*, 1996; Atkinson, 1999). It is also important to characterise the wastewater quantitatively and qualitatively when designing a wastewater treatment plant for biological phosphorus removal (Mkhize, 2002).

Traditionally, physical-chemical analyses are used to monitor and characterise wastewater quality (Sedlak, 1991). Hence, it is necessary to monitor the wastewater quality extensively before designing a wastewater treatment plant. Fluctuation in wastewater quality and flow rates may affect the potential of wastewater treatment plant to achieve consistent biological phosphorus removal (Mkhize, 2002). The evaluation of wastewater quality by means of physical-chemical analyses and the use of information to apply the correct technology and

corrective plan in the treatment of wastewater plays an important role in designing a wastewater treatment plant for biological phosphorus removal (Schroeder, 1977).

Water is a major utility in the edible oil industry, which results in the significant effluent volumes being generated hence the challenge of its disposal cannot be ignored. The volume of effluent arising from the edible oil industry is dependent on the type of product being processed and the degree of water management being exercised. The effluent of edible oil industry may contain high concentrations of oil (FOG) as oil is of concern due to their adverse effects on the environment, such as toxicity to the living organisms (Adam and Marjanovic, 1996; Bekir, 2001). In addition wastewaters from the oil industry may be high in organic content (BOD and COD), dissolved solids, sodium, sulphates and phosphates (Steffen *et al.*, 1989; WBG, 1998). Vegetable oils are the principal sources of linoleic and linolenic acids (ISEO, 1999).

BPR processes are among the most economic methods to remove phosphorus from wastewater; however, not all wastewater seems suitable for BPR. If the wastewater is poorly characterised, a BPR process may not be feasible or may be improperly designed (Park *et al.*, 2001).

3.2 MATERIALS AND METHODS

This part of the study was conducted to characterise and quantify the wastewater and their sources from a typical edible oil refining plant at Company A. Company A is situated in Pietermaritzburg, in the middle of KwaZulu-Natal Province, South Africa. The factory consists of three production plants, viz. the refinery plant, which produces refined oil; the acid oil plant, which produces soap stock and acid oil and the soap plant, which produces soap from soap stock and candles.

Field survey and sampling of the refinery oil effluent was carried out every second week for the first four months of the study to familiarise one with the processes employed at Company A. Composite wastewater (untreated effluent) samples were collected from the discharge channel of the refinery plant every second week for the first four months and thereafter once a month over a period of ten months. The samples were collected in 25 litre containers and then immediately transported to the Centre for Water and Wastewater Research (CWWR) testing laboratory at the Durban Institute of Technology, formerly known as Technikon Natal, in Durban. The analyses were performed immediately on arrival to the laboratory to determine effluent characteristics. The bulk of the samples after analysis were stored at temperatures below 4 °C in the cold room to prevent biological activity that could results in the deterioration in the effluent quality.

The samples were analysed for the parameters considered to be necessary for wastewater characterisation for the design of a bio-P removal system. The results of parameters analysed for are summarised in table 3.1. Total suspended solids and Fats, oil and grease (FOG) were analysed according to Standard methods (APHA, 1989) (APPENDICES 7 and 8). COD, ammonium, nitrates and sulphates were analysed for by a colourimetric method, using SQ 118 spectrophotometer during the first eight months of the study (APPENDICES 1, 4, 5 and 6). NOVA 60 spectrophometer from Merck was used for the other five months period of the study. Total phosphorus and soluble P were measured using the vanadate-molybdate (VM) method (APPENDICES 2 and 3) at 470nm absorbance by UV/VIS spectrophometer. All samples were analysed in triplicate. pH and temperature were measured using a bench scale pH meter with a temperature probe.

3.3 RESULTS

During the survey, wastewater was quantified and characterised, results for the characterisation of the wastewater are summarised in table 3.1 and graphically presented in figures 3.1 – 3.7.

Table 3.1: Wastewater characterisation of the untreated oil effluent from Company A refinery plant.

Parameters (mg/L)	Low	High	Mean
pH	9.66	12.16	11.17
COD_t	3631	8523	5990
COD_s	1651	7427	4601
PO_4-P	112	530	355.7
NO_3-N	0.98	1.63	1.29
NH_4-N	0.39	0.88	0.57
Suspended solids	1060	5240	3137
FOG	11130	80110	44399
SO_4	47.1	285.5	112.2

Total and soluble COD concentrations for the untreated oil effluent from Company A over 12 months period are presented in figure 3.1. Total COD concentrations for the untreated oil effluent ranged from 3631 mg/L to 8523 mg/L. Minimum and maximum soluble COD concentrations of 1651 mg/L and 7427 mg/L were obtained, respectively over the twelve months period of the characterisation of the untreated oil effluent as illustrated in figure 3.1. Raw data for the characterisation of the untreated oil effluent over twelve months period is presented in APPENDIX 12A.

FOG profile for the untreated oil effluent presented in figure 3.2 was determined on the basis that all the FOG substances contained in the effluent were similar in physical characteristics and their common solubility in diethyl ether. That is FOG measured was presented as material recovered or substance soluble in diethyl ether. This method of FOG analysis is suitable for most industrial wastewaters including vegetable oil effluent (APHA, 1989). FOG

concentrations ranged from minimum of 11.13 g/L to maximum of 80.11 g/L. FOG concentrations were presented as mg/L as illustrated in figure 3.2. FOG concentration during this experimental period was averaged at 44399 mg/L.

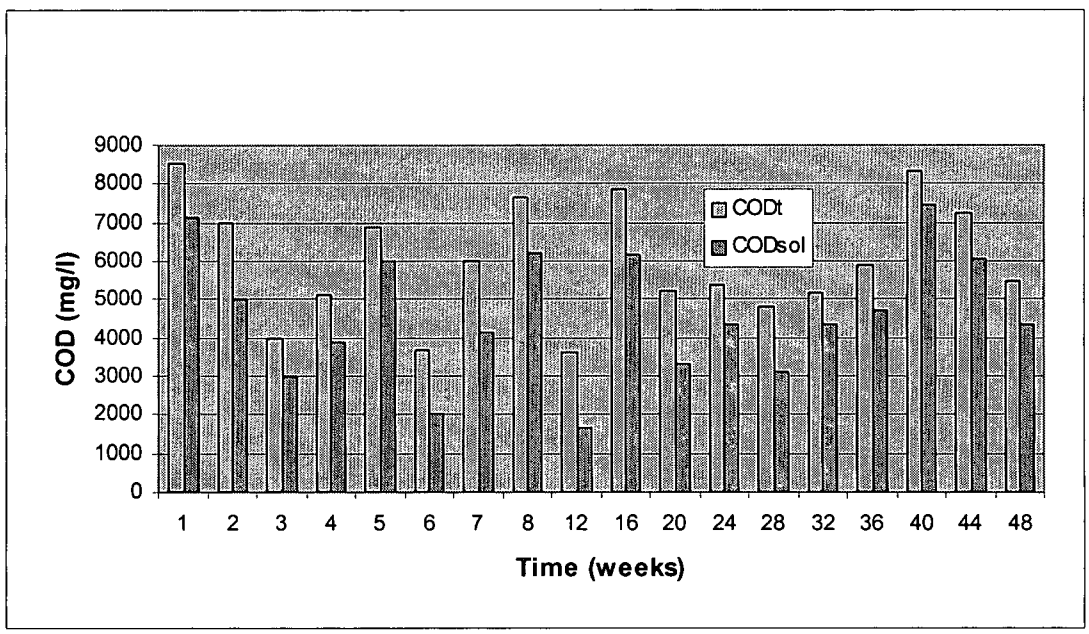


Figure 3.1: Total and soluble COD profiles of untreated effluent over a period of twelve months.

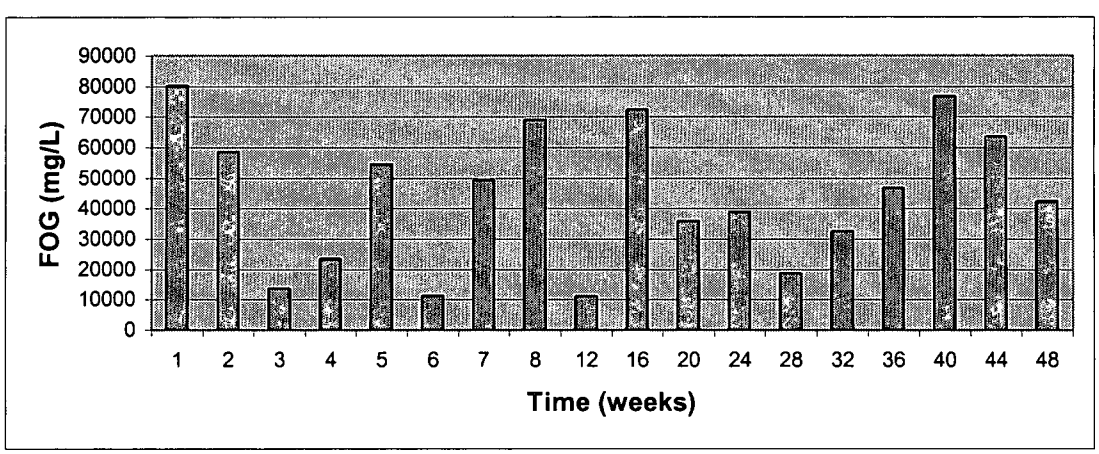


Figure 3.2: FOG profile of untreated effluent over a period of twelve months.

Total phosphorus concentrations of untreated effluent presented in figure 3.3 were measured using the vanadate-molybdate (VM) method (APPENDIX 2). The TP concentrations of untreated effluent presented in figure 3.3 ranged from minimum P concentration of 112 mg P/L to maximum P concentration of 530 mg P/L, respectively over the first three months of

the experimental work. Average P concentration of 355.7 mg P/L was achieved during this period.

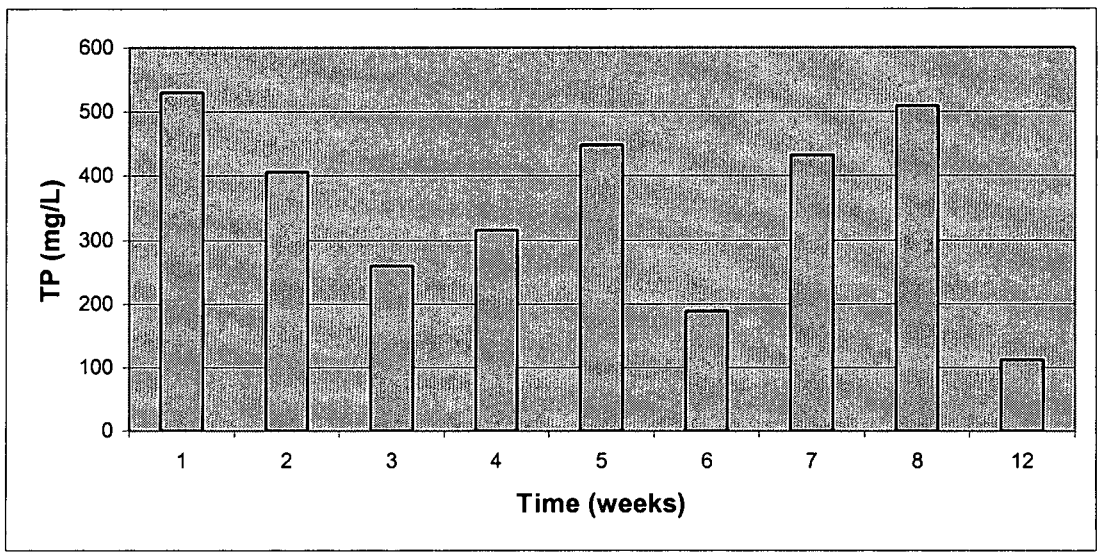


Figure 3.3: TP profile of untreated effluent over the first three months period.

Nitrate and ammonia profiles of untreated effluent presented in figure 3.4 ranged from average low concentrations of 0.98 mg/L and 0.39 mg/L to average high concentrations of 1.63 mg/L and 0.88 mg/L, respectively over the twelve months period of experimental work.

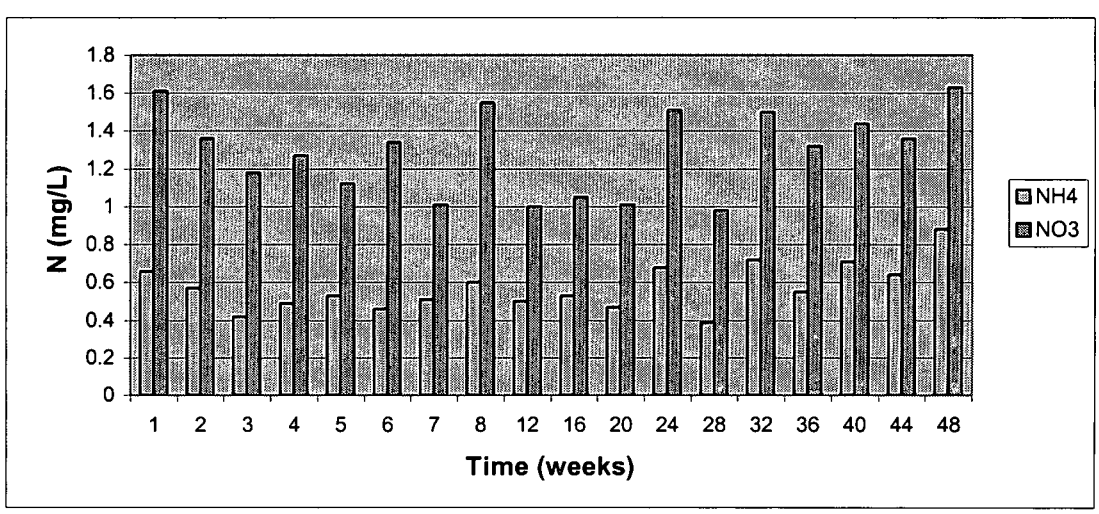


Figure 3.4: Nitrate and ammonia profiles of untreated effluent over a period of twelve months.

pH values, sulphate and total suspended solids concentrations for the untreated oil effluent from Company A over 12 months period are presented in figures 3.5, 3.6 and 3.7, respectively. Raw data for the characterisation of the untreated oil effluent over twelve months period is presented in APPENDIX 12A and summarised in table 3.1 above.

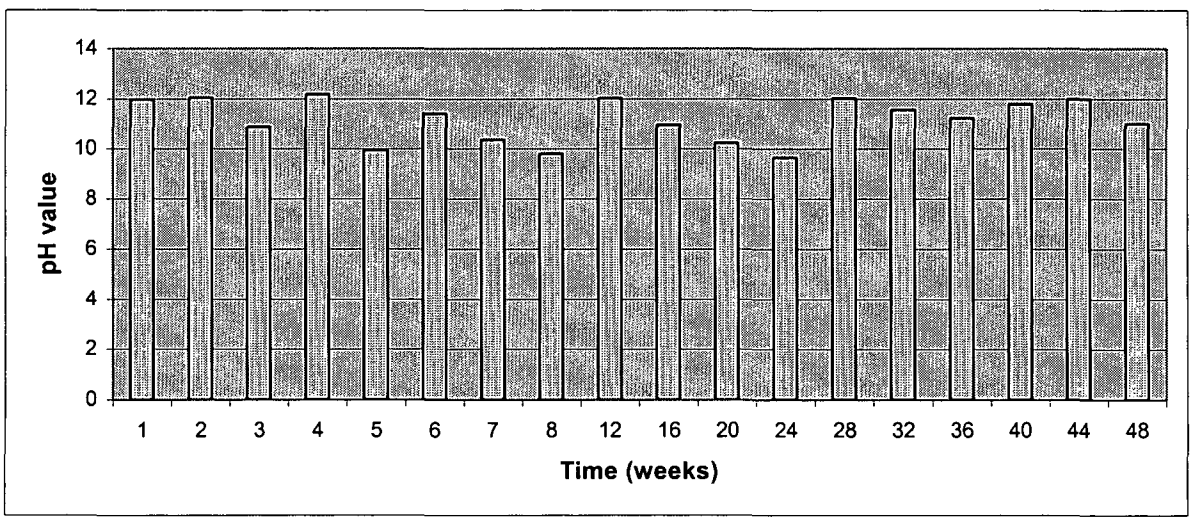


Figure 3.5: pH profile of untreated effluent over a period of twelve months.

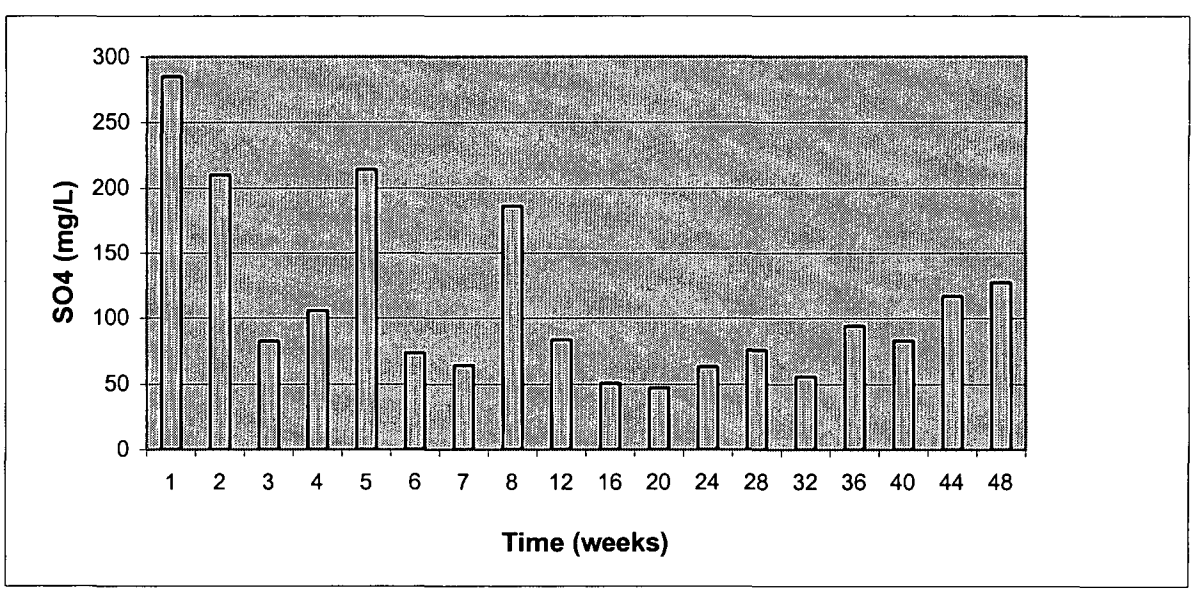


Figure 3.6: Sulphate profile of untreated effluent over a period of twelve months.

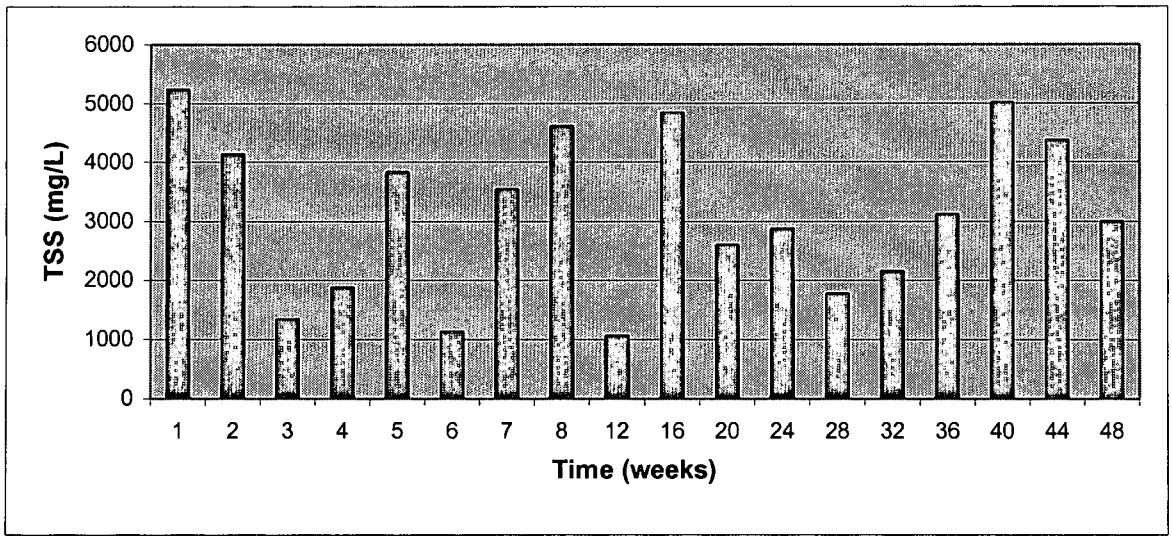


Figure 3.7: Total suspended solids profile of untreated effluent over a period of twelve months.

3.4 DISCUSSION

Monitoring of the effluent discharged from Company A was not carried out due to lack of onsite monitoring programme. The treatment and disposal of industrial effluents and their proper management is of cardinal importance in controlling pollution effectively (Elliot, 1991). The disposal of edible oil effluents can be managed by three methods, i.e. direct discharge to municipal sewer, partial treatment prior to discharge into municipal sewer and industry based recycled of effluent for reuse as process water (Khan and Akhtar, 1998; WBG, 1998). Onsite effluent treatment method was employed at Company A prior to discharge into municipal sewerage system. The effluent plant comprised of two large holding tanks, pH correction tank, dissolved air floatation (DAF) unit and two settling tanks, which were operated in series.

The effluent that was discharged into the municipal sewerage system from Company A varied considerably in quality and quantity (a daily average effluent of approximately 96 tons was discharged from the refinery plant) over a twenty four hour period depending on the refining method being employed, i.e. whether physical or chemical method. As a means of good

pollution prevention practices, the edible oil industry prefer physical refining rather chemical refining, as it has lower environmental impact than the chemical refining (WBG, 1998). The choice of refining method solely depends on the particular oil type and its quality (Hui, 1996).

Data presented in table 3.1, and in figures 3.1 – 3.7 for the untreated effluent samples collected from the refinery plant resembles wastewater from a typical edible oil industry, which contains high quantities of FOG, sulphates, phosphates, suspended solids and COD. Studies conducted by Steffen *et al.*, 1989; Adam and Marjanovic, 1996; Hwu, 1998; Khan and Akhtar, 1998; WBG, 1998 also point out that FOG, sulphates, phosphates, suspended solids and COD as the main pollutants in a typical edible oil producing industry.

The untreated effluent profiles presented in figures 3.1 and 3.2 for total COD and FOG, respectively indicate variation in both COD and FOG concentrations, ranging from average lows of 3631 mg/L and 11130 mg/L to average highs of 8523 mg/L and 80110 mg/L. Investigations by Ozturk *et al.*, 1990 showed that there are strong correlations between COD-oil and grease parameters, i.e. removal of oil-grease content in the wastewater will also reduce the COD loadings.

It is evident from the results presented in table 3.1 that the effluent contains high quantities of both organic (BOD and COD) and inorganic loads. The high organic load is attributed to the presence of large quantities of FOG contents in the effluent and the chemical nature of the edible/vegetable oil. The hydrolysis products of the edible oil, which are the long chain fatty acids, glycerol and glycerides, and the protein component, all add to the organic load of the effluent (as cited by Mkhize, 2002). Eroglu *et al.*, 1990; Ozturk *et al.*, 1990, estimated the organic load of wastewater from a typical the edible oil refining plant to range between 0.85 and 1.42 kg BOD_5/ton of the oil produced.

Wastewater is a complex substrate consisting of compounds of differing biodegradability (Dold *et al.*, 1980; Naidoo and Buckley, 2000). The fatty contaminants in the edible oil effluent can be characterised by their biodegradability (as cited by Mkhize, 2002). Biodegradable COD consists of two sub-fractions, slowly biodegradable (SB) COD (particulate) and readily biodegradable RBCOD (soluble) (Dold *et al.*, 1991). A 76.8 % of the untreated effluent total COD was found to be soluble, i.e. a ratio of total to soluble untreated effluent COD expressed as a percentage from data presented in table 3.1. It can thus be conceptually suggested that the effluent is biodegradable. Studies by Henze, 1992; Orhon *et al.*, 1994; 1997; Orhon and Ubay Cokgör, 1997 indicate that the slowly biodegradable COD accounts for the bulk of organic content of industrial wastewaters. From experiments conducted by Mkhize, 2002 it is clear that the edible oil effluent can be successfully treated using biological methods, which suggest that the oil effluent is biodegradable, i.e. 75% COD reduction was achieved. This also suggests that the bulk of the COD component may be slowly biodegradable, hence the low P removal efficiency of only 17% achieved. Biological phosphorus removal is strongly influenced by the influent characteristics, especially RBCOD during anaerobiosis to simulate P release (Degrémont, 1991; Park *et al.*, 1997).

The inorganic constituents of concern were phosphates and sulphates. Effluent phosphate was mainly originating from the use of phosphoric acid during the degumming stage and to a minor extent from the hydrolysis of phospholipids. Hui, 1996, reported that crude oil, particularly soybean oil, contains considerably quantities of organic phosphorus in the form of phosphatides, which are subsequently translocated from the oil phase to the water phase during the refining process. After acidification of the refinery wastewater, phosphatides get hydrolysed and the resulting phosphates are then released to the water phase (as cited by Mkhize, 2002).

Phosphoric acid was used at Company A for the removal of non-hydrated gums during the degumming stage for the first five months of the project inception. Results obtained during

this period of experimental work are presented in figure 3.3. The use of citric acid in the degumming operations was implemented during the November 2001 month, i.e. six months after the project inception period. This resulted in refinery plant effluent with very low or no traces of P. Subsequent to the introduction of citric acid in the degumming operations, hydrogen potassium phosphate (K_2HPO_4) solution was spiked into the pre-treated effluent, which was treated with C-40 flocculent for experimental purposes (see chapter 4, section 4.2.2. for detailed information). The presence of high phosphorus loads in the oil effluent could be addressed by changing the use of phosphoric acid during the degumming phase of crude oil refining (Khan and Akhtar, 1998; WBG, 1998). This could mean that the reduction in the phosphorus content in the edible oil effluent would result in less complex treatment methods being employed for the treatment of edible oil effluent.

It is evident from the results presented in figure 3.4 that the untreated effluent N concentrations were very low. The nitrate-nitrogen component was present in high concentrations as compared with the ammonia-nitrogen component. This may be of concern when designing a bio-P removal system. The results obtained here, show the complexity of the composition in the refinery oil effluent.

The data presented in figure 3.1 – 3.7 were collected over a period twelve months to assist in the design and operation of a biological phosphorus removal process. Several design models have been used for a number of years to demonstrate biological phosphorus removal. All these models are based on the circulation of activated sludge through the anaerobic and aerobic zones. Sometimes anoxic zones are incorporated for denitrification, mainly because the presence of nitrate is assumed to inhibit phosphorus removal (Rensink, 1991; Kuba *et al.*, 1994). Because of the low nitrogeneous material found in the oil effluent, a bio-P removal process without nitrification-denitrification was considered to be more suitable for this study. A/O SBR process has found extensive application in phosphorus removal and is considered the least complicated bio-P process (Chang *et al.*, 1996; Henze, 1996).

CHAPTER 4

LABORATORY SCALE SEQUENCING BATCH REACTOR DESIGN FOR BIOLOGICAL PHOSPHORUS REMOVAL

4.1 INTRODUCTION

Since early 1970s significant developments have taken place in the activated sludge method of treating wastewaters. The function of the single sludge system has expanded from carbonaceous energy removal to include progressively nitrification, denitrification and phosphorus removal by biological means. These extensions have been accommodated through manipulation of the system configuration – incorporation of multiple in-series reactors. Not only have the system configuration and its operation increased in complexity, but concomitantly the number of biological processes influencing the effluent quality and the number of compounds involved in these processes, have increased. With such complexity, it is no longer possible to make a reliable quantitative or qualitative prediction as to effluent quality to be expected from a design, or to assess the effect of a system or operational modification, without some model that stimulates the system behaviour accurately (Wentzel *et al.*, 1992).

EBPR systems should be designed to biologically reduce the phosphorus content of the wastewater to a practical minimum and to meet the prescribed effluent limits (Park *et al.*, 1997). The process design for a given biological phosphorus removal process should, among other factors, specify aspects such as reactor volume, sludge concentrations and wastage rates, recycle flows where applicable, oxygen requirements in the aeration basin, sludge handling and disposal (Toerien *et al.*, 1990).

In recent years, a number of phosphorus removal processes using SBR principle have been developed (Keller *et al.*, 2000). SBRs have proven to be effective and efficient for plants requiring biological phosphorus removal (Shamskhorzani and Norcross, 2000). The operational conditions for SBR to develop the enhanced bio-P culture depend on wastewater characteristics. The key feature of SBR is its flexibility to adjust the anaerobic/aerobic retention time depending on the type of wastewater (Novotny, 1998).

In most cases, bio-P removal is combined with nitrification-denitrification in a single mainstream process. Bio-P removal without nitrification-denitrification is the least complicated bio-P removal process but requires nitrification to be suppressed and is little used (Henze, 1996). This research study is based on the A/O process for biological phosphorus removal without nitrification-denitrification using SBR.

4.2 MATERIALS AND METHODS

The experimental work was carried out in two phases, i.e. phase I (SBR design for BPR) and phase II (optimisation and evaluation of the SBR performance for BPR). Phases I is discussed in detail in this chapter, whilst phase II will be covered in the next chapter, i.e. chapter 5. The effect of SRT, HRT and sludge loading were used to determine the treatability of the refinery oil effluent for the BPR SBR design.

4.2.1 Unit set-up

A 25L plastic container was used as a storage tank, as shown in the schematic layout of the experimental lab-scale SBR unit in figure 4.1 below. The storage tank was filled with untreated oil effluent from Company A, refinery plant once to twice a day, depending on the number of cycles per day and rinsed at least once a month. A mechanical stirrer was used to ensure that the contents of the tank were continuously mixed. A ø 25 mm hole was drilled and

fitted with a straight connector, which was connected to ø 20 mm silicone tubing. The other end of the tubing ran through the Masterflex type peristaltic pump (model 752010). The peristaltic pump consisted of a single roller head that was used to pump influent from the storage tank to the SBR reactor. The pump was calibrated to check the accuracy of the feed flow rate. The feed flow rate was found to fall within 0.2% accuracy.

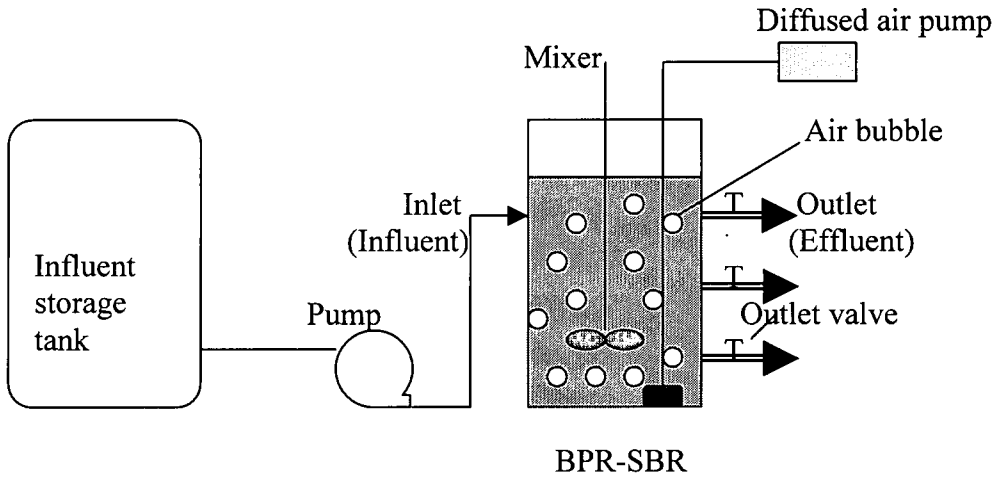


Figure 4.1: Schematic diagram of the experimental lab-scale SBR set-up.

A (volume of cylinder10L) cylindrical PVC reactor with 8L working volume was used as an SBR reactor. The SBR reactor had one inlet and three outlets ports (ø 190 mm), each controlled by a solenoid clamp (valve), which provided discharge of the treated effluent. A mechanical stirrer was used for mixing, running at 70 rpm, a velocity, which provided adequate mixing without surface turbulence. An aquarium air pump with two nozzles was used to pump diffused air into the reactor.

4.2.2 Sequencing Batch Reactor design for Biological Phosphorus Removal

4.2.2.1 Effect of HRT

Three lab-scale SBR units were used to evaluate the effect of HRT on biological phosphorus removal from the refinery oil effluent. The units were run at 12, 8 and 6 hours HRT for 40 days. Each SBR unit was similar to the SBR unit described in section 4.2.1 above. The experiments were carried out at room temperature, which ranged from 22°C to 30°C. pH was maintained at 7,0 ± 0,5 by using dilute hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions.

Each operational cycle had five basic periods (fill, react, settle, draw, and idle) in a time sequence. The operational conditions and characteristics of the SBR units are outlined in table 4.1. A 12 days acclimatisation period was allowed for the three SBR units by adjusting the influent COD concentration to ~ 1500 mg/L with tap water prior to the fill period.

The SBR units were marked SBR_1, SBR_2 and SBR_3. SBR_1 was operated at 12 h cycle (2 cycles per day), SBR_2 at 8 h cycle (3 cycles per day) and SBR_3 at 6 h cycle (4 cycles per day) respectively. The sludge age was controlled and the mixed liquor suspended solids concentration was kept constant for all three SBR units, by wasting the required amount of activated sludge at the end of the aerobic period of the cycle.

All three SBR units were fed with untreated refinery effluent (influent) from Company A after seeding with mixed liquor from an aerobic basin of Darvill Wastewater Treatment Works in Pietermaritzburg, South Africa, which was configured for biological nutrient removal as a modified Johannesburg 5-stage activated sludge system. The sludge volume before fill was kept at 2 L. At the beginning of each cycle, the oil influent was continuously

pumped at a constant flow rate of 200 mL/min into the SBR units over a period of 0.5 hours. The mixed liquor volume in the reactors at the end of the fill period was 8 L.

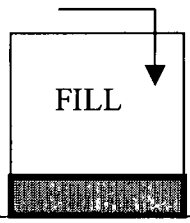
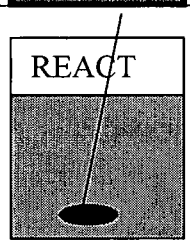
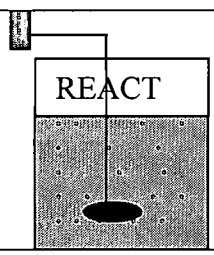
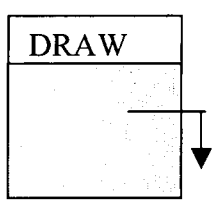
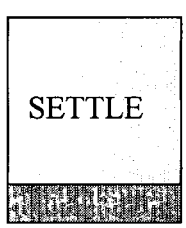
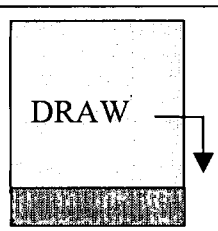
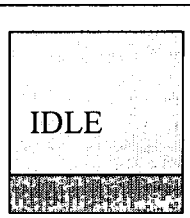
In the subsequent period, anaerobic reaction stage, the reactor contents (mixed liquor) were stirred by a mechanical stirrer and maintained for 3, 2 and 1 hours for SBR_1, SBR_2 and SBR_3 respectively. In the aerobic react stage, the mixed liquor was aerated by bubbling air through an air diffuser for 6.5, 3.5 and 2 hours for SBR_1, SBR_2 and SBR_3. The dissolved oxygen concentration was maintained constantly in the range of 2 to 5 mg/L.

SBR units were operated with MLSS concentration of 4 000 mg/L to maintain a sludge age of about 15 days by wasting 267 mL of mixed liquor at the end of the aeration period. The reactors contents were then allowed to settle for 1 hour. After the settle period, 5733 ml of clarified supernatant were drawn from the reactors at 191 ml/min for 30 minutes. Subsequent to draw period the reactors were subjected to the idle phase for 30 minutes to do general maintenance and cleaning of the reactors. During this phase no activity takes place in the reactors. The settled sludge volume was kept at 2 L after the end of each cycle.

4.2.2.2 Effect of SRT

The fourth SBR unit (SBR_4) was set up and allowed to acclimatise for 12 days on day 28. SBR_4 was operated under the same conditions as the SBR_1 during the acclimatisation period. On day 41 operational conditions for all four SBR units were altered and run at 12 hours HRT at various SRT for 32 days to evaluate the effect of SRT on biological phosphorus removal from the refinery oil effluent. The SBR units were then operated at 12 h cycles (2 cycles per day), SRT of 5, 10, 15 and 20 days for SBR_1, SBR_2, SBR_3 and SBR_4, respectively and HRT of 12 hours under same laboratory conditions as outlined in section 4.2.2.1.

Table 4.1 SBR unit operation.

Volume	Cycle Time	Diagram	Purpose	Operation
Initial volume 2L (seeded sludge)	30 minutes		Add substrate (feeding)	Pumped influent @ 200mL/min and stir
8L active volume	3 hours 2 hours 1 hour		Anaerobiosis react phase	Mixing, restrict O_2 and NO_3 from entering the system
8L active volume	6.5 hours 3.5 hours 2 hours		Aerobiosis react phase	Supply O_2 and maintain between 2mg/L and 5mg/L
267mL sludge	2 minutes		Waste sludge to maintain steady state in the reactor for 15 day sludge age	Waste 267mL of sludge per cycle
7733mL active volume	1 hour		Settle solids	No mixing and supply of O_2
5733mL effluent	30 minutes		Discharge effluent	Discharge effluent @ 191mL/min
2L settled sludge	30 minutes		General maintenance and cleaning	Everything off

The cycles were then divided into fill (30 min), anaerobic (mixing) (3h), aerobic (6.5h), settling (1h), draw (30 min) and idle (30 min). Prior to the fill stage the refinery effluent was pre-treated with a flocculent (C-40), a silica compound.

1350 g of C-40 was added to the 100 L container filled with the refinery oil effluent after the pH adjustment to achieve ~ 90 % FOG removal. After the flocculation process the supernatant was then spiked with hydrogen potassium phosphate (K_2HPO_4) solution containing 450 g/L (K_2HPO_4), i.e. 1 ml of solution per 80 L was equivalent to 1mg P/L. The P content in the feed effluent was adjusted to ~ 40 mg P/L by adding 40 ml of the K_2HPO_4 solution in 80 L.

4.2.2.3 Effect of Organic loading

On day 74 the SBR_4 was discontinued and the other three SBR units (SBR_1, SBR_2 and SBR_3) altered to 10 day SRT at 12 h HRT to determine the P removal efficiency at various organic loading, i.e. 0.1, 0.15 and 0.25 kg COD/kg MLSS respectively. The SBR units were operated under the same laboratory conditions as outlined in section 4.2.2.1 for 30 days.

Influent and effluent samples were collected on a daily basis at the beginning and end of each cycle and analysed for COD, P, MLSS, VSS and SVI respectively. COD (APPENDIX 1) and total phosphorus concentrations (APPENDIX 2) were determined using the spectroquant Nova 60 (Merck) and Standard Methods, respectively. MLSS, VSS and SVI (APPENDICES 9 and 10) were performed in accordance with standard methods (APHA, 1989).

4.3 RESULTS

After the 12 days acclimatisation period, both influent and effluent samples were collected at the beginning and during the draw phase of each cycle. Only COD and TP samples were analysed for during the first 40 days of the experimental period to evaluate the effect of HRT at various operational conditions. Results obtained are shown in APPENDIX 13A, B and C. Graphical presentations of data obtained in APPENDIX 13A, B and C are shown in figures 4.2 and 4.3.

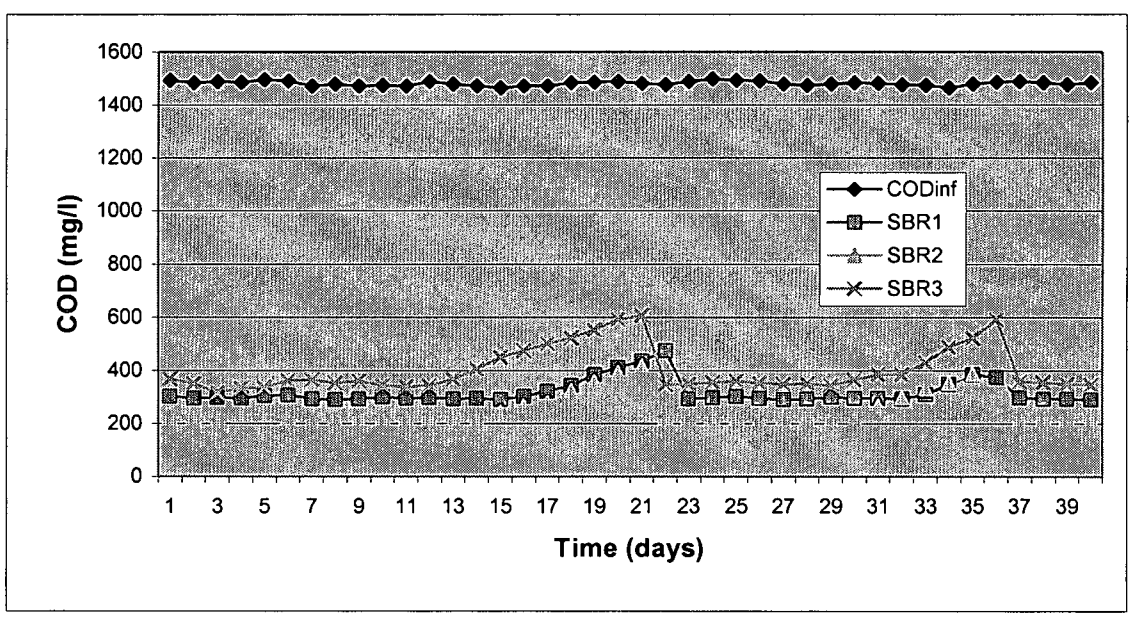


Figure 4.2: COD removal at various HRT during the first 40 days of experimental period.

The SBR units were operated at the average influent COD of 1482 mg/l, ranging from a cyclic average low of 1466 mg/l to a cyclic high of 1498 mg/l over a period of 40 days as shown in figure 4.2. COD removal efficiency was averaged at 79.93 % for the SBR_1 over the first 15 days of operation. COD removal efficiency deteriorated from 79.93 % to 68.00 % from day 16 to day 22 for SBR_1. Fresh sludge was then inoculated into the reactor to restore the initial removal efficiency on day 22. The removal efficiency achieved from day 23 onwards was then averaged at 80.01 % until the system collapsed again on day 32. The COD

concentration decreased from day 33 onwards until the SBR unit was inoculated with fresh sludge again to restore the initial removal efficiency of approximately 80.00 %.

The SBR_2 and SBR_3 also followed the same trend as the SBR_1 unit. In the SBR_2 unit COD removal efficiency deteriorated (from 85.05 % to 71.95 %) from day 14 onwards until the system was inoculated with fresh sludge on day 22. The removal efficiency was then restored to 85.09 % from day 23 onwards until it dropped on day 31 to 36. The system was inoculated with sludge on day 36 to restore the original condition. The system was run further for another four days until day 40 at the average COD removal efficiency of 85.14 %.

76.42 % COD removal efficiency was achieved in the first 13 days of operation for the SBR_3 unit. The removal efficiency dropped (59.07 %) from day 14 onwards to day 21 until the system was inoculated with fresh sludge as in the SBR_1 and SBR_2 units respectively. 76.24 % COD removal was achieved from day 22 to day 30 until it started to deteriorate on day 31. The SBR_3 was also inoculated on day 36 to adjust the removal efficiency. 76.27 % removal efficiency was achieved from day 37 onwards until day 40 when the system was abandoned.

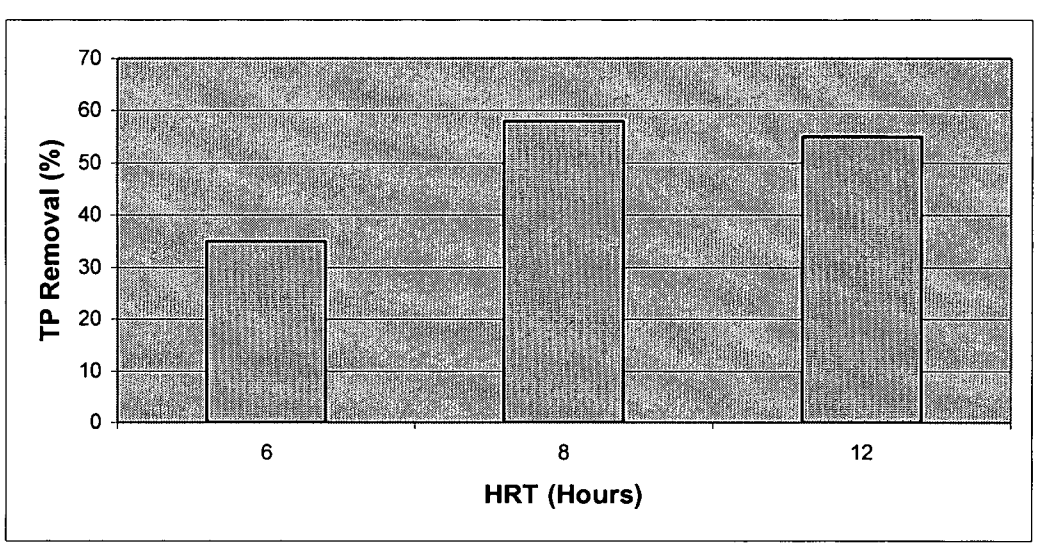


Figure 4.3: % TP removal at various HRT during the first 40 days of experimental period.

The P removal efficiency also followed the same trend as the COD removal graph as shown in figure 4.2. The percentage P removal efficiency is showed graphically in figure 4.3. As shown in figure 4.3, the removal efficiency of P observed was ranging between 35 and 58 %. The percentage P removal efficiency for SBR_2 was higher than those in SBR_1 and SBR_3. A high rate of % P removal, i.e. 58 % was achieved for SBR_2. It is evident from the results that 8 hour HRT yield higher % P removal efficiencies than at 6 and 12 hours HRT.

The results on the effect of SRT on biological phosphorus removal at various SRT are presented in figure 4.4. The average percentage P removal efficiencies at various SRT over the 32 days period, i.e. day 41 to day 73 were used to determine the effect of SRT on biological phosphorus removal.

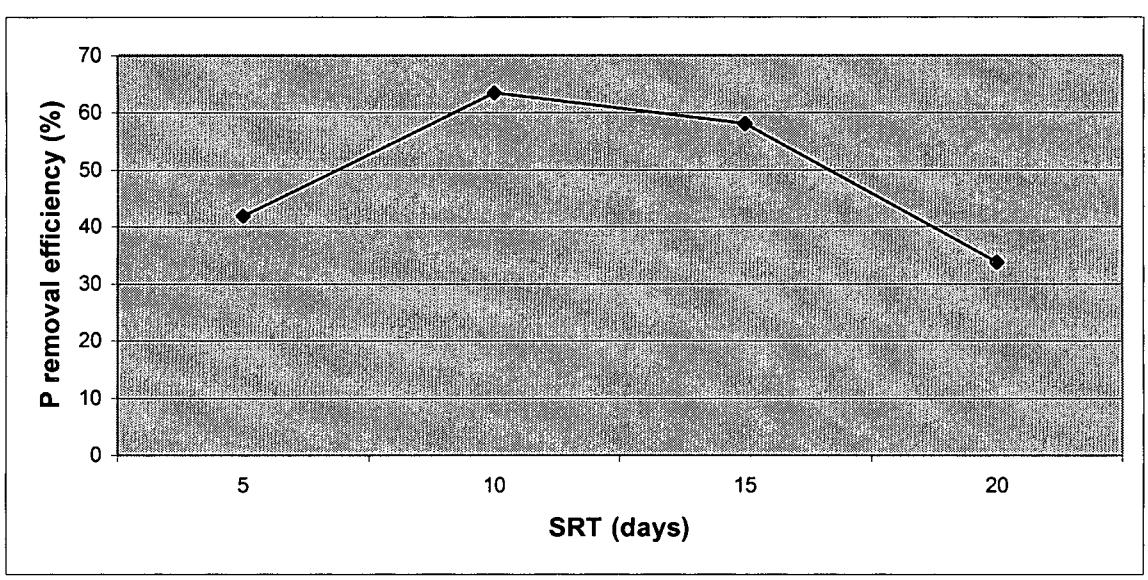


Figure 4.4: Relationship between SRT and % P removal efficiency.

The results on the effect of organic loading on P removal efficiency are presented in figure 4.5.

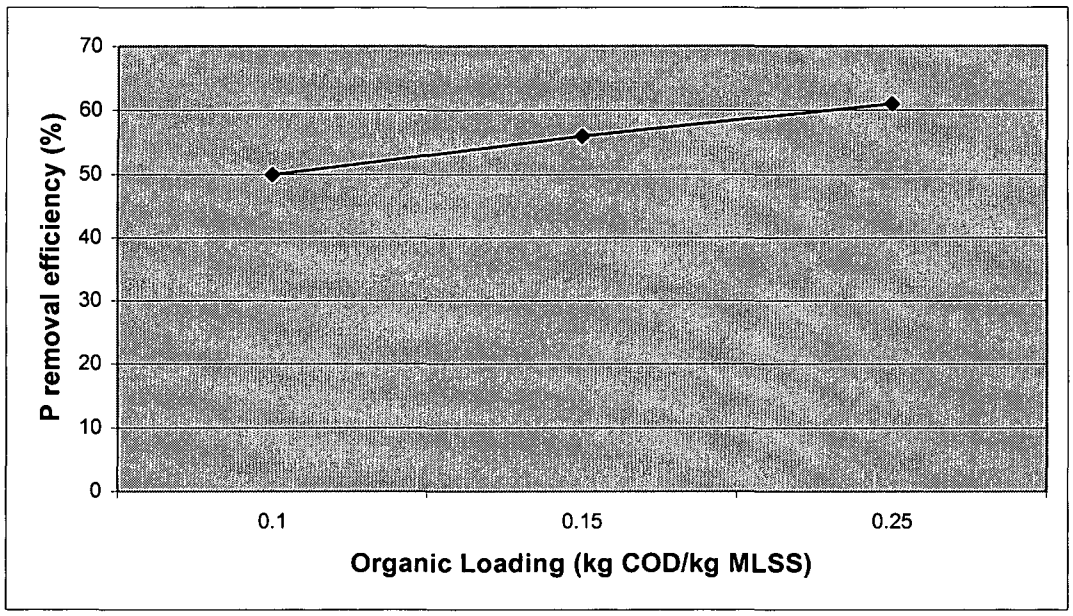


Figure 4.5: Relationship between Organic Loading and % P removal efficiency.

The results on the relationship between organic loading and sludge volume index are summarised in table 4.2.

Table 4.2: Relationship between sludge volume index and organic load.

SBR unit	Organic Load (kg COD/kg MLSS.day)	Average SVI (ml/g)
SBR_1	0.1	71
SBR_2	0.15	64
SBR_3	0.25	50

4.4 DISCUSSION

Determination of optimum HRT is one of the most fundamental and important decisions in the design and operation of EBPR system. The overall efficiency of bio-P removal system depends on the VFA production and HRT in the anaerobic zone (Danesh and Oleszkiewicz, 1997). It is evident from the results presented in figure 4.3 that at 2 hours HRT in the anaerobic zone SBR_2 yielded the highest P removal than SBR_1 and SBR_3 at 1 and 3 hours

HRT, respectively. Studies conducted by Danesh and Oleszkiewicz, 1997 also indicated that short HRT does not provide sufficient time for VFA production and long HRT causes secondary release of phosphorus, both having negative effects on the efficiency of phosphorus removal. EPA, 1987 and Droste, 1997 also found that in EBPR system, the anaerobic zone is typically sized to provide HRT between 0.5 and 2 hours based on the process influent flow.

Maximum P removal efficiency was achieved at 3.5 hours HRT in the aerobic phase in this experiment. Contrast to this study Muyima *et al.*, 1997 found that EBPR systems are designed to operate at HRT between 4 and 12 hours in the aerobic zone.

High P removal efficiencies were observed at 8 hours HRT than at 6 and 12 hours HRT during this experiment. Studies by Gray, 1990; Horan, 1990 found that in conventional activated sludge systems the HRT is usually operated at least 5 hours and 10 hours at the most at dry weather flow (DWF). Sedlak, 1991 also found that for some wastewaters with relatively low RBCOD content, an HRT at the upper end of this range can result in the greater phosphorus removals.

It was observed during the experiment that high FOG contents have adverse effects on biological phosphorus removal. It was evident on days 14 to 16 for all three SBR units and again on days 31 and 32, respectively when both COD and P removal efficiencies deteriorated. Atkinson, 1999 cited that the discharge of effluent from edible oil refining industries is posing a serious threat to biological wastewater treatment. Mkhize, 2002 cited that effluent stream from this industry should be pre-treated prior to discharge into municipal sewerage system.

It was observed that at high FOG, above 1400 mg/l chemical treatment might be applied as a pre-requisite to achieve effective biological phosphorus removal from refinery oil effluents. Seng, 1980 also found that edible oil effluents can be treated by either chemical or biological

means, or by combination of both methods. The flocculent, C-40 was used to pre-treat the untreated oil effluent prior to biological treatment to remove the oil content, which inhibited the bio-P removal as observed during the first 40 days of the experimental period.

Prior to the fill stage the untreated refinery effluent was pre-treated with C-40 from day 41 onwards. A series of bench tests were carried out to determine the optimal point(s) for the flocculent dosage. Results and procedure are presented in APPENDICES 11 and 12B. 1350 g of C-40 was added to the 100 L container filled with the untreated refinery oil effluent after the pH adjustment to achieve ~ 90 % FOG removal.

After the flocculation process the supernatant was then spiked with hydrogen potassium phosphate (K_2HPO_4) solution containing 450 g/L (K_2HPO_4), i.e. 1 ml of solution per 80 L was equivalent to 1mg P/L. The P content in the feed effluent was adjusted to ~ 40 mg P/L by adding 40 ml of the K_2HPO_4 solution in 80 L. This was as a result of Company A changing from using phosphoric acid to citric acid. The P content in the untreated refinery oil effluent was found to be low as result of the new changes. There are growing demands for the use of citric acid instead of phosphoric acid in edible oil refining processes from the viewpoint of water quality conservation and environmental protection (WBG, 1998).

The results presented in figure 4.4 on effect of SRT on biological phosphorus removal were obtained over a period of 32 days, i.e. from day 41 to day 73. It was observed that the pre-treatment of the untreated refinery oil effluent with C-40 had a significant influence on P removal efficiency. It was further observed that P removal efficiency was consistent in all four SBR units during this period of the experiment. The results presented in figure 4.4 are an indicative that optimum P removal efficiencies for oil effluent can be achieved at 10 days SRT. The average P removal efficiency at 10 days SRT was 63.5%, which was higher than at 5, 15 and 20 days SRT. In contrast to this Rodrigo *et al.*, 1996 when studying the effect of various sludge ages on an EBPR system, found that an optimum SRT would be 3-5 days.

However, Ekama and Marais, 1984 found that where nitrification is obligatory, such short SRT values are not possible, and SRT values greater than 10-15 days are required, depending on anaerobic mass fraction, temperature, and nitrifier maximum specific growth rate. Grunebaum and Dorgeloh, 1992 found that the presence of nitrates affects P removal in biological nutrient removal processes. Since the microbial diversity of the activated sludge was not analysed and it was presumed that PAOs were the dominant bacterium, nitrification could have taken place. Anoxic conditions were not carried out and therefore nitrate elimination could not be ensured.

The 5 days SRT yielded average P removal efficiencies of 41.9% from the SBR_1 unit. It was found that the initially the soluble P levels during anaerobiosis were similar to that of the influent P in the SBR_1, but as time progressed P release did take place. A large P uptake during the aerobic phase was further observed. It was also found that lower SRT's require higher WAS (Waste Activated Sludge) values. 1600ml of sludge had to be wasted per day in order to maintain a constant sludge volume for 5days SRT as compared to 800ml, 533ml and 400ml for 10, 15 and 20 days SRT, respectively. Gray, 1990 demonstrated that to maintain a constant sludge concentration (MLSS) in the EBPR system reactor the sludge wastage rate must be increased, to achieve reduced SRT values. As the plant SRT is reduced, the biomass becomes more active, resulting in greater rate of phosphorus uptake and quantity of sludge removed, or wasted from the system.

Multiple-reactor-type plants with A/O process for biological P removal can be operated at sludge age of 8 days or less (Ekama and Marais, 1984; Toerien *et al.*, 1990). Fukase *et al.*, 1985 found in an anaerobic-aerobic pilot plant system treating municipal wastewaters that the COD/P removal ratio increased from 19 to 26 as the SRT was increased from 4.3 to 8 days. At the same time the phosphorus content of the activated sludge decreased from 5.4 to 3.7%. It was found in this study that at SRT of 5 to 10 days the COD/P removal ratio was increased

from 24 to 30. This indicate that system designs requiring longer SRTs need a greater amount of COD removal to meet low effluent phosphorus concentrations (Sedlak, 1991).

There was a decrease in P removal for the 15 days SRT as compared to the 10 days SRT. P removal efficiency of 58.2 % was achieved at 15 days SRT for SBR_3 unit. It was observed that during the initial 10 days P-release took place under anaerobiosis and P-uptake occurred under the aerobic phase. However as the experiment progressed both P-release and P-uptake decreased. There was an increase in the MLSS and VSS for the 15 days SRT in comparison to the 5 and 10 days SRT. MLSS and VSS represent the amount of biomass in the system. The. Growth of the microorganisms therefore is increased (Crocetti *et al.*, 2000). Contact time of the microorganisms with the substrate was longer and it can therefore be assumed that this is the reason for the high MLSS and VSS results.

Viability of the cells were not established and this could therefore be one of the reasons for the decrease in P removal efficiency. As observed by Lee *et al.*, 1996 a longer sludge age results in decreased stability of the sludge properties (i.e. the composition of the sludge). The presence of filamentous bacteria contribute to the low food to microorganisms (*f:m*) ratio, which therefore affects the bio-P organisms. It was also observed that COD decomposition, which is required for VFA production, did occur in the anaerobic phase. However, a further decrease in COD in the aerobic zone did not take place. It can therefore be hypothesised from these observations that insufficient COD utilisation could have been the cause for the decrease in P removal efficiency.

The phosphorus removal efficiency of the 20 days SRT was less compared to the 5, 10 and 15 days SRT. Studies by Droste, 1997 indicated that higher SRT cause the sludge to undergo more endogenous decay. This has an effect on the settleability of the sludge as well as on the total amount of sludge produced in the system. As stated previously a longer SRT designs result in lower sludge production, which results in a lower amount of biological phosphorus

removal, since the phosphorus is removed with the waste sludge (Lee *et al.*, 1996; Rodrigo *et al.*, 1996). It was observed that P-release did not take place during anaerobiosis at 20 days SRT, but P-uptake did take place during the aerobic phase and this was observed by a decrease in P levels in the effluent. It can be assumed that the anaerobic phase could have served as an anoxic zone. This could therefore mean that nitrates could have been present in the system and P- uptake took place instead of P-release during anaerobiosis. Since nitrates affect P removal this observation will therefore explain better P removal in the 20 days SRT in comparison to the 10 and 15 days SRT.

The low P release in the anaerobic zone could be due to insufficient COD utilisation and this could have in turn been due to of insufficient ATP production for VFA uptake. Under anaerobic conditions COD is required to produce VFA's for polyhydroxybutyrate formation (Wentzel, 1995). It can therefore be stated that P release and uptake is largely dependant on the COD utilisation. A series of trial tests run by Sheer and Seyfried, 1996 demonstrated that the soluble COD has the greatest influence on biological P removal. Maximum fermentation of biodegradable substrates (COD), (which is used for the formation of VFA's) during anaerobiosis occurs when there is sufficient anaerobic contact time (Sheer and Seyfried, 1996).

EBPR systems depend greatly on sludge age, i.e. sludge age affects the width of the phosphorus removal range, which shifts towards higher values as sludge age decreases (Rodrigo *et al.*, 1996). This phenomenon was evident with SRT values that ranged from 10 to 15 days as presented in figure 4.4.

The effect of organic loading on biological phosphorus removal was expressed as a relationship between % P removal efficiency and organic loading in figure 4.5. The experiment was carried out over a period of 30 days, i.e. from day 74 to day 104 at 10 days SRT. It was observed during this period that an increase in sludge mass resulted in a

proportional increase in phosphorus removal, i.e. organic loading is linearly related to the phosphorus removal as shown in figure 4.5. Toprak, 2001 defined a process with 0.25 organic load and SRT of between 5 and 15 days as being ideal for nutrient removal. SBR_3 yielded the highest P removal efficiency as compared to the SBR_1 and SBR_2, respectively, as presented in figure 4.5, i.e. 61 % of P removal was achieved at 0.25 organic loading and 10 days SRT. This confirms the relationship, which exists between organic loading and SRT as predicted in studies conducted by Gray, 1990. The sludge loading is related to SRT because the sludge activity increases if the organic loading is increased, resulting in an increased rate of sludge growth. This study demonstrated that phosphorus removal depends on organic load, which corresponds to the findings by Temmink *et al.*, 1996.

It was further observed that settleability of sludge increases with the organic load as summarised in table 4.2. The results obtained suggest that SVI is inversely proportional to the organic loading, which is in agreement with the experimental work that was carried out by Ramalho, 1983 and Cuevas-Rodríguez *et al.*, 1998. The values obtained for all three SBR units were below 80 ml/g, which is considered excellent. This was further confirmed by the low suspended solids concentrations in the final effluent, i.e. < 1mg/L. It was also observed that MLSS and MLVSS followed the same trend as in phosphorus removal efficiency, although the MLVSS was lower since it represents the volatile fraction of the MLSS.

It is evident from this study as well as others conducted by Ekama *et al.*, 1984; Wentzel *et al.*, 1990; Brdjanovic *et al.*, 1997; Tasli *et al.*, 1999 that to achieve biological phosphorus removal the process requires an anaerobic and aerobic sequence. SBR technology has further proved to be suitable for treating edible oil effluent for biological phosphorus removal in this experiment. Cuevas-Rodríguez *et al.*, 1998 used SBRs successfully for biological phosphorus removal because they allow easy control of mean cellular retention time (sludge age) and other control parameters, such as HRT and organic loading.

CHAPTER 5

OPTIMISATION AND PERFORMANCE EVALUATION OF SEQUENCING BATCH REACTOR FOR BIOLOGICAL PHOSPHORUS REMOVAL

5.1 INTRODUCTION

Increased concern over a clean environment by authorities is forcing the implementation of stringent regulations in improving the quality of the environment in South Africa (Pitman and Boyd, 1999). The current National Water Act requires that industrial water-users treat their effluent to some extent before discharging it into the sewerage system (as cited from Atkinson, 1999). The effluent stream from the industrial water-users should thus be pre-treated prior to discharge to municipal sewerage system (Hui, 1996). Pietermaritzburg-Msunduzi Transitional Local Council (Msunduzi local municipality) has promulgated industrial effluent by-laws and implemented discharge limits on phosphate emissions, which push for the industrial water users to pre-treat their wastewater prior to discharge into the municipal sewerage system. Phosphorus discharge limit of 20 mg/l has been introduced to regulate P emissions into the municipal sewerage system (IEB, 1996). The imposition of such laws and regulations has greatly stimulated research and development work for finding more cost effective methods for biological P removal processes (Wiechers, 1987).

These stringent environmental standards have promoted the development of new intensive biological processes for treating edible oil effluents (Bekir, 2001). These developments has prompted this study and in Company A also taking steps towards investigating viable biological

treatment processes to be incorporated on-site to their existing physical-chemical effluent treatment methods.

Company A discharges its final treated effluent into municipal sewerage system for further biological treatment at Darvill wastewater works. Darvill wastewater works is primarily a domestic wastewater works, which also treats some industrial waste from Pietermaritzburg, South Africa. Darvill wastewater works is prone to trade effluent problems, primarily in the form of vegetable oil waste from the local producers of cooking oil, margarine and soap. These wastes cause serious primary sedimentation and scum removal problems, as well as being directly implicated in drastic increase in secondary effluent P concentrations. High influent and effluent P concentrations are as a direct result of these liquid wastes entering the plant as the industries concerned use phosphoric acid in their refining process (De Haas, 1998).

A number of operating parameters have a profound influence on the degree of biological phosphorus removal from wastewater treatment process. Manipulation of design and process operating parameters can be used to optimise the bio-P removal process performance (Elliot, 1991; Mkhize, 2002).

The aim of this part of the study was to optimise and evaluate the performance of the laboratory-scale SBR pilot plant that was designed and modelled upon the anaerobic-aerobic configuration to enhance biological phosphorus removal as previously discussed in chapter 4. The main objective was however to achieve effluent P concentrations below 20 mg/L.

5.2 MATERIALS AND METHODS

Phase II of the study consisted of the optimisation and evaluation of the SBR performance for BPR. This phase was carried out over a period of 158 days. Based on the results obtained from the characterisation of the untreated oil effluent in chapter 3 and in section 4.2.2 on SBR design for BPR system, a laboratory-scale SBR pilot plant was designed and modelled upon the anaerobic-aerobic configuration to enhance biological phosphorus removal. The set up for the lab-scale SBR pilot plant was similar to the one discussed previously in figure 4.1.

The influent storage tank was filled daily with pre-treated refinery oil effluent (influent) from day 105 and fed directly to the SBR unit. The untreated oil effluent was treated with C-40 flocculent to reduce FOG content prior to biological treatment and then spiked with hydrogen potassium phosphate (K_2HPO_4) solution. The P content in the feed influent was adjusted to ~ 40 mg P/L.

The SBR unit was run for 158 days after 12 days of acclimatisation period, i.e. from day 105 to day 263. During this part of the experiment the SBR unit was marked BPR-SBR. The BPR-SBR operation was adjusted to three cycles a day (cycle time, t_c = 8 h). Each cycle involved the regular consecutive sequence of fill phase, anaerobic and aerobic react phase, settle phase, draw and idle phases. The operational conditions and characteristics of the BPR-SBR unit are outlined in table 5.1.

Table 5.1: Operational conditions and characteristics of BPR-SBR.

Run	Duration (days)	Operational conditions								Reactor characteristics			
		m	t_F (h)	t_{AN} (h)	t_{Ae} (h)	t_s (h)	$t_{D=}$ (h)	t_I (h)	t_c (h)	V_O (*l*)	V_F (*l*)	V_O/V_F	V_F/V_T
Optimisation	158	3	0.5	2	3.5	1	0.5	0.5	8	2	6	0.33	0.75
m: cycle number in a day; t_F: fill time; t_{AN}: anaerobic react time; t_{AE}: aerobic react time; t_S: settle time; t_D: draw time t_I: idle time; t_C: total cycle time; V_O: initial volume; V_F: fill volume; V_T: total volume													

During the fill phase the unit was fed with pre-treated refinery oil effluent (influent) from the storage tank after seeding with mixed liquor from an aerobic basin of Darvill Wastewater Treatment Works in Pietermaritzburg, South Africa, which was configured for biological nutrient removal as a modified Johannesburg 5-stage activated sludge system. The sludge volume before fill was kept at 2 L. At the beginning of each cycle, the influent was continuously pumped at a constant flow rate of 200 mL/min into the BPR-SBR unit over a period of 0.5 hours. The mixed liquor volume in the reactors at the end of the fill period was 8 L.

In the subsequent phase, anaerobic reaction stage, the reactor contents (mixed liquor) were stirred by a mechanical stirrer and maintained for 2 hours. The anaerobic react stage was then followed by aerobic react stage. During the aerobic react phase the mixed liquor was aerated by bubbling air through an air diffuser for 3.5 hours. The dissolved oxygen concentration was maintained constantly in the range of 2 to 5 mg/L.

The BPR-SBR unit was operated at 10 days SRT by wasting 267 ml of mixed liquor at the end of the aeration period. After wasting sludge the reactor contents were allowed to settle for 1 hour. Subsequent to the settle phase, 5733 ml of clarified supernatant were drawn from the reactors at 191 ml/min for 30 minutes and then allowed to idle for 30 minutes. Parameters design for the lab-scale BPR-SBR system are summarised in table 5.2.

Table 5.2: Parameters design for the lab-scale pilot plant for bio-P removal.

Variable	Units	Range
Influent fill time	Hrs	0.5
Anaerobic react	Hrs	2.0
Aerobic react	Hrs	3.5
F/M ratio	kg COD/kg MLSS.day	0.3 – 0.5
SRT	days	10
HRT	Hrs	8
COD_{sol}/P_{sol}	-	30
MLSS	mg/L	3500 – 4000
Temperature	°C	22 – 30

Total suspended solids (TSS), FOG and MLSS were analysed according to Standard methods (1989) (APPENDICES 7, 8, 9 and 10). Total and soluble COD, nitrates and ammonia were analysed for by a colourimetric method, using SQ 118 spectrophotometer during the first eight months of the study (APPENDIX 1). NOVA 60 spectrophometer from Merck was used for the other five months period of the study. All samples were analysed in triplicate. pH and temperature were measured using a bench scale pH meter with a temperature probe. Raw data is presented in APPENDICES 14A and B.

Phosphate and total phosphorus were measured using the vanadate-molybdate (VM) method. For P determinations, a 5 ml sample was mixed with 5 ml colour reagent. Samples were left to stand for 30 minutes at room temperature to allow for full colour to development. Absorbance was read at 470nm using a UV/VIS spectrophometer with a flow cell of 1 cm path-length against a distilled water blank (APPENDICES 1 and 2).

All the experimental work was carried out at room temperature, which ranged from 22°C to 30°C. pH was maintained at 7,0 ± 0,5 by using dilute hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions. The dissolved oxygen concentration was maintained constantly in the range of 2 to 5 mg/L.

5.3 RESULTS

The BPR-SBR unit was operated at the average influent COD of 1388 mg/l, ranging from a cyclic average low of 1292 mg/l to a cyclic high of 1495 mg/l over a period of 158 days as shown in figure 5.1. COD removal efficiency was averaged at 80.16 % during the experimental period for the BPR-SBR operation. It is evident from figure 5.1 that the effluent COD remained fairly constant throughout the experimental period.

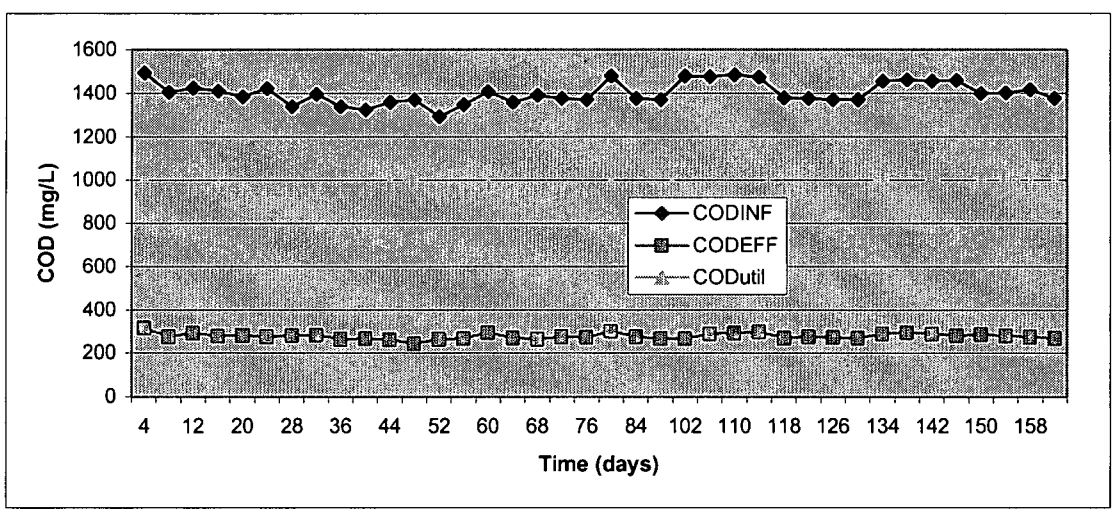


Figure 5.1: Total influent and effluent COD profiles for the BPR-SBR over the 158 days of operation.

The COD utilisation (COD_{util}) fraction was estimated at about 68.10 % of the total influent COD. The COD utilisation fraction was determined by calculation from the difference obtained between the amount of the soluble influent COD that was used up by the PAOs during the react phase and soluble effluent COD produced. The COD_{util} ranged between 904 mg/L and 1063 mg/L with an average mean of 972 mg/L as shown in figures 5.1 and 5.2. The soluble COD fraction was made up to an average mean of 87.92% of the total influent COD. The soluble COD profile is presented in figure 5.2.

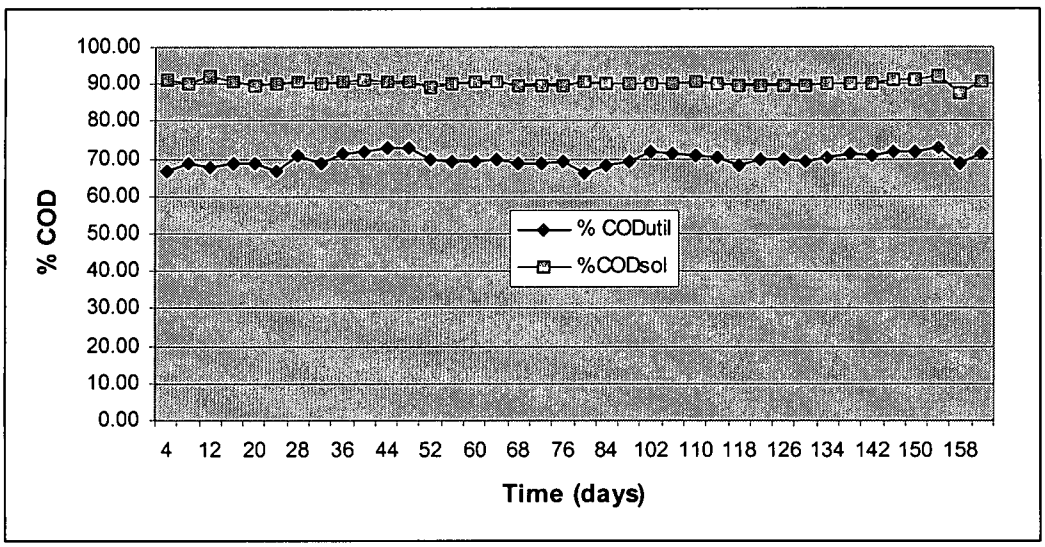


Figure 5.2: COD_{util} and soluble COD profiles for the BPR-SBR during the 158 days of operation.

A series of standards ranging from 5 mg P/L to 50 mg P/L were prepared and run against the samples for P determination. A typical calibration curve for the P standards is presented in figure 5.3.

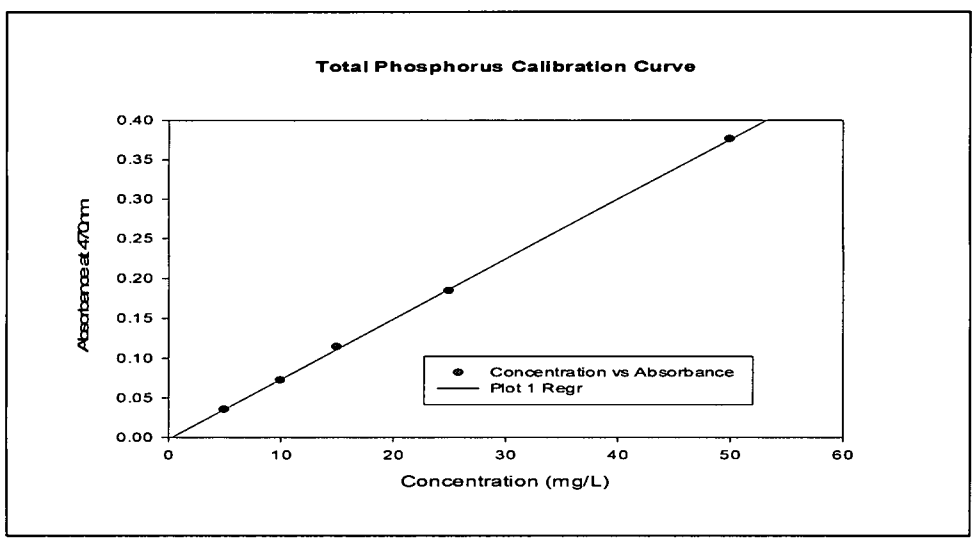


Figure 5.3: Calibration curve for P standards

Figure 5.4 represents the influent and effluent total phosphorus profiles during the 158 days of the experimental period. The influent and effluent TP concentrations ranged from average lows of 48.65 mg/L and 9.98 mg/L and average highs of 50.03 mg/L and 11.79 mg/L, respectively. The overall P removal efficiency achieved was 77.95 %, i.e. 10.48 mg P/L. As observed in the COD removal efficiency results in figure 5.1, it is also evident from figure 5.4 that the effluent P remained fairly constant throughout the experimental period. This suggests that the steady state with regard to COD and TP removal has been achieved.

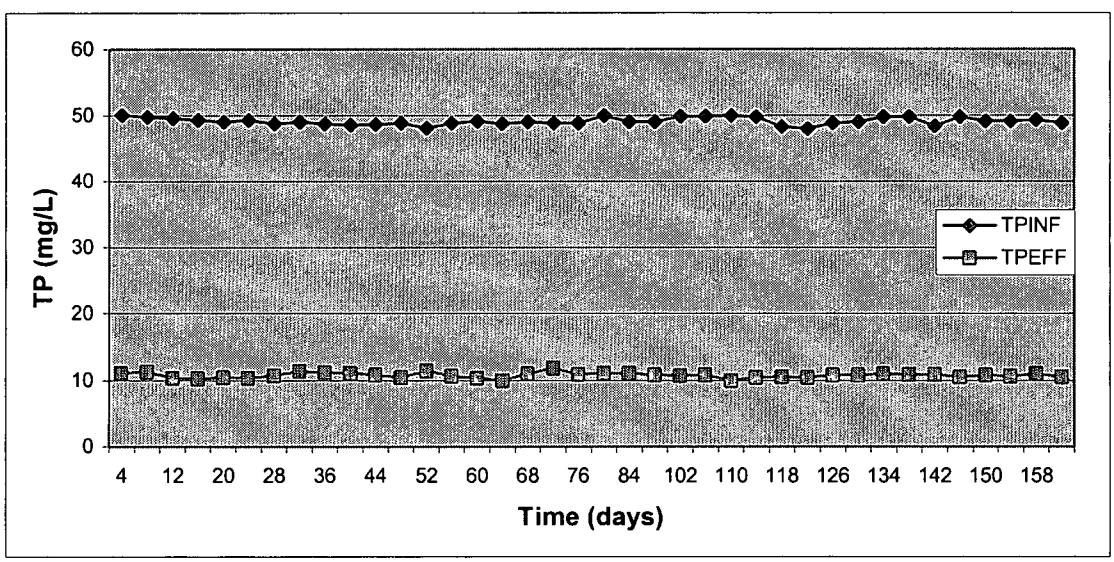


Figure 5.4: Influent and effluent TP profiles for the BPR-SBR over the 158 days of operation.

Figures 5.5 and 5.6 graphically illustrate the relationships between influent COD and P, P-uptake and P-release for the BPR-SBR over the 158 days of operation.

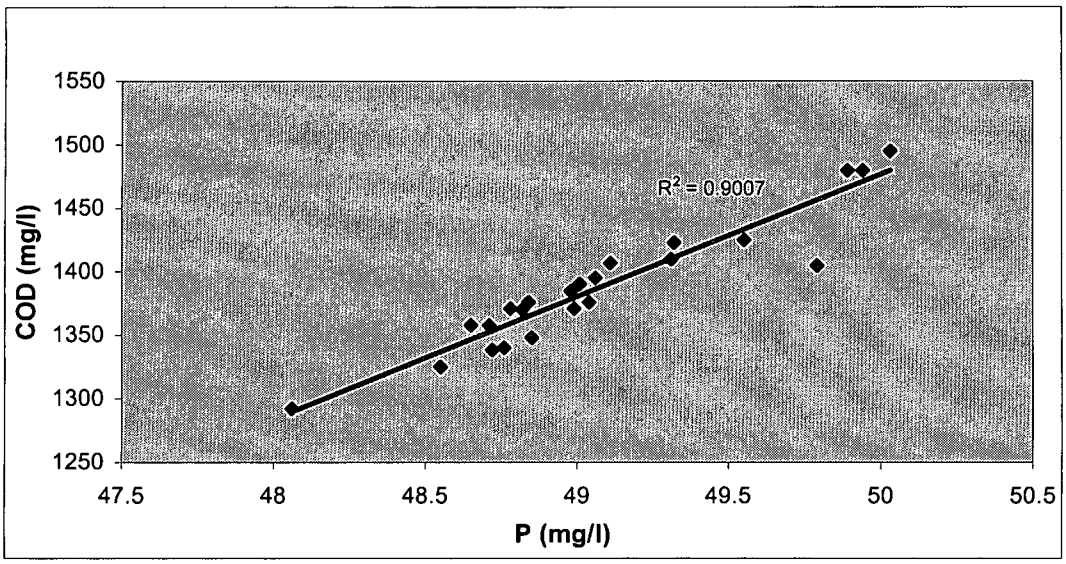


Figure 5.5: Relationship between influent COD and P over the 158 days of operation.

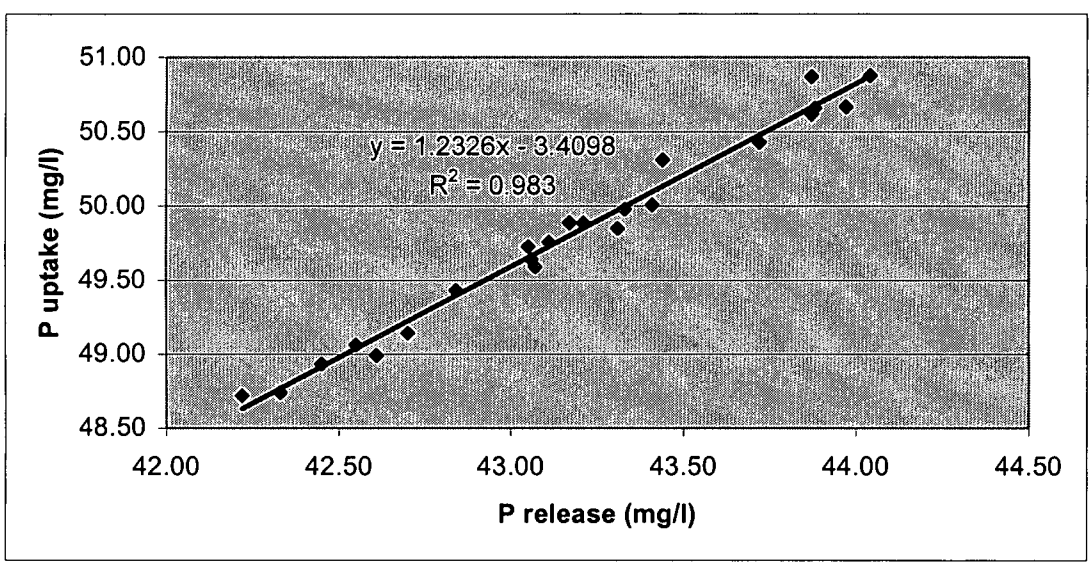


Figure 5.6: Linear relationship between P uptake and P release over the 158 days of operation.

The percentage FOG removal efficiencies presented in figure 5.7 varied from average low FOG concentrations of 70.33 % to average high of 74.76 % during the experimental period. The % COD removal varied from minimum of 78.08 % to maximum of 82.19% during this period. The mean % COD of 79.98% and % FOG of 72.39% removal efficiencies were achieved, respectively from the linear curves as illustrated in figure 5.7.

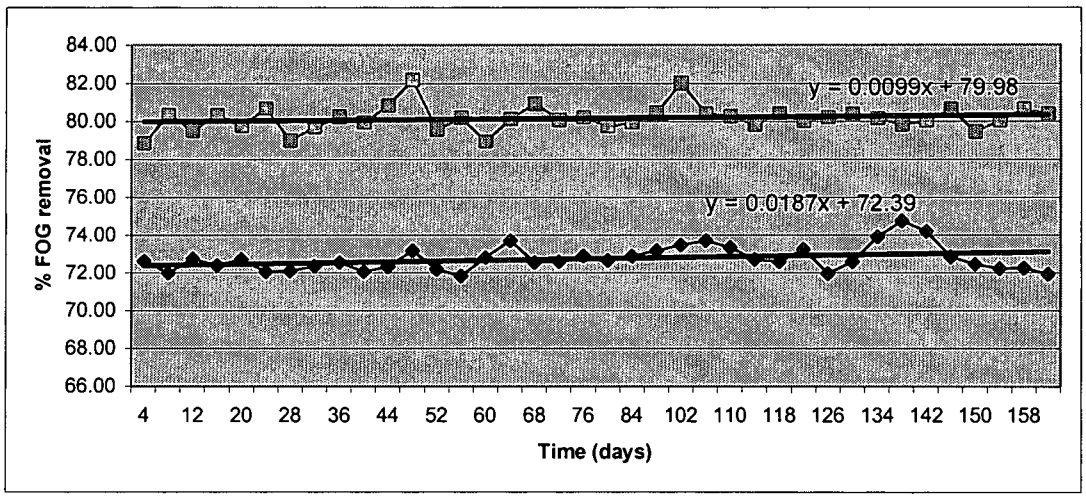


Figure 5.7: % FOG and % COD removal profiles for the BPR-SBR over the 158 days of operation.

The effluent total suspended solids (TSS) and sludge volume index (SVI) profiles for the BPR-SBR unit over the 158 days of operation are presented in figure 5.8. The effluent TSS concentrations obtained were < 1mg/L, whilst the SVI ranged from average low of 50.43 ml g^{-1} to average high of 57.56 ml g^{-1}. The mean SVI of 53.78 ml g^{-1} was achieved during the experimental period as shown in the linear equation in figure 5.8.

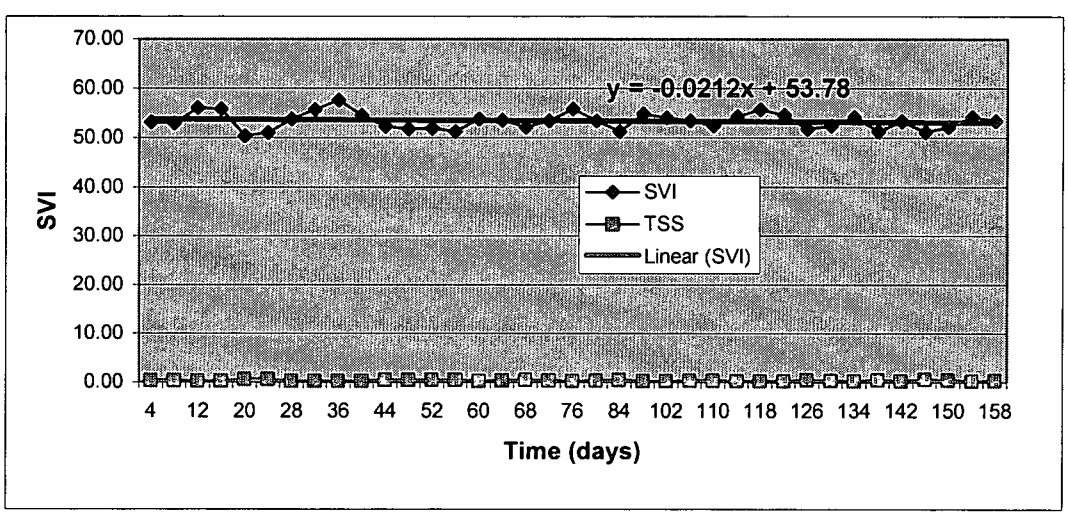


Figure 5.8: SVI and effluent TSS profiles for the BPR-SBR unit over the 158 days of operation.

The relationship between VSS and effluent P over the 158 days of the experimental period is presented in figure 5.9.

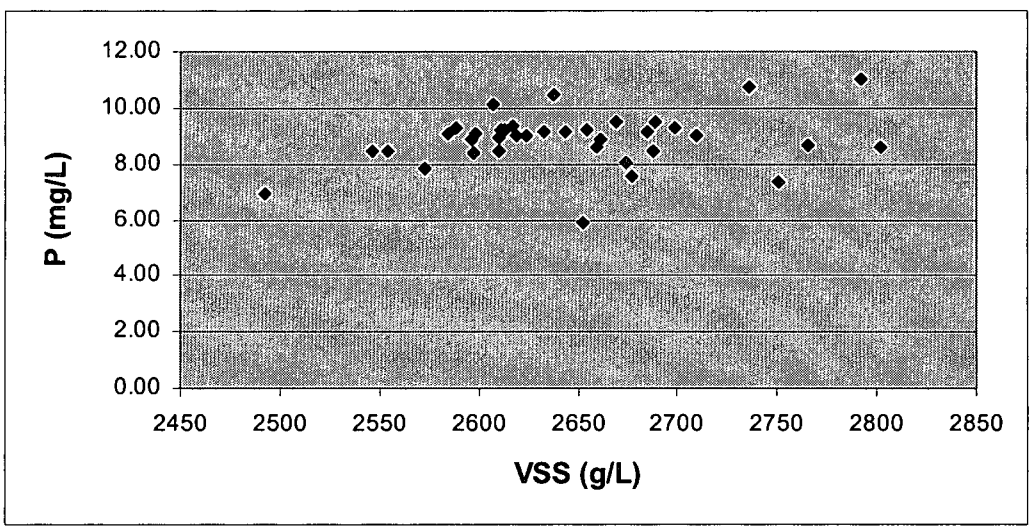


Figure 5.9: Relationship between VSS and P over 158 days of experimental period.

5.4 DISCUSSION

The addition of C-40, flocculent during the pre-treatment stages was to effectively reduce the FOG content in the untreated refinery oil effluent prior to the biological treatment process. Approximately 90 % FOG content was removed with the addition of C-40, flocculent during the pre-treatment stages throughout the experimental period.

Tasli *et* al., 1999 found that the magnitude of the available organic substrate and its readily biodegradable fraction is likely to have a decisive impact on the extent of biological phosphorus removal and that such an impact can be visualised within an SBR system. The total influent and effluent COD concentrations were determined in order to estimate organic substrate utilisation as shown in figure 5.1. Results presented in figures 5.2 and 5.4 on the profiles of % COD_{util} fraction and P removal efficiencies, respectively are indicative that the organic substrate has an impact on

biological phosphorus removal efficiency. Satoh *et* al., 1992 also reported that biological phosphorus removal is dependent on the presence of readily biodegradable substrate.

Novotny, 1998 found that the influent COD fractions can be accurately used to describe the behaviour of the biological phosphorus removal process. The soluble influent COD was used in this experiment to estimate the magnitude of the bio-P removal efficiency. Lemos *et al.*, 1998 also found that the efficiency of the BEPR process is influenced greatly by the carbon source and concentration. The mean soluble COD fraction for the total influent COD found in this experiment was 90.36% as shown in figure 5.2. The high P removal efficiency (77.95 %) and COD utilisation (70 %) suggest that the bulk of the soluble COD fraction is biodegradable. This confirms the findings that were made earlier in chapter 3 and the results that were achieved by Mkhize, 2002. Khan and Akhtar, 1998 also reported that wastewater from edible oil and fat industry contains a substantial amount of biodegradable organics.

Figure 5.5 graphically illustrates the relationship between influent COD and P. The degree of closeness of the plots to the straight line indicates that there is a direct linear relationship between COD and P with small dispersion, i.e. a strong positive linear correlation exists between COD and P. This implies that the COD can confidently (with 90.07% level of confidence) be used to estimate the P removal efficiency. Sedlak, 1991, illustrated that effluent phosphorus is related to influent COD/P ratio.

It is generally accepted that COD/P ratio greater than 35 is feasible to achieve effluent P concentration of <1mg TP/l (Randall *et al.*, 1992). An average COD/P ratio of 30.84 was observed in this experiment during the158 days of operation for the BPR-SBR. Effluent P concentrations ranged from average low of 9.98 mg/L and average high 11.79 mg/L, respectively. The overall P removal efficiency achieved was 77.95 %, i.e. 10.48 mg P/L at COD/P ratio of

30.84 as presented in figure 5.4. The objective of this experiment was to achieve effluent P concentrations below 20 mg P/l. Effluent P concentrations of below 20 mg P/L were obtained throughout the experimental period.

Experimental observations by Wentzel *et al.*, 1985 and 1989; Abu-ghararah and Randall, 1991; Karlovich, 1994, and Rubens, 1994 indicated that the magnitude of biological excess phosphorus uptake is strongly linked to the magnitude of phosphorus release in the anaerobic phase. It was observed in this experiment that a linear relationship between P uptake and P release exist as shown in figure 5.6. The values of the phosphorus uptake-to-phosphorus release ratio reported in literature are presented in table 5.3. From the observed linear relationship between phosphorus uptake and release, the relationship that was proposed by Wentzel *et al.*, 1985, can be expressed in the form of an equation as follows:

$$P\ (uptake) = \alpha P\ (release) + P\ (metabolic)$$

$$y = 1.2326x - 3.4098$$

where

P (uptake) = y = total phosphorus uptake, mg P/L

α = constant:= 1.2

P (release) = x = phosphorus released during anaerobic conditions, mg P/L

P (metabolic) = phosphorus requirement for removing COD, mg P/L.

Table 5.3: Linear relationship between phosphorus uptake and phosphorus release

Source	EBPR System	Substrate	Ratio	Correlation
Wentzel *et al.* (1985)	Modified UCT	Municipal wastewater	1.15 –1.12	0.99
Abu-ghararah and Randall (1991)	UCT	Municipal wastewater with SCFA addition	1.2	0.99
Wentzel *et al.* (1989)	Modified Bardenpho	Acetate	1.16 – 1.2	-
Rubens (1994)	A^2/O	Municipal wastewater	1.1	0.99
Karlovich (1994)	Modified UCT	Municipal wastewater	1.12	0.98
This experiment (2002)	A/O	Edible oil effluent	1.2	0.98

Findings of this experiment suggests that as MLSS increases a proportional increase in P removal efficiency will occur, i.e. at 3502 mg/L of MLSS 10.07 mg P/L was achieved whilst at 3964 mg/L MLSS 5.88 mg P/L was achieved. This was presumed to be influenced by the presence of more viable poly-P organisms in the sludge, which was achieved by stimulating the growth of PAOs, which store large quantities of phosphorus internally as poly-P. Studies by Gray, 1990 and Mark *et al.*, 1997 also found that the greater the proportion of biodegradable COD the PAOs obtain, the greater their fraction in the mixed liquor, the greater the percent phosphorus content of the mixed liquor, and the greater the phosphorus removal efficiency.

VSS was further used as measure of MLSS to determine sludge activity used for biological phosphorus removal as shown in figure 5.9. It is illustrated in figure 5.9 a direct linear relationship with greater dispersion exists between VSS and effluent P concentration. This suggests that for any given value of VSS, the range in effluent P concentrations is larger than expected. Values of 0.03 to 0.04 mg P/mg VSS were obtained in this experiment to achieve effluent P concentrations below 20mg P/L. Findings by Wentzel *et al.*, 1989 and 1990 demonstrated that the amount of phosphorus incorporated in the sludge is increased from the "normal" value of approximately 0.03 mg P/mg VSS to values of approximately 0.06 to 0.15 mg P/mg VSS.

DO concentrations of 1.75 to 3.0 mg/L were found to be optimal for phosphorus uptake in this experiment. Studies by Pipes, 1979; Sedlak, 1991 found that DO concentrations in the range 1.5 to 3.0 mg/L are considered to be adequate for biological phosphorus removal processes. It was further observed in this experiment that DO is a function of sludge loading, i.e. as the organic load increase there was an increase in DO concentration. This also resulted in good sludge settleability and clear effluent produced from the BPR-SBR system, i.e. mean SVI of 53.78 ml g^{-1} and effluent TSS concentrations of below 1mg/L were achieved as illustrated in figure 5.8. Research findings by Gray, 1990 suggests very good sludge settleability at 50 ml g^{-1}.

Very low nitrates and ammonia concentrations were found in the untreated refinery oil effluent prior to the physical-chemical treatment with the application of C-40 flocculent. Nitrates concentrations were below 1.0 mg/L and ammonia concentrations ranged between 1.0 and 1.6 mg/L (APPENDIX 12A). After the application of the C-40 flocculent, no traces of nitrates or ammonia could be found. Research findings by Ekama *et al.*, 1984 suggested that if nitrates are positively excluded from the anaerobic reactor, excess P can be achieved if RBCOD fraction is in excess of 50 mg COD/L. studies by Comeau *et al.*, 1990; Jenkins and Tandoi, 1991; Sedlak, 1991; Kuba *et al.*, 1994; Tasli *et al.*, 1999 further found that the introduction of nitrates or nitrite depletes the readily biodegradable substrate, which is necessary for the PAOs. They also found that the presence of nitrates in the recycled stream significantly inhibits the biological phosphorus removal potential. This further confirms that the bulk of the available soluble COD fraction is biodegradable as presumed earlier. This may lead to conclude, in the support of the experimental data that in the absence of nitrates, RBCOD fraction was sufficient to achieve bio-P removal.

Due to the reasonably stable influent COD load and constant sludge age, the f/m ratio was controlled within the range of 0.34 to 0.43 kg COD/kg MLSS.d. Most significant was the nearly steady concentrations of residual P, COD and FOG in the effluent within this range. It can thus be

presumed that P was completely removed within this range to guarantee the presence of the poly-P organisms in the BPR-SBR system.

The pre-treatment of edible oil effluent with C-40 flocculent has tremendously improved the bio-P removal efficiencies resulting in high P removal as presented in figure 5.4. The purpose of this study was to ensure effluent P values below 20 mg P/L and elimination of problematic constituents such as COD and FOG from the edible oil effluent prior to discharge into municipal sewerage system by biological treatment method. A relationship between FOG and COD can be drawn from the results presented in figure 5.7, i.e. as the FOG content is removed from the oil effluent so does the COD content. It was observed that % COD removal follows the same trend as the % FOG removal efficiency as illustrated in figure 5.7. Ozturk *et al.*, 1990 also found that there is a strong correlation that exists between COD and FOG content in wastewater. The effluent FOG concentrations achieved in this experiment ranged from average low of 296 mg/L to average high of 348 mg/L.

It was observed from the study that bio-P removal efficiency is highly influenced by operating parameters such as sludge age, hydraulic retention time and organic load especially when using SBR technology. The environmental factors such as pH, temperature and dissolved oxygen under which this experiment was carried out were relatively stable and their variations can not be considered important factors to affect the performance of the SBR system for bio-P removal.

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Findings of this study suggests that edible oil effluents can be successfully treated by biological methods using SBR technology prior to discharge into municipal sewerage system for further treatment. Pre-treatment of the oil effluent prior to biological treatment with flocculent proved to be a necessary treatment step when high FOG concentrations were experienced. This also suggests that successful P removal efficiencies may be realised by employing a biological treatment process only if FOG concentrations are below 1500mg/L. It is therefore, important that the outflow effluent from the oil industry be monitored for parameters considered to be of concern, such FOG, phosphorus, COD, pH and TSS. This should be carried out at least weekly or more frequently, if the flows vary significantly.

The final refinery oil effluent that was discharged from Company A did not comply with the local municipality's effluent discharge limits, even though the effluent was treated on-site. Physico-chemical treatment methods were practised on-site by using DAF unit to remove FOG and solids and the addition ferric chloride for phosphorus removal, respectively. The optimisation of the DAF unit operation and chemical dosage should be investigated to achieve acceptable effluent quality.

The single staged BPR-SBR treatment sequence proposed in this study with the use of the combination of chemical-biological treatment methods proved to be successful. Effluent TP, COD and FOG mean concentrations obtained were in compliance with the effluent discharge

limits as regulated by the local municipality. Effluent P concentrations as total phosphorus below 20 mg/L were achieved. Mean value of 77.95 % P removal efficiency was achieved, i.e. influent P concentration was reduced from average high of 49.16 mg/L to effluent average P concentration of 10.84 mg/L.

Effluent quality from refinery oil plant is highly influenced by the type of crude oil and refining process employed, whether physical or chemical. It is recommended that where appropriate, preference be given to physical refining rather than chemical refining of crude oil as active clay has a lower environmental impact than the chemicals generally used. The presence of high P loads in the final effluent could also be addressed by using citric acid instead of phosphoric acid, where feasible during the degumming process.

Space constraints or availability has been identified as one of the limiting factor in the application of biological treatment methods in most industries in treating their effluent. Because of their high operational flexibility SBRs are practical methods of treatment, which industries can install to treat their effluent. Research has proved that a typical SBR plant produces about 25% reduction in capital cost and about 10% reduction in running cost over conventional activated sludge process. The many advantages offered by the SBR systems may justify the recent increase in the implementation of this technology in industrial and municipal wastewater treatment. It is evident from this study that the SBR technology is suitable for treating wastewater from edible oil producing industry.

It can thus be concluded that many factors could have influenced the treatability of the refinery oil effluent in achieving good P removal efficiencies. SBR systems require optimisation to ensure perfect growth conditions to achieve biological phosphorus removal. From the experimental work conducted, the following conclusions can be reached:

- In the design of an activated sludge process for biological phosphorus removal, the characterisation of wastewater to be treated was found to be of prime importance. Wastewater characteristics govern both the selection of the process to be employed and the removal efficiency attainable in a process for biological P removal.
- The untreated refinery oil effluent may require pre-treatment prior to biological treatment depending on the quality or composition of the untreated effluent. It was further observed during the experimental period that high FOG content (above 1500mg/L) inhibits or interferes with bio-P removal. That is high P removal efficiencies are only possible at FOG concentrations below 1500mg/L.
- Biological phosphorus removal could replace partially or completely chemical phosphorus precipitation, depending on the phosphorus standard, wastewater and plant characteristics.
- The results of the bench scale experiment confirm that the majority of the constituents of the edible oil effluent are readily biodegradable, hence the high removal rate of COD, TP and FOG profiles. This should suggest that the biological treatment of edible oil effluent is feasible.
- To achieve successful bio-P removal efficiencies using SBR system the process should be modelled upon an anaerobic-aerobic configuration and optimised at SRT of 10 days, HRT maintained at 8 h and organic load ranging from 0.3 to 0.45 kg COD/kg MLSS.d.
- The biological phosphorus removal without nitrification-denitrification has proven to be least complicated bio-P removal process, but requires nitrification to be suppressed and adequate RBCOD fraction for P transformation.
- The laboratory-scale study has demonstrated that the SBR technology is suitable for treating wastewater from edible oil producing industry. The BPR-SBR system showed a consistent P removal efficiency of up to 77.95 %.

- There is a correlation between FOG and COD, i.e. as the FOG content is removed from the oil effluent so does the COD content.
- Adopting a pollution prevention programme (on-site treatment) is a way of doing business, which can provide a number of significant benefits to a company by avoiding unnecessary municipal environmental fines.

It should be noted, however that the multitude of reactions taking place between C-40 and the solids or other constituencies present in the oil effluent could not be established as this did not form part of the study. The use of the C-40 flocculent was to achieve a supernatant liquid with FOG concentration below 1500 mg/L prior to biological treatment process.

Due to the high percentage of oil removal in the application of C-40, it is recommended that investigation be carried out on the:

- possibility of recovering oil from the chemical sludge cakes produced
- treatment of sludge produced
- reuse or disposal of the sludge

The SBR technology has proven a practical option for the treatment of edible oil effluent. It can thus be recommended that the SBR technology be applied at full scale to treat edible oil effluents due to their:

- Reliable, consistently high effluent quality
- Inherent phosphorus removal capacity
- Flexible and adaptable process operation
- Less process equipment maintenance
- Low capital and operational costs
- Low land requirement.

REFERENCES

APHA (1989). *Standard Methods for the Examination of Water and Wastewater*, 17th ed. American Public Health Association, Washington, DC.

AWWA (2000). *Phosphorus in waterways.* Australian Water and Wastewater Association. http://www.awwa.asn.au.html. 30/01/2000

Abou-Elela, S.I. and Zaher, F. (1998). Pollution prevention in the oil and soap industry: A case study. *Wat. Sci. Tech.* **38** (4-5), 139-144.

Abrams, L. (2000). *The South African Water Scene.* The Water Page, URL internet http://www.thewaterpage.com/south_africa.htm.

Abreu L.H. and Estrada, S. (1998). *Sequencing batch reactors. An efficient alternative to wastewater treatment.* URL http://www.rpi.edu/dept/env-energy-eng/WWW/STEPS/EnvSysSBR.html.

Abu-ghararah, Z.H. and Randall, C.W. (1991). The effect of organic compounds on biological phosphorus removal. *Wat. Sci. Tech.*, (**23**), 585.

Adam, N. and Marjanovic, P. (1996). *Oil Degradation using activated sludge.* Umgeni Water communications.

Adamse, A.D. (1968). Bulking of dairy waste activated sludge. *Water Research*, (**2**), 715-722.

Addiscott, T.M., Whitmore, A.P. and Powlson, D.S. (1991). *Farming, fertilizers and the nitrate problem.* C.A.B. International, Wallingford, Oxford.

Andrews, J. F., and Pearson, E. A. (1965). Kinetics and Characteristics of Volatile Acid Production in Anaerobic Fermentation Process, *Int. J. Air Water Pollut.* (G. B.) **9**, 439 – 461.

Appeldoorn, K.J., Kortsee, G.J.J. and Zehnder, A.J.B. (1992). Biological phosphate removal by activated sludge under defined conditions. *Wat. Res.*, **26** (4), 453-460.

Artan, N., Wilderer, P., Orhon, D., Morgenroth, E. and Özgur, N. (2000). The mechanism and design of sequencing batch reactor system for nutrient removal – The state of the art. *Converence Proceedings : 2nd International symposium on Sequencing Batch Reactor Technology.* 10-12 July, 2000, Narbonne, France.

Arun, V., Mino, T. and Matsuo, T. (1988) Biological mechanism of acetate uptake mediated by carbohydrate consumption in excess phosphorus removal systems. *Wat. Res.*, **22** , 565 – 570.

Arun, V., Mino, T., Matsuo, T. (1989). Metabolism of carboxylic acids located in and around the glycolytic pathway and the TCA cycle in the biological phosphorus removal process. *Wat. Sci. Tech*, **21** (4-5), 363 – 374)

Arvin, E (1985), Biological removal of phosphorus from wastewater. *CRC. Crit. Rev. Environ. Control*, **15**: 25-64.

Atkinson, B.W. (1999). *Identification of polyphosphate accumulating bacteria from pilot-and-full scale nutrient removal activated sludge.* Mtech Biotech, dissertation Technikon Natal, Durban, South Africa.

Auling, G., Pilz, F., Busse, H.J., Karrasch, S., Streichan, M. and Schön, G. (1991). Analysis of the polyphosphate accumulating microflora in phosphosrus-eliminating, anaerobic-aerobic activated sludge systems by using diaminopropane as a biomarker for rapid estimation of *Acinetobacter* spp. *Appl. Environ. Microbiol.*, **57**: 3585-3592.

Baetens D., Vanrollenghem, P. A., van Loosdrecht, M. C. M., and Hosten, L. (1999). Temperature effects in bio-p removal. *Wat. Sci. Tech.* **39** (1) pp 215 – 225.

Baetens, D. (2001). *Enhanced biological phosphorus removal: modeling and experimental design.* Thesis submitted in fulfillment of the requirements for the degree of Doctor (Ph.D) in Applied Biological Science, Gent University.

Banerjee, A, Elefsiniotis, P. and Tuhtar, D. (1998). Effects of HRT and temperature on the acidogenesis of municipal primary sludge and industrial wastewater. *Wat. Sci. Tech.,* **38** (8-9): 417-423.

Barnard, J.L., (1974). Cut P and N without chemicals. *Water Wastes Eng.*, **11** : 33-44.

Barnard, J.L., (1976). A review of biological phosphororus removal in the activated sludge process. *Water SA*, **2:** 136-144.

Barnard, J.L. (1993). Prefermentation in biological nutrient removal plants. *Proc. of Joint CSCE-ASCE Nat. Conf. on Environmental Engineering*, July 12-14, Montreal, QC, 1785-1792.

Barnard, J.L. (1994) Alternative Prefermentation Systems. *Paper presented at 67th Annu. Water Environ Fed. Tech. Exposition Conf.*, 1994 Chicago, III.

Barnard, J.L., Wilson, A.W. and Rabinowitz, B. (1997). BNR applications in colder temperature climates. *Presented at: 26th Annual WEAOT Technical Symposium and OPCEA Exhibition*, city of London, Ontario April 13-15, 1997.

Barnes, D., and P. J. Bliss. (1983). *Biological control of nitrogen in wastewater treatment.* E. and F. N. Spon Inc, London.

Bath, *A.* J., (1989). Phosphorus Transport in the Berg River, Western Cape. *Department of Water Affairs and Forestry Report TR143*, Pretoria.

Bayly, R. C., Duncan. A., May, J. W., Schembri, M., Semertjis, A., Vasiliadis, G. and Raper, W.G. C. (1991). Microbiological and genetic aspects of the synthesis of polyphosphate by species of Acinetobacter. *Wat. Sci. Tech.*, **23** (4 – 6), 747 – 754.

Beacham, A.M, Seviour, R.J., Lindrea, K.C and Livingston, I (1990). Genospecies diversity of *Acinetobacter* isolates from a biological nutrients removal pilot plant of a modified UCT configuration. *Wat. Res.* **24** (1): 23 – 29.

Beacham, A,M., R.J. Seviour, and K.C. Lindrea. (1992) Polyphosphate accumulating abilities of *Acinetobacter* isolates from a biological nutrient removal pilot plant. *Wat. Res.* **26** : 121- 122.

Bekir, C. (2001). Membrane bioreactors (MBR) and application of MBR's to oily wastewaters. URL http://www.cce.iastate.edu/courses/ce521/ersu.

Benefield, L.,Kim, S. and Randall, C. (1990). The effect of pentachlorophenol on enhanced biological phosphorus removal in SBR systems. *44th Purdue industrial Waste Conference Proceedings*, 1990 Lewis Publishers, Inc., Chelsea, Michigan.

Bond, P.L., Hugenholtz, P., Keller, J. and Blackall, L.L., (1995). Bacterial community structures of phosphate removing and non-phosphate-removing activated sludges from sequencing batch reactors. *Appl. Environ, Microbiol.*, **61** : 1910-1916.

Bond, P.L., Erhart, R., Wagner, M., Keller, J. and Blackall, L.L., (1999). Identification of some of the major groups of bacteria in efficient and nonefficient biological phosphorus removal activated sludge systems. *Appl. Environ. Microbiol.*, **65** (9): 4077-4084

Bosch, M. and Cloete, T.E. (1993). Taxonomy and phosphorus uptake of *Acinetobacter* in activated sludge mixed liquor. *Water Research Commission Report No. 314/1/93.*

Boughton, W.H., Gottfried, R.J. Sinclair, N.A. and Yall, I. (1971). Metabolic factors affecting enhanced phosphorus uptake by activated sludge. *Appl. Microbiology*, **22** (4), 571-577.

Bosh, M., (1992). *Phosphorus uptake kinetics of Acinetobacter in activated sludge mixed liquor.* M.Sc. thesis. University of Pretoria, South Africa.

Brdjanovi, D., van Loosddrecht, M.C.M., Hooijmans, C.M., Alaerta, G.J. and Heijnen, J.J., (1997). Temperature effect on physiology of biological phosphorus removal. *J.Env. Eng.*, 144-153.

Brdjanovic, D., Slamet, A., van Loosdrecht, M.C., Hooijmans, C.M., Alaerts, G.J. and Heijnen, J.J., (1998a). Impact of excessive aeration on biological phosphorus removal from wastewater. *Water Res.* **32**: 200.

Brdjanovic, D., van Loosdrecht, M.C., Hooijmans, C.M., Mino, T., Alaerts, G.J. and Heijnen, J.J., (1998b). Effect of polyphosphate limitation on the anaerobic metabolism of phosphorus accumulating organisms. *Appl. Microbiol. Biotechnol.*, **50**: 272.

Brodisch, K.E.U. and Joyner, S.J., (1983). The role of micro-organisms other than *Acinetobacter* in biological phosphate removal. *Wat. Sci. Tech.*, **15** (3-4): 117-125.

Brodisch, K.E.U., (1985). Interaction of different groups on microorganisms in biological phosphate removal. *Wat. Sci. Tech.*, **17** (11-12): 89-97.

Brommele, (1999) Chemical or biological phosphorus removal. *Scope Newsletter* **14/15** (32), 40 – 41.

Buchan, L. (1983). Possible biological mechanism of phosphorous removal. *Wat. Sci. Tech.*, **15** : 87 – 103.

CSS, (2000). *Central Statistics Services Food Manufacturers report, 1999/2000.*, Pretoria.

CST, (1997). *Phosphorus*. CRC Handbook of chemistry and physics and the American Chemical Society. CST Information Services Team. URL internet http://pear/1.lan/gov/periodic/elements/15.html.

CYB, (2001). *Edible oils*. URL internet http://cybgroup.co.uk/oils.html.

Carberry, J. B. and Tenney, M. W. (1973). Luxury uptake of phosphate by activated sludge. *Journal of the Water pollution Control Federa.*, **45**: 2444-2462.

Carucci, A., Majone, M., Ramadori, R. and Rossetti, S. (1997). Biological phosphorus removal with different organic substrates in an anaerobic/aerobic sequencing batch reactor, W*at. Sci. Tech.*, **35** (1), 161.

Cavaseno, V. (1980). *Industrial wastewater and solid waste engineering*. Chemical Engineering, McGraw-Hill Publication, Inc., New York, US.

Cech, J. and Hartman, P., (1993). Competition between polyphosphate and polysaccharide accumulating bacteria in enhanced biological phosphorus removal systems. *Water Res.*, **27**: 1219-1225.

Chambers, B. and Thomlinson, E.J., (1982). *The cost of chemicals treatment to control the bulkinn of activated sludge. I Bulking of activated sludge.* (ed.), Chambers, B. and Tomlinson, E.J., Ellis-Horwood, Chichester, pp. 264-70.

Chang, W. C., Chiou, R. .J., and Ouyang, C.F. (1996). The effect of residual substrate utilization on sludge settling in an enhanced biological phosphorus removal process. *Wat. Sci. Tech.* **34** (1-2) 425 – 430.

Chapman, D. (1992). *Water quality assessment: A guide to the use of biota, sediments and water in environmental monitoring.* UNESCO/WHO/UNEP, University Press, Cambridge, GB ISBN O 41245610 9.

Christensson, M. (1997). *Enhanced Biological Phosphorus Removal – Carbon Sources, Nitrate as Electron Acceptor, and Characterisation of the Sludge Community.* Doctoral Dissertation, Department of Biotechnology, Centre for Chemistry and Chemical Engineering, Lund University.

Chuang, S. H., Ouyang, C. F., Yuang, H. C. and You, S. J. (1998). Phosphorus and Polyhydroxyalkanoates Variation in a Combined Process with Activated Sludge and Biofilm. *Wat. Sci. Tech.,* **37** (4 – 5), 593 – 597.

Chudoba, P., Capdeville, B. and Chudoba, J., (1992). Explanation of biological meaning of the S·/X· ratio in batch cultivation. *Wat. Sci. Tech.*, **26** (3-4): 743-751.

Cloete, T.E. and Steyn, P.L., (1987). *A combined fluorescent antibody-membrane filter technique for enumerating Acinetobacter in activated sludge.* In Ramadori, R. (ed.), Biological phosphate removal from wastewaters. Pergamon Press, Oxford, United Kingdom, pp 335-338.

Cloete, T.E. and Steyn, P.L. (1988a). A combined membrane filter-immunofluorescent technique for the *in situ* identification and enumeration of *Acinetobacter* in activated sludge. *Wat. Res.*, **22**: 961-969.

Cloete, T.E. and Steyn, P.L. (1988b). The role of *Acinetobacter* as polyphosphate removing agent in activated sludge. *Wat. Res.*, **22**: 971-976.

Comeau, Y., Hall, K.J., Hancock, R.E.W. and Oldham, W.K. (1986). Biochemical models for enhanced biological phosphorus removal. *Water Res.*, **20** (12), 1511-1521.

Comeau, Y., Hall, K.J., Rabinowitz, B. and Oldham, W.K. (1987). Phosphorus release and uptake in enhanced biological phosphorus removal from wastewater. *J Water Pollut. Control Fed.* **59** 707-715.

Comeau, Y., Hall, K.J. and Oldham, W.K. (1990). Indirect polyphosphate quantification in activated sludge. *Water Poll. Res. J. Canada*, **25**: 161-174.

Corbin, K., (2001). *Biogeochemical cycles: soil microbiology.* BIOL/CSES 4684. URL internet http://www.bsi.vt.edu/changtedor/boil 4684/Cycles/Pcycle.html

Crocetti, G.R. (1999). *The microbiology of enhanced biological phosphorus removal.* PhD thesis University of Queensland, Australia.

Crocetti, G.R, Hugenholtz, P, Bond, P.L., Schuler, A Keller, J, Jenkins, D and Blackall, L.L., (2000). Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Appl. Environ. Microbiol.* **66** (3): 1175 – 1182.

Cuevas-Rodgriguez, G., Gonalex-Barcelo, O. and Gonzalez-Martinez, S. (1998). Wastewater fermentation and Nutrient Removal in sequencing Batch Reactors. *Wat.Sci.Tech.* **38** (1), 255-264.

DEAT, (1999). *White Paper on Environmental Management Policy.* Department of Environmental Affairs and Tourism, South Africa, Pretoria.

DEAT, (2000). *White Paper on integrated pollution and waste management for South Africa.* Department of Environmental Affairs and Tourism, South Africa, Pretoria.

DWAF, (1986). *Management of the water resources of the Republic of South Africa.* Department of Water Affairs and Forestry, South Africa, Pretoria.

DWAF, (1997). *Water quality management orientation course: Integrated Catchment Management.* Department of Water Affairs and Forestry, South Africa, Pretoria.

DWAF, (2000). *The development of a Charge System for Discharging Waste into water resources.* URL internet http://www/africanwater.org/sapollution.htm.06/13/2000.

Dallas, H.F. and Day, J.A. (1993). The effect of water quality variables on riverine ecosystems: A review. *Water Research Commission Rreport TT61/93.*

Danesh, S. and Oleszkiewicz, J.A. (1997). Use of a new anaerobic-aerobic sequencing batch reactor system to enhance biological phosphorous removal. *Wat. Sci. Tech.,* **35** (1):137-144.

Daniel, S., Skalsky, Glen T., Daigger. (1995). Wastewater solids fermentation for volatile acid production and enhanced biological phosphorus removal. *Water Environment Research.* **67** (2)

Davelaar, D., Davies, T.R. and Wiechers, S.G., (1978). The significance of an anaerobic zone for the biological removal of phosphate from wastewater. *Water SA*, **4**: 54-60.

Davies, B and Day, J. (1998). *Vanishing Waters*, UCT Press, Cape Town.

Degrémont, (1991). *Water Treatment Handbook.* Vol. (1-2). 6th (ed.), Lavoisier Publishing, Secaucus, USA.

de Haas, D.W. (1998). *The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting enhanced biological phosp*horus removal. PhD thesis, Department of Civil Engineering University of Cape Town, South Africa.

de Haas, D.W., Lötter, H.L. and Dubery, I.A. (1990). An evaluation of the methods used for the determination of orthophosphate and total phosphate in activated sludge extracts. *Water SA*, **16** (1), 55-65.

de Haas, D.W. and Adam, N. (1995). Use of simple titration procedure to determine H_2CO_3 alkalinity and volatile fatty acids for process control in wastewater treatment. *Water SA*, **21** (4), 307-318.

de Haas, D.W., Wentzel, M.C. and Ekama, G.A. (2000). The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal. Part 1:Literature review. *Water SA*, **26** (4): 439-452.

Deinema, M.H., Habets, L.H.A., Scholten, J., Turkstra, E., and Webers, H.A. (1980). The accumulation of polyphosphate in *Acinetobacter* spp. *FEMS Microbiology Letters,* **9**: 275 –27 9.

Deinema, M.H., van Loodsdrecht, M. and Scholten, A. (1985). Some physiological characteristics of Acinetobacter spp. accumulating large amounts of phosphate. *Wat. Sci. Tech.*, **17** (11-12), 119-125.

de Silva, D.G.V. and Rittmann, B.E. (2000). Nonsteady-state modeling of multispecies activated sludge processes. *Water Environment Research*, **72** (5), 554-565.

Dick, R.I. and Vesilind, P.A. (1969). Sludge volume index-what is it? *Journal of the Water Pollution Control Federation*, **41**: 1285-1291.

Dold, P.L., Ekama, G.A. and Marais, G.v.R., (1980). A general model for activated sludge process. *Prog. Wat. Tech.*, **12** (6), 47-54.

Dold, P.L., Wentzel, M.C., Billing, A.E., Ekama, G.A. and Marais, G.v.R., (1991). Activated sludge simulation programs. *Water Research Commisssion report*, Pretoria, South Africa.

Droste, R.L. (1997). *Theory and practice of water and wastewater treatment.* John Wiley & Sons, Inc. 545-621.

Drysdale, G.D., Atkinson, B.W., Mudaly, D.D., Kasan, H.C. and Bux, F. (2000). Investigation of the microbial contribution to nutrient removal in an activated sludge wastewater treatment process. *Water Research Commission report, no. 822/1/00.* Pretoria, South Africa.

du Plessis, B.J., Bruwer, C.A. and Howard, M.R. (1990). Eutrophication: The tropic status of selected South African Reservoirs. *Department of Water Affairs and Forestry report no. N3/0704.*

EPA. (1984). Technical guidance manual for performing waste load allocations. Book IV: Lakes and impoundments, nutrients/eutrophication impacts. *United States Environmental Protection Agency, report no. EPA 440/4-84-019.*

EPA, (1987). Design Manual – Phosphorus Removal, *Environmental Protection Agency Report EPA/625/1-87/001, September, 1987.*

EPA, (1998). How wastewater treatment works...the basics. *Environmental Protection Agency Report, EPA 833-F-98-002.*

EWPCA, (1985). Lakes pollution and recovery. *proceedings of International Congress of the European Water Pollution Control Association*, Rome, 15-18 April 1985.

Eastman, J.A. (1977). *Solubilisation Of Organic Carbon During The Acid Phase Of Anaerobic Digestion*, Ph.D. Thesis, University of Washington.

Ekama, G.A., Marais, G.v.R. and Siebritz, I.P. (1984). Biological excess phosphorus removal. In theory, design and operation of nutrient removal activated sludge processes. *Water Research Commission report*, Pretoria, South Africa.

Ekama , G.A., Dold, P.L. and Marais G.v R. (1986). Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Wat. Sci. Tech.*, **81** : 91-114.

Ekama, G.A., Wentzel, M.C. and Marais, G.v.R. (1992). Modelling and design of single sludge activated sludge systems for biological removal of carbon, nitrogen and phosphorus. *First IAWQ Technical Tour Nutrient Removal and Anaerobic Digestion in South Africa.* Volume One: Nutrient Removal.

Ekama, G. A. and Wentzel, M.C. (1997). Denitrification kineties in biological N & P removal activated sludge systems treating municipal wastewaters. *Journèes Internationales d'Etude du Cebedeau,* Liège, Palais des Congrès, 22 – 23 May, 1997.

Ekama, G.A. and Wentzel, M.C. (1999). Difficulties and development in biological nutrient removal technology and modeling. *Wat. Sci. Tech.* **39** (6), 1-11.

Elefsiniotis, P. and Oldham, W.K. (1991). The effect of operational parameters on the acid-phase anaerobic fermentation in the biological phosphorus removal process. *Proc.: ASCE Natl Conf. in Environ. Eng., Reno,* Nov, 1991.

Elefsiniotis, P. and Oldham, W.K. (1994). Anaerobic acidogenesis of primary sludge: The role of solids retention time. *Biotech. Bioeng.*, **44**: 7-13.

Elliot, S.A. (1991). *Effluent treatment for small businesses.*

Eroglu,V., Ozturk, I., San, H.A. and Demir, I. (1990). Comparative evaluation of treatment alternatives for wastewaters from an edible oil refining industry. *Wat. Sci. Tech.*, **22** (9), 225-234.

Fox, P. and Pohland, F.G. (1994). Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environment Research,* **66** (5), 716-724.

Frese, H., Robison, L. and Kelly, H.G. (1993). Biological sludge thickening using a rotating drum thickener. *Proc. BCW and Annu. Conf.*, Vernon, April, 1993.

Fuhs, G.W. and Chen, M. (1975). Microbiological basis of phosphate removal in activated sludge process for the treatment of wastewater. *Microbial Ecol.*, **2**, 119-138.

Fukase, T., Shibata, M. and Miyaji, Y., (1985). The role of an anaerobic stage on biological phosphorus removal. *Water.Sci.Technol.*, **17** (2-3), 69-80.

GCIS, (2000). *South African Yearbook, 2000/01.* Government Communication and Information System, Government Printers, Pretoria, South Africa.

Garzón-Zúñiga, M.A. and González-Martínez, S. (1996). Biological phosphate and nitrogen removal in a biofilm sequencing batch reactor. *Wat. Sci. Tech.*, **34** (1-2), 293-301.

Gerber, A., Monstert, E.S., Winter, C.T. and Villiers, R.H.d. (1986). The effect of acetate and other short-chain carbon compounds on the kinetics of biological nutrient removal. *Water SA*, **12** (1), 7-12.

Gerber, A., Monstert, E.S., Winter, C.T. and Villiers, R.H.d. (1987). Interactions between phosphate, nitrate and organic substrate in biological nutrient removal process. *Wat. Sci. Tech.*, **19**: 183 – 194.

Ghosh, S., Conrad, J.R. and Klass, D.L. (1975). Anaerobic acidogenesis of wastewater sludge. *J. Water Pollut. Control Fred.* **47**: 30-45

Graja, S. and Wilderer, P.A. (2000). Characterization and treatment of the liquid effluents from an anaerobic reactor treating putrescible solid waste. *Conference Proceedings : 2nd International symposium on Sequencing Batch Reactor Technology.* 10-12 July, 2000, Narbonne, France.

Grant, P.E. (1980). *Treatment of fatty effluents*. In Food Industry Wastes: Disposal and Recovery. A . Herzka and R.G. Booth (Eds). Applied Science Publishers. London and New Jersey.

Gray, N.F.1989. *Biology of wastewater treatment*. Oxford University Press: New York.

Gray, N.F., 1990. *Activated sludge: theory and practice.* Oxford University Press: New York.

Gray, M.G. (1999). *Recent environmental law developments in SA: The Constitution, the national Environmental Management Act and the National Water Act: Impacts, impressions and industry.*

Grobler, D. C., (1985). Phosphorus budget models for simulating the fate of Phosphorus in South African reservoirs. *Ecological Modelling,* **31**: 219 – 230.

Grobler, D. C. and Silberbauer, M. J., (1985). The combined effect of geology, phosphate sources and runoff on phosphate export from drainage basins. *Wat. Res.*, **19** (8) : 975 – 981.

Gruenebaum, T and Dorgeloh, E. (1992). Biological phosphorus elimination combined with precipitation and flocculation. *Wat. Sci. Tech.* **25**(4 – 5): 219 – 224.

Gujer, W., Henze, M., Mino, T., Matsuo, T., Wentzel, M.C. and Marais, G.v.R. (1995). The activated sludge model no. 2: Biological phosphorus removal. *Wat. Sci. Tech.*, **31** (2), 1-11.

Harris, J., van Vliet, H.R. and MacKay, H.M. (1997). *Water resources quality policy*: The approach adopted by DWAF under the Water Law Principles.

Helmer, C., and Kunst, S., (1998). Low temperature effects on phosphorus release and uptake by microorganisms in EBPR plant. *Wat. Sci. Tech.,* **37** (4 – 5) 531 – 539.

Henze, M., Grady, C.P.L. Jr, Gujer, W., Marais, G.v.R. and Matsuo, T. (1987). A general model for single-sludge wastewater treatment system. *Wat. Res.*, **21** (5), 505-515.

Henze, M. (1992). Characterisation of wastewater for modeling of activated sludge processes. *Wat. Sci. Tech.*, **25** (6), 1-15.

Henze, M., (1996). Biological phosphorus removal from wastewater. *Processes and technology. In: Proc. 10th Forum for Applied Biotechmol.* 26-27 September 1996. University of Gent, Belgium.

Hiraishi, A., Masamune. K. and Kitamura. H. (1989). Characterization of the bacterial pollution structure in an anaerobic-aerobic activated sludge systems on the basis of respiratory quinone profiles. *Appl. Envirom. Microbiol.* **55.** 897 – 901.

Hiraishi, A, Ueda, Y and Ishihara, J-I (1998). Biomarker and molecular approaches to the microbial community analysis of activated sludge. *Proceedings of Microbial Community and Functions in Wastewater Treatment Processes.* The International Symposium of the Centre of Excellence, Department of Urban Engineering, School of Engineering, The University of Tokyo, 10-11 March, 1998.

Holhs,B.C., Quibell, G., du Plessis, B.J. and Belcher, T. (1998). Assessment of the implementation of the phosphate standard at the Baviaanspoort and the Zeekoegat water care works. *Institute for Water Quality Studies Report, Department of Water Affairs and Forestry, N/A230/01/DEQ0797.*

Hong, S., Krichten, D., Best., and Rachwal, A. (1984). Biological phosphorus and nitrogen removal via the A/O process: recent experience in the United States and United Kingdom. *Wat. Sci. Tech.,* **16** (10-11), 151 – 172.

Horan, N.J., (1990). *Biological Wastewater treatment Systems: Theory and Operations.* John Wiley & Sons: Chichester, England.

Hui, H.Y. (1996). *Edible oil and fats products: Processing technology.* In: Bailey. Industrial oil and fats products. John Wiley & Sons, Inc.

Hwu, C.S. (1998). Biosorption of long-chain fatty acids in UASB treatment process. *Water Research*, **32** (5), 1571-1579.

ISEO, (1999). Food Fats and Oils. *Institute of Shortening and Edible Oils report.* New York Avenue, NW, Washington, DC.

ITC, (1997). The South African Market for food and beverages. *International Trade Centre report*: African Buyers/Sellers Meeting, 21-23 January 1997, Durban, South Africa.

Irvine, R.L. and Busch, A.W. (1979). Sequencing batch biological reactors – An overview. *J. Water Pollut. Contr. Fed.*, **15**: 235-243.

Irvine, R.L., Wilderer, P.A. and Flemming, H.C. (1997). Controlled unsteady state processes and technologies – An overview. *Wat. Sci. Tech*, **35** (1), 1-10.

Jenkins, D., Richard, M.G. and Daigger, G.T., (1984). Manual on the cause and control of activated sludge bulking and foaming. *Water Research Commission report*, Pretoria, South Africa.

Jenkins, D. and Tandoi, V. (1991). The applied microbiology of enhanced biological phosphorus removal-accomplishments and needs. *Wat.Res.*, **25** (12), 1471-1478.

Jeon, C.O., Lee, D.S. Lee, M.W. and Park, J.M., (2001). Enhanced biological phosphorus removal in an anaerobic-aerobic sequencing batch reactor: Effect of pH. *Water Environment Research.*, **73** (3), 301-306.

Jeyanayagam, S., Rodieck, A. and Husband, J. (2000). Catch up with a quick, comprehensive biological nutrient removal refresher. *WE & T*, 85-88.

Jones, P. H., Tadwalker, A.D., and Hsu, C.L. (1987). Enhanced uptake of phosphorus by activated sludge – effect of substrate addition. *Water Research,* **21,** 301 – 8.

Jones, M. and Stephenson, T. (1996). The effect of temperature on enhanced biological phosph*ate* removal. *Env. Techn.,* **17,** 965 – 976.

Juni, E., (1984). *Genus III. Acinetobacter.* Brisou and Prevot, 1954. In: Krieg, N.R. and Holt, J.G. (ed.), Bergey's manual of systematic bacteriology, The Williams and Wilkens Co., Baltimore, **1**: 303-307.

Karlovich, W.M., (1994). *Pilot scale study of biological phosphorus removal using Modified UCT process.* M.Sc. thesis. Dept. Civil Environmental Engineering, University of Wisconsin.

Kasper, H.F. and Wuhrmann, K. (1978). Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. *Applied and Environmental Microbiology,* **36** (1), 1-7.

Kavanaug, R.G. and Randall, C.W. (1994). Bacterial populations in a biological nutrient removal plant. *Wat, Sci. Tech.* **29** (7): 25 – 34.

Keefer, C.E. (1963). Relationship of sludge density index to the activated sludge process. *Journal of the Water Pollution Control Federation*, **35**, 1166-73.

Keller, J., Watts, Battye-Smith, W. and Chong, R. (2000). Full-scale demonstration of biological nutrient removal in a single tank SBR process. *Conference Proceedings: 2nd International symposium on Sequencing Batch Reactor Technology.* 10-12 July, 2000, Narbonne, France.

Khan, M.N. and Akhtar, W. (1998). Wastes from edible oil and fat industry of Karachi. *Proceedings: 24th WEDC Conference, Sanitation and Water for All*, I998, Islamabad, Pakistan.

Kuba, T., Smolder, G., A., van Loosdrecht, M.C.M. and Heijnen, J.J. (1993). Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. *Water Sci. Tech.*, **27** (5-6), 241-252.

Kuba, T., Watchtmeister, A., van Loosdrecht, M.C.M. and Heijnen, J.J. (1994). Effect of nitrate on phosphorus release in biological phosphorus removal systems. *Water Sci. Tech.*, **30** (6), 263-269.

Lau, A.O., Storm, P.F. and Jenkins, D., (1984). Growth kinetics of *Sphaerotilus natans* and a floc-former in pure and dual continuous culture. *Journal WPCF*, **56** (1): 41-51.

Laughlin D.K., Edwards, F.G., Egemen E., and Nirmalakhandan, N. (1998). Importance of dissolved oxygen in sequencing batch reactors treating high cod industrial wastes. *Fourth International Symposium on Waste Management Problems in Agro-Industries*, Istanbul-Turkey 23 – 25 September.

Lemos, P.C., Viana, C., Salgueiro, E.N., Ramos, A.M., Crespo, J..P.S.G. and Reis, M.A.M. (1998). Effect of carbon source on the formation of polyhydroxyalkanoates (PHA) by a phosphate-accumulating mixed culture. *Enzyme and Microb. Tech.*, **22**: 662-671.

Lilley, I.D., Wentzel, M.C., Loewenthal, R.E. and Marais, GvR. (1990). Acid fermentation of primary sludge at 20°C. *Res. Rep. W64,* Department of Civil Engineering, University of Cape Town.

Lilley, I.D., Pybus, P.J. and Power, S.P.B. (1997). Operating manual for biological nutrient removal wastewater treatment works. *Water Research Commission report TT 83/97.*

Little, K. and Zander, B. (1996). Optimal trading between point and non-point sources of phosphorus in the Chatfield Basin, Colorado. URL http://www.epa.gov/OWOW/watershed/Proceed/little.html.

Liu, W,-T.; Mino, T.; Nakamura, K.; and Matsuo, T. (1994). Role of Glycogen in Anaerobic Uptake and Polyhydroxyalkanoate Synthesis in Anaerobic-Aerobic Sludge with a Minimized Polyphosphate Content. *J. Ferment. Bioeng.*, **77**, 5, 535.

Liu, W.-T.; Mino, T.; Matsuo, T.; and Nakamura, K. (1996). Biological Phosphorus Removal Process – Effect of pH on Anaerobic Substrate Metabolism. *Water Sci. Technol.*, **34**: 25.

Liu, W.-T., Nakamura, K., Matsuo, T. and Mino, T., (1997). Internal energy-based competition between polyphosphate- and glycogen-accumulating bacteria in biological phosphorus removal reactors – Effects of P/C feeding ratio. *Water Res.*, **31**: 1430.

Lory, J.A. (1999). *Managing manure phosphorus to protect water quality.* Department of Agronomy and Commercial Agriculture Program. Agricultural publication G9182.

Lötter, L.H., (1985). The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process. *Wat. Sci. Tech.*, **17**: 127-138.

Lötter, L.H., and Murphy, M., (1985). The identification of heterotrophic bacteria in an activated sludge plant with particular reference to polyphosphate accumulation. *Water SA*, **11** (4): 179-184.

MPCA, (2000). MPCA phosphorus strategy. Minnesota Pollution Control Agency. URL http://www.pca.state.mn.us/water/phosphorus.html.

Mamais, D. and Jenkins, D., (1992). The effects of MCRT and temperature on enhanced biological phosphorus removal. *Wat. Sci. Tech.*, **26**: 955.

Mamais, D, Jenkins, D and Pitt, P. (1993). A rapid physical-chemical methods for the determination of readily biodegradable soluble COD in municipal wastewater. *Wat. Res.*, **27** (1):195-197.

Manson, A.D., Miles, N., Roberts, V.G. and Katusic, V. (2000). *Soil fertility and analytical services.* Course notes, 2000. URL http://agriculture.kzntl.gov.za/d... ent/soilfertility/phosphorus.htm. 12/21/2000.

Manz, W., Wagner, M., Amann, R. Schleifer, K.-H., (1994). *In situ* characterization of the microbial consortia active in two wastewater treatment plants. *Water Res.*, **28**: 1715-1723.

Marais, G.V.R.; Loewenthal, R.E.; and Siebritz, I.P. (1983). Review: Observations Supporting Phosphate Removal by Biological Excess Uptake. *Water Sci. Techno.*, **15,** 15.

Mark, C., Wentzel, M.C. and Ekama, G.A., (1997). Principles in the design of single-sludge activated sludge systems for biological removal of carbon, nitrogen and phosphorus. *Water Environment Research*, **69** (7): 1222-1231.

Marklund, S. and Morling, S., (1994). Biological phosphorus removal at temperatures from 3°C to 10°C – A full-scale study of a sequencing batch reactor unit. *Can. J. Civil Eng.*, **21**:81.

Maszenan, A.M., Seviour, R.J., Patel, B.K.C., Rees, G.N. and McDougall, B., (1998). The hunt for the G-bacteria in activated sludge biomass. *Wat. Sci. Tech.*, **37** (4-5): 65-69.

Matsuo, Y. (1994). Effect of the Anaerobic Solid Retention Time on Enhanced Biological Phosphorous Removal. *Water Sci. Technol.*, **30**:193

Maurer. M., Gujer, W., Hany, R. and Bachmann, S. (1997). Intracellular carbon flow in phosphorus accumulating organisms from activated sludge system. *Wat. Res.* **31** (4). 907 – 917.

Maurer, M. and Gujer, W. (1997). *Biological Phosphorus Removal in Activated Sludge Systems*, provided by the Eurospider Information Retrieval System.

Maurer, M. and Gujer W. (1998). Dynamic modeling of enhanced biological phosphorus and nitrogen removal in activated sludge systems. *Wat. Sci. Tech.*, **38** (1), 203 – 210.

Mbewe, A., Wentzel, M.C. and Ekama, G.A., (1995). Characterisation of municipal wastewaters. *UCT Report No. W 84*, Dept. Civil Eng. University of Cape Town, Rondebosch, South Africa.

McClintock, S.A., Randall, C.W. and pattarkine, V.M., (1993). Effect of temperature and mean cell residence time on biological nutrient removal processes. *Water Environ. Res.*, **65**:110.

McInerney, M.J., Bryant, M.P., and Costerton, J. W. (1981). "Syntrophomonas wolfei gen. Nov., an Anaerobic, Syntrophic, Fatty Acid-Oxidizing Bacterium," *Appl. Environ. Microbiol.*, **41**: 1029 – 1039.

Metcalf and Eddy (1991). *Wastewater engineering: Treatment, disposal and reuse*. 3rd Ed. McGraw Hill, Inc. New York.

Mino, T., Arun. V, Tsuzuki, Y. and Matsuo, T. (1987). *Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process*. In: *Biological Phosphate Removal from Wastewater*. R Ramadori (ed.) Pergamon Press, Oxford. Pp. 27 – 38.

Mino, T., Liu, W.T., Kurisu, F. and Matsuo, T., (1995). Modelling glycogen storage and denitrification capability of microorganisms in enhanced biological phosphate removal processes. *Wat. Sci. Tech.*, **31**: 25-34.

Mino, T., van Loosdrecht, M. C. M. and Heijnen, J. J. (1998). Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research,* **32** (11), 3193 – 3207.

Mkhize, S.P. (2002). *Assessment of biological nutrient removal process for the remediation of edible oil effluent*. Mtech Biotech, dissertation Technikon Natal, Durban, South Africa.

Momba, M.N.B. and Cloete, T.E., (1996). The relationship of biomass to phosphate uptake by *Acinetobacter junii* in activated sludge mixed liquor. *Wat. Res*. **30** (2): 364-370.

Momberg, G.A. (1991). Phosphate crystallization in activated sludge systems. *Water Research Commission report.*

Momberg, G.A. and Oellermann, R.A. (1993). A comprehensive study on an iron-phosphate removal system. *Water Research Commission report 430/1/93.*

Moore, C.A., van Veelen, M., Ashton, P.J. and Walmsley, R.D. (1991). Preliminary water quality guidelines for the Kruger National Park Rivers. *Kruger National Park Rivers Research Programme*, Report no.1:91.

Moser-Engeler, R., Udert, K.M., Wild, D. and Siegrist, H. (1998). Products from primary sludge fermentation and their suitability for nutrient removal. *Wat. Sci. Tech.*, **38** (1): 265-273.

Moss, B. (1998). Shallow lakes, biomanipulation and eutrophication. *Scope Newsletter*, **29**: 20-27.

Műnch E. and Koch F. A. (1997a). A survey of prefermenter design, operation and performance in Australia and Canada. *Wat Sci. Tech.* (in press).

Műnch E. and Pollard P.C. (1997b). Measuring bacterial biomass-COD in wastewater containing particulate matter. *Water Res.* **31** (10), 2550 – 2556.

Muench, E.V., Keller, J., Newell, R.B. and Lant, P.A. (1996). Application of prefermenters to aid biological nutrient removal from domestic wastewater. *Proc. of the Asia-Pacific Conference on Sustainable Energy and Environmental Technology*, Singapore, 41-48.

Mullis, M. K., and E. D. Schroeder. (1971). A rapid biochemical oxygen demand test suitable for operational control. *Journal of Water Pollution Control Federation* **43** (2): 209 – 15.

Muñoz-Colunga, A. and Gonzalez-Martinez, S., (1996). Effects of population displacements on biological phoshorus removal in a biofilm SBR. *Wat. Sci. Tech.*, **34** (1-2), 303-313.

Murray, K.A. (1987). Wastewater treatment and pollution control. *Water Research Commission*, Report, Pretoria.

Muyima, N.Y.O., Momba, M.N.B. and Cloete, T.E. (1997). Biological methods for the treatment of wastewaters. In: Cloete, T.E. and Muyima, N.Y.O. (eds) Mecrobial Community Analysis: The key to the design of biological wastewater treatment systems. *IAWQ Scientific and Technical Report No. 5, IAWQ*, London. pp1-24.

Muyzer, G. and Ramsing, N.B., (1995). Organisation of microbial communities. *Wat. Sci. Tech.*, **32** (8): 1-9.

NCSU, (1998). *Water resources characterization DSS-phosphorus*. URL internet http://h2osparc.wq.ncsu.edu/ info/pho.html. 02/03/1998.

Nakamura, K., Masuda, K. and Mikami, E. (1989). *Polyphosphate accumulating bacteria and their ecological characteristics in activated sludge process.* In recent Advances in Mecrobial Ecology, Pro. 5th International Symposium on Microbial Ecology, ISMES (Edited by Hattori, T., Ishioda, Y., Maruyama, Y,. Morita, R. and Uchida,A.), pp. 427-431. Japan Scientific Societies Press, Tokyo.

National Environmental Management Act, (1998). *National Environmental Management Act, No.107 of 1998.* Department of Environmental Affairs and Tourism, Cape Town, South Africa.

NIWR, (1985). The limnology of Hartbeespoort Dam. South African Scientific Programmes, *National Institute of Water Research Report No. 110.*

National Water Act, (1999). *National Water Act, No. 36 of 1998.* Department of Water Affairs and Forestry, Cape Town, June, 1999.

Natuscka., M. Lee., Carlsson., H., Aspegren, H., Welander, T., and Anderson, Bengt. (1996). Stability and variation in sludge properties in two parallel systems for enhanced biological phosphorus removal operated with and without nitrogen removal. *Wat Sci Tech.* **34** (1-2) 101 – 109.

Nicholls, H. A., Pitman, A. R., and Osborn, D. W. (1985). The readily biodegrable fraction of sewage: Its influence on phosphorus removal and measurement. *Wat. Sci. Tech* **17,** (73 – 87).

Nicholls, H. A., Osborn, D. W. and Pitman, A. R. (1986). Biological phosphorus removal at the Johannesburg Northern and Goudkoppies wastewater purification plant. *Water SA* **12,** 13 – 18.

Norcross, K.L. (1992.) Sequencing Batch Reactors – An Overview. *Wat. Sci. Tech.*, **26** (9-11), 2523-2526.

Novotny, G. (1998). Wastewater characterization for evaluation of biological phosphorus removal wastewater fraction. Internet URL http://www.dnr.state.wi.us/org/water/wm/ww/biophos/3fract.htm. 1-18.

Nitshudisane, B.M., Oosthuizen D.J., Ehlers, T.E. (2000). Biolog for the determination of microbial species diversity and evenness in activated sludge systems. *Water Research Commission. Report No 933/1.*

Orhon D., Ates, E., Sözen, S. and Ubay Cokgör, E. (1994). Characterisation and COD fractionation of domestic wastewaters. *Envir. Pollut.* **95** (2), 191-204.

Orhon, D. and Ubay Cokgör, E. (1997). COD fractionation in wastewater characterization – the state of the art. J. Chem. Tech. Biotech., **68**: 294-302.

Orhon, D., Tasli, R. and Sözen, S. (1998). Experimental basis of activated sludge treatment for industrial wastewaters. The state of the art. *Fourth International Symposium on waste management problems in Agro-industries,* 23 – 25 September. Istanbul – Turkey.

Orhon, D., Ubay Cokgör, E. and Sözen, S. (1999). Experimental basis for the hydrolysis of slowly biodegradable substrate in different wastewaters. *Wat. Sci. Tech.*, **39** (1): 87-95.

Osborn, D.W., Lötter, L.H., Pitman, A.R. and Nicholls, H.A. (1986). Enhancement of biological phosphate removal by altering process feed composition., *Water Research Commission, Report 137/1/86.*

Osifo, P.O., (2000). *Development and assessment of a membrane bioreactor for wastewater treatment.* Mtech Biotech, dissertation Technikon Natal, Durban, South Africa.

Ozturk, I., San, H.A. and Eroglu, V, (1990). Pilot and full scale treatability studies on wastewater from an edible oil refining industry. *44th Purdue Industrial waste Conference Proceedings*, Lewis Publishers, Inc. Chelsea, Michigan, USA.

PMTLC, (1996). *Industrial Effluent By-laws, Council's Drainage Bylaws, Municipal Notice 267.* Pietermaritzburg-Msunduzi Transitional Local Council. South Africa, Pietermaritzburg.

Palm, J.H. Jenkins, D., and Parker, D.S. (1980). Relationship between organic loading, dissolved oxygen concentration and sludge settleability in the completely-mixed activated sludge process. *Journal of the Water Pollution Control Federation*, **52**:2484-506.

Park, J.K., Wang, J. and Novotney, G. (1997). Wastewater characterization for biological phosphorus removal. *Wisconsin Department of Natural Resources Research Report 174*, August 1997.

Park, J.K, Whang, L.M., Wang, J.C. and Novotny, G. (2001). A biological phosphorus removal potential test for wastewaters. *Water Environment Research*, **73** (3), 374-382.

Pauss, A. and Nyns, E.J. (2000). Biology of methane formation: methanogenesis. *Handout notes*, 2000, Department of Biotechnology, Technikon Natal.

Pereira, H., Lemos, P. C., Carrondo, M. J. T., Crespo, J. P. S. G., Reis, M .A. M. and Santos, H. (1996) Model for carbon metabolism in biological phosphorus removal processes based on *in vivo* NMR labeling experiments. *Water Res.* **30,** 2128 - 2138

Pillay, M. (1994). *Detergent phosphorus in South Africa: Impact on eutrophication with specific reference to the Mngeni catchment.* MSc Engineering dissertation, Department of Chemical Engineering, University of Natal, Durban.

Pipes, W.O. (1967). Bulking of activated sludge. *Advances in Applied Micro-biology*, **9**: 185-234.

Pipes, W.O., (1979). Bulking, deflocculation and pin-point floc. *Journal Water Pollution Control Federation*, **51**: 62-70.

Pitman, A. R. (1984). Settling of nutrient removal activated sludges. *Wat. Sci. Tech.* **17** (4/5), 493 – 504.

Pitman, A. R., Trim, B. C. and van Dalsen, L. (1998). Operating experience with biological nutrient removal at the Johannesburg Bushkoppie works. *Water Science and Technology,* **20** (4 – 5), 51- 62.

Pitman, A. R. (1991). Design considerations for nutrient removal plants. *Water Science and Technology,* **23** (4 - 6), 781 – 790.

Pitman, A.R., Loetter, L.H., Alexander, W.V. and Deacon, S.L. (1992). Fermentation of raw sludge and elutriation of resultant fatty acids to promote excess biological phosphorus removal. *Wat. Sci. Tech.*, **25** (4-5): 185-194.

Pitman, A.R. (1995). Practical experiences with biological nutrient removal on full-scale wastewater treatment plants in South Africa. *Bio-P Conference, Hanover 95*, Germany.

Pitman, A.R. and Boyd, L.A. (1999). Transforming local government wastewater departments from adversary to industrial partner. *Wat. Sci. Tech., **39*** (10-11): 39-45.

Quibell, G., van Vliet, H., and Merwe van der, W. (1997). Characterising cause-and-effect relationships in support of catchment water quality management. *Water S.A*, **23** (3).

Rabinowitz, B. and Oldham, W.K. (1985a). Excess biological P removal in the activated sludge process using primary sludge fermentation. *Annu. Conf. Of the Canadian Soc. for Civil Engineering, Saskatoon,* SK.

Rabinowitz, B. and Oldham, W.K. (1985b). The use of primary sludge fermentation in the enhanced biological phosphorus removal process. *Proc. ASCE/CSCE Conf. on New Directions and Research in*

Waste Treatment and Residuals Management, 23-28 June, University of British Columbia, Vancouver. 347-363.

Rae, B., Moollan, R.W. and Clark, R.C. (1999). Algal toxins in drinking water supplies. *Water Research Commission Report, No. 549/1/99.*

Ramalho, R.S. (1983). *Introduction to wastewater treatment processes.* Second edition. Academic Press, New York.

Randall, C.W., Barnard, J.L. and Stensel, H.D. (1992). *Design and retrofit of wastewater treatment plants for biological nutrient removal.* Technomic Publishing, Lancaster, Pa.

Randall, A.A., Benefield, L.D. and Hill,W.E. (1996). Enhanced biological phosphorus removal: the variation in location and form of intracellular phosphate induced by different substrates and observed with ^{31}P-NMR. Internet URL http://www.sfo.com/~aer/518-96.html.

Randall, A. A., Benefield L. D., Hill, W. E., Nicol, J-P., Boman, G. K., and Jing, S-R. (1997). The effect of volatile fatty acids on enhanced biological phosphorus removal and population structure in anaerobic sequencing batch reactors. *Wat. Sci. Tech* **35** (1) 153 – 160.

Rensink, JH. (1991). Principle of phosphorus and nitrogen removal processes, incorporated in activated sludge treatment systems. *proc. of "The 1st Netherlands-Japan workshop on sewage treatment" in the Netherlands.*

Rodrigo, M.A., Seco, A., Penya-roja, J.M. and Ferrer, J., (1996). Influence of sludge age on enhanced phosphorus removal in biological systems. *Wat. Sci. Tech.*, **34** (1-2), 41-48.

Ross, W.R. Novella, P.H. Pitt, A.J. Lund, P. Thomson, B.A. King, P.B. and Fawcett, K.S. (1992). Anaerobic digestion of wastewater sludge : Operating Guide. *Water Research Commission, report TT55/92.*

Rubens, T.F., (1994). *Pilot plant demonstration of the A2O Process.* Masters thesis, Dept. Civil Environmental Engineering, University of Wisconsin, Madison.

Rusnik, I. And Wanner, J. (2000). Biological nutrient removal in SBR systems. *Conference Proceedings: 2nd International symposium on Sequencing Batch Reactor Technology.* 10-12 July, 2000, Narbonne, France.

Rustrian, E., Delgenes, J.P. Bernet, N. and Moletta, R. (1997). Nitrate reduction in acidogenic reactor: Influence of wastewater DCO/N-NO3 ratio on denitrification and acidogenic activity. *Envir. Techn.,* **18**: 309-315.

STI, (2000). *Dynamic simulator for prefermenters.* Science Traveler International. Internet URL http://www.scitrav.com/wwater/dsp.htm. 05/20/2000.

Sampson, I. (2001). Introduction to a legal framework to pollution management in South Africa. *Water Research Commission, report TT 149/01.*

Sanchez, W.A., Kennedy, K., Hamoda, M.F. and Droste, R.L. (1990). A study of anaerobic SBRs for the treatment of soluble wastes. *44th Purdue Industrial Waste Conference Proc.* Lewis Publishers, Inc. Chelsea, Michigan, USA.

Satoh, H., Mino, T. and Matsuo, T., (1992). Uptake of organic substrates and accumulation of polyhydroxyalkanoates linked with glycolysis of intracellular carbohydrates under anaerobic conditions in the biological excess phosphate removal processes. *Wat. Sci. Tech.*, **26**: 933-942.

Satoh, H., Mino, T. and Matsuo, T., (1994). Deterioration of enhanced biological phosphorus removal by the domination of microorganisms without polyphosphate accumulation. *Wat. Sci. Tech.*, **30**: 203-211.

Satoh, H., Koch, F.A. Mino, T. and Matsuo, T., (1996). Anaerobic substrate uptake by the enhanced biological phosphorus removal activated sludge treating real sewage. *Wat. Sci. Tech.* **34** (1-2), 9-16.

Satoh, H., Mino, T. and Matsau, T. (1997). Anaerobic uptake of glutamate and asparate by the enhanced biological phosphorus removal activated sludge. *Proceedings Second International Conference on Microorganisms in Activated Sludge and Biolfilm Processes.* IAWQ, 21st- 23rd July, 1997, Berkeley, California.

Sawyer, C.N., McCarty, P.L. and Parkin, G.F. (1994). *Chemistry for environmental engineering.* 4th ed. McGraw-Hill, Inc.

Sawyer, J.E., and Mallarino, A. (2000) *Why manage phosphorus?* Internet URL http://www.ent.iastate.edu/ipm/icm/2000/4-24-2000/phosphorus.html

Scheer, H. and Seyfried, C.F. (1996). Enhanced biological phosphate removal: Modelling and design in theory and practice. *Water Sci. Tech.*, **34** (1-2), 57-66.

Schreiner, B. (1999). Water everywhere. *Water Sewage and Effluent.* **19** (19), 10-11.

Schroeder, E.D. (1977). *Water and wastewater treatment.* McGraw-Hill Publication, Inc. New York, US.

Sedlak, R. (1991). *Phosphorus and nitrogen removal from municipal wastewater.* 2nd ed. Lewis.

Sell, R. L., Krichten, D. J., Noichl, O.J. and Hartzog, D. G. (1981). Low temperature biological phosphorus removal. *Proc., 54th WPCF Conf.*

Seng, W.C., (1980). Wastewater treatment for edible oil refineries. *Journal of American Oil Chemistry Society*. **57** (12): 926A-928A.

Serafim, L.S., Lemos, P.C., Crespo, J.G. and Reis, M.A.M. (2000). Effect of pH control on EBPR. *2nd International Symposium on SBR Technology*, July, 2000. Narbonne, France.

Shahnaz, D. and Oleszkiewicz, J.A. (1997). Use of a new anaerobic-aerobic sequencing batch reactor systems to enhance biological phosphorus removal, *Wat. Sci. Tech.*, **35,** No.1, pp 137 – 197.

Shamskhorzani, R. and Nocross, K.L. (2000). SBR systems: An attractive BNR process. *2nd International Symposium on SBR Technology*, July, 2000. Narbonne, France.

Shao, Y.J., Abkain, V., Crosse, J., Horestein, B. and Jenkins, D. (1991). The effect of MCRT on biological phosphorus removal. *California Annual WPCF Conference*, Pasadenea, CA.

Sharpley, A. (1995). RCA III fate and transport of nutrients: Phosphorus. *Working paper no. 8. USDA, Agricultural Research Service, National Agricultural Water Quality Laboratory*, Durant, Oklahoma. Internet URL http://www.nhq.nrcs.usda.gov/RCA papers/WP08text.html.

Sidat, M., Kasan, H.C. and Bux, F., (1999). Laboratory-scale investigation of biological phosphate removal from municipal wastewater. *Water SA*, **25** (4), 459-462.

Siebritz, I. P, Ekama, G. A. and Marais, G v R (1983). A parametric model for biological excess phosphorus removal. *Wat. Sci. Tech,* **15,** (3/4), 127-152.

Silberbauer. M. J. (1992). The effect of a proposed sewage works in the Moreleta Spruit catchment on the phosphate and nitrate loading to Roodeplant Dam. Hydrological Research Institute, *Department of Water Affairs and Forestry. Report number B33/2/123/7.*

Smit, M.R., Zinder, S.H. and Hah, R.A. (1980). Microbial methanogenesis from acetate. *Proc. Biochem.* **15:** 34-39.

Smolders, G.J.F.; van der Meij, J.; van Loosdrecht, M.C.M.; and Heijnen, J.J. (1994a). Model of the Anaerobic Metabolism of the Biological Phosphorus Removal Process: Stoichiometry and pH influence. *Biotech. Bioeng*, **43**: 461-470.

Smolders, G. J. F., Van Loosdrecht M.C. M. and Hiejnen J. J. (1994b). A metabolism model for the biological phosphorus removal process. *Wat. Sci. Tech.* **31** (2), 79 – 93.

Smolders, G. J. F., van Loosdrecht. M. C. M. and Heijnen. J. J. (1995). A Metabolic Model for the Biological Phosphorus Removal Process. *Water Science Technology.*, **31** (2). 79 – 93.

Spatzierer, G., Ludwig, C. and Matsche, N. (1985). Biological phosphorus removal in combination with simultaneous precipitation. *Wat. Sci Tech.*, **17** (11/12), 163 – 176.

Srinath, E.G., Sastry, C.A. and Pillai, S.C. (1959). Rapid removal of phosphorus from sewage by activated sludge. *Experientia,* **15**: 339-340.

Steffen, Robertson and Kirsten Inc. Consulting Engineers. (1989). Natsurv 6: Water and wastewater management in the edible oil industry. *Water Research Commission, report 145, TT40/89.*

Steyl, I. (1999). Strategic environmental assessment for stream flow reduction activities in South Africa – A way of enhancing decision-making. *Paper presented: African environment past and present conference,* Oxford, UK, July 1999.

Streichan, M., Golecki, J.R. and Schon, G., (1990). Polyphoshpate accumulating bacteria from sewage plants with different processes for biological removal. *FEMS Microbiological Ecology*, **73**: 113-124.

Stryer, L. (1981). *Biochemistry*. W. H. Freeman and Co., San Francisco, CA.

Suresh, N., Warburg, R., Timmerman, M., Wells, J., Coccia, M., Roberts, M.F. and Halvorson, H.O, (1985). New strategies for the isolation of microorganisms responsible for phosphate accumulation. *Wat. Sci. Tech.*, **17**:99-111.

Sutton, C. (1994). *Survey of industrial effluents produced in KwaZulu Natal.* Btech Biotech, Technickon Natal, Durban.

Tasli, R., Orhon, D. and Artan, N. (1999). The effect of substrate composition on the nutrient removal potential of sequencing batch reactors. *Water SA*, **25** (3), 337-344.

Tchobanoglous, G. and Burton, F.L., (1991). *Water engineering, treatment, disposal and re-use.* McGraw-Hill series in water resources and environmental engineering 3rd ed. Metcalf and Eddy. pp. 359-434.

Temmink, H., Petersen, B., Isaacs, S. and Henze, M. (1996). Recovery of biological phosphorus removal after periods of low organic loading. *Wat. Sci. Tech.*, **34** (1-2), 1-8.

Toerien, D.F., Hyman, K.L. and Bruwer, M.J. (1975) A preliminary trophic status classification of some South African impoundments. *Water SA*, **1**: 15 – 23.

Toerien, D. F., (1977). A view of eutrophication and guidelines for its control in South Africa. *Special Report Wat.* **48,** National Institute for Water Research, CSIR, Pretoria, Republic of South Africa.

Toerien, D. F., Hyman, K.L. & Bruwer, M.J., (1978). A preliminary trophic status classification of some South African impoundments. *Water SA* **1.**

Toerien, D.F., Gerber, A., Lötter, L.H. and Cloete, T.E. (1990). Enhanced biological phosphorus removal in activated sludge systems. In *Advances in Microbial Ecology* (Edited by Marshall, K.C.), pp. 173-230. Plenum Press, New York.

Tomlinson, E.J. and Chambers, B. (1984). Control strategies for bulking sludge. *Water Science and Technology*, **16**: 15-34.

Toprak, (2001). *Design considerations for secondary wastewater treatment.* Internet URL http://www.deu.edu.tr/atiksu/toprak/ani4061.html

Tracy, K.D. and Flamino, A. (1987). Biochemistry and energetics of biological phosphorus removal. Advances in Water Pollution Control. Biological Phosphate Removal from Wastewaters, *Proc. Of an IAWPRC specialized Conference*, Rome.

Ubisi, M.F., Jood, T.W., Wentzel, M.C. and Ekama, G.A., (1997). Activated sludge mixed liquor heterotrophic active biomass. *Water SA*, **23** (3), 239-248.

v. Münch, E.V., (1998). Prefermentation technology for BNR plants. *The Activated Sludge Pages*, Issue 1:9.

Vacker, D. (1967). Phosphate removal through municipal wastewater treatment at San Antonio, Texas. *Jour. Water Pollut. Control Fed.*, **37**: 800.

van der Merwe, W. and Grobler, D.C. (1990). Water quality management in the RSA: Preparing for the future. Water SA, **16** (1),49-53.

van der Merwe, B. and Quibell, G. (1993). The impact of phosphorus load reduction on eutrophication related water quality in the Klipfontein Dam. Hydrological Research Institute, *Department of Water Affairs and Forestry Report No. N/W200/DPQ/2493.*

van Dijk, J.C. and Braakensiek, H. (1985). Phosphate removal by crystallization in fluidized bed. *Wat. Sci. Tech.*, **17** (2-3), 133-142.

van Loosdrecht, M.C., Smolders, G.J., Kuba, T. and Heijnen, J.J. (1997). The metabolism of micro-organisms responsible for phosphorus removal. *Scope Newspaper*, **71**: 109-116.

van Loosdrecht, M.C.M. (2001). *Role of biological processes in phosphate recovery.* Kluyver Institute for Biotechnology, Delft University of Technology. Internet URL http://www.nhm.ac.uk/mineralogy/pho/loosdr.htm 02/01/2001.

van Niekerk, A.M. (2000). Technological perspectives on the new South African effluent (waste) discharge standards. *presented @ WISA 2000 Biennial Conf.*, Sun City, SA. 28 May-1 June 2000.

Venter, S.N., Lötter, L.H., de Haas, D.W. and Mac Donald, L., (1989). The use of the analytical profile index in the identification of activated sludge bacteria: problems and solutions. *Water SA*, **15** (4), 265-267.

Viessman, W. and Hammer, M.J., (1985). *Water supply and pollution control.* 4th ed. Harper and Row, New York.

Voss, R. and Griffith, B. (1999). *Phosphorus and surface water project for Illinois agriculture.*

WBG, (1998). Vegetable oil processing, Pollution Prevention and Abatement, *Handbook, World Bank Group.*

WRC. (1996a). WRC 25 years (1971-1996), *SA Waterbulletin*, Water Research Commission, Pretoria.

WRC. (1996b). *WRC Technical Annual Report*, Water Research Commission, Pretoria.

Wagner, M., Amann, R., Lemmer, H. and Schleifer, K.-H., (1993). Probing activated sludge with oligonucleotides specific for proteobacteria: inadequancy of culturedependent methods for describing microbial community structure. *Appl. Environ. Microbiol.*, **59**: 1520-1525.

Wagner, M., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wedi, D. and Schleifer, K-H., (1994). Development of an rRNA-targeted oligonucleotide probe specific for the genus *Cinetobacter* and its application for *in situ* monitoring in activated sludge. *Appl. Environ. Microbiol.*, **60**: 792-800.

Walden, (1997) *SBR Technology and Walden innovation: A winning combination.* Internet URL http://www.mint.net/walden/sbr.html.

Walmsley, R. D. and Butty, M., (1980). The limnology of some selected South African impoundments. *A collaborated report by the Water research Commission and the National Institute for Water Research of the Council for Scientific and Industrial Research,* Pretoria: 229 pp.

Walmsley, R.D. and Thornton, J.A. (1984). Evaluation of OECD-type phosphorus eutrophication models for predicting the trophic status of Southern African man-made lakes. *SAJS*, **80**: 257-259.

Walmsley, R. D. (2000). Perspective on eutrophication of surface waters. *Water Research Commission, Report No 129.*

Wanner, J., Ottava, V. and Grau, P., (1987). Effect of an anaerobic zone on settleability of activated sludge. In *Advances in Water Pollution Control (IAWPRC)*, Biological phosphorus removal from wastewaters, Ed. R. Ramadori, Pergamon, Oxford, England, 155-164.

Wanner, J. (1997). Microbial population dynamics in biological wastewater treatment plants. In: Cloete, T.E. and Muyima, N.Y.O. (eds) Microbial Community Analysis: The key to the design of biological wastewater treatment systems. *IAWQ Scientific and Technical Report No. 5, IAWQ*, London, pp. 35-95.

Water Services Act, (1997). *Water Services Act, No. 108 of 1997.* Department of Water Affairs and Forestry, Cape Town, December 1997.

Watts, J. (1996). *Industrial biological wastewater treatment plants.* Internet URL http://www.demon.co.uk/industry.html. 06/14/2000.

Weddepohl, J.P. and Meyer, D.H. (1992). Utilisation of Models to simulate phosphorus loads in Southern Africa catchments. *Water Research Commission report 197/1/92.*

Wentzel, M.C., Dold, P.L., Ekama, G.A. and Marais, G.v.R., (1985). Kinetics of biological phosphorus release. *Wat.Sci.Tech.*, **17** (11-12), 57.

Wentzel, M.C., Lötter, L.H., Loewenthal, R.E. and Marais, G.vR. (1986). Metabolic behaviour of Acinetobacter spp. in enhanced biological phosphorus removal – a biochemical model. *Water SA*, **12** (4), 209-224.

Wentzel, M.C., Loewenthal, R.E., Ekama, G.A. and Marais, G.v.R. (1988). Enhanced polyphosphate organism cultures in activated sludge systems – Part 1: Enhanced culture development. *Water SA*, **14** (2): 81-92.

Wentzel, M.C., Ekama, G.A., Loewenthal, R.E., Dold, P.L. and Marais, G.v.R., (1989). Enhanced Polyphosphate Organism Cultures in Activated Sludge Systems. Part II: Experimental Behaviour. *Water SA*, **15** (2),71.

Wentzel, M.C., Ekama, G.A., Dold, P.L. and Marais, G.v.R., (1990). Biological excess phosphorus removal – Steady state process design. *Water SA*, **16** (1), 29-48.

Wentzel, M.C., Lötter, L.H., Ekama, G.A., Loewenthal, R.E. and Marais, G.v.R., (1991). Evaluation of biochemical models for biological excess phosphorus removal. *Wat. Sci. Tech.*, **23** (4-6), 567-576.

Wentzel, M.C. (1992). Phosphorus removal from sewage in activated sludge systems. *First IAWQ Technical Tour Nutrient Removal and Anaerobic Digestion in South Africa.* Volume One : Nutrient Removal, 11-23 October, 1992.

Wentzel, M.C., Ekama, G.A. and Marais, G.v.R. (1992). Processes and modeling of nitrification denitrification biological excess phosphorus removal systems – A review. *Wat. Sci. Tech.*, **25** (6), 59-82.

Wentzel, M.C., Mbewe, A. and Ekama, G.A. (1995). Batch test for measurement of readily biodegradable COD and active organism concentrations in municipal wastewaters. *Water SA,* **21** (2), 117-124.

Wentzel, M.C., Ekama, G.A. and Marais, GvR. (1997). Consolidation of activated sludge research II. *Water Research Commission, report 356/1/97.*

Wentzel, M.C., Mbewe, A., Lakay, M.T. and Ekama, G.A., (1999). Batch test for characterization of the carbonaceous materials in municipal wastewaters. *Water SA,* **25** (3),327-335.

Wepener, V., van Vuuren, J.H.J. and du Preez, H.H. (2000). Application of the equilibrium partitioning method to derive copper and zinc quality criteria for water and sediment: A South African perspective. *Water SA*, **26** (1).

West, E. (1998). *Innovative partnership develops Europe's largest Sequencing Batch Reactors*. Internet URL http://www.cegelecproj.co.uk/ce2240a.htm.

Wiechers, H.N.S. and Heynike, J.J.C., (1986). Sources of phosphorus which give rise to eutrophication in South African waters. *Water SA,* **12** (2), 99-.102.

Wiechers, H.N.S. (1987). Guidelines for chemical phosphate removal from municipal wastewaters. *Water Research Commission, report.*

Wilderer, P.A., Irvine, R.L. and Doellerer, J. (1997). Sequencing batch reactor technology – Batch application of periodic processes. *Wat. Sci. Tech.*, **35** (1).

Wilkes University, (2001). *Total phosphorus and phosphate impact on surface waters.* Centre for Environmental Quality, Geo-Environmental Science and Engineering Department. Internet URL http://Wilkes.edu/~eqc/phosphate.htm 06/02/2001.

Wilen, B-M. and Balmer, P. (1998). Short term effects of dissolved oxygen concentration on the turbidity of the supernatant of activated sludge. *Wat. Sci. Tech.*, **38** (3), 25 – 33.

Ydstebø, L., Bilstad, T. and Barnard, J. (2000). Experience with biological nutrient removal at low temperatures. *Water Environment Research*, **72** (4), 444-454.

Zhao Q.L. (2000). Wastewater Treatment by Biofilm Processes. *China Architecture & Building Press*, Beijing (in Chinese).

APPENDICES

APPENDIX 1

DETERMINATION OF CHEMICAL OXYGEN DEMAND (COD)

Overview

The chemical oxygen demand (COD) of wastewater is a measure of the oxygen equivalent of the organic matter content, which can be oxidized by a strong chemical oxidant, i.e. potassium dichromate. Samples are heated up in strongly acidic solutions with known excess of potassium dichromate. After digestion with potassium dichromate and silver sulphate catalyst in strong sulphuric acid, the remaining unreduced potassium dichromate is measured using a photometer.

Method:

SQ 118 Merck, photometer was used for the determination of COD concentrations. Spectroquant analysis methods 14541 for 100 - 1500 mg/l COD and 14555 for 500 -10000 mg/l COD were used.

APPENDIX 2

TOTAL PHOSPHATE DETERMINATION

Overview

Total phosphorus (TP) determination of a wastewater sample includes all orthophosphate and polyphosphate, both dissolved and particulate, organic and inorganic. To release organically bound phosphorus, a digestion and oxidation procedure is necessary. Samples are digested using a persulphate digestion technique and an autoclave. All forms of phosphorus are then converted to orthophosphate, the concentration of which is determined colorimetrically.

Reagents

A. Vanadomolybdate solution

Dissolve 20 g ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and 1 g ammonium meta-vanadate (NH_4VO_3) in ~ 500 mL distilled water. Add 140 mL conc. nitric acid (HNO_3; 55 %) and mix well. Allow to cool and make up to 1 L with distilled water. Store reagent in the dark and prepare weekly. Discard if precipitate or growth occurs.

B. Digestion reagent -7.1% (w/v) potassium persulphate

Dissolve 7.1 g potassium persulphate ($K_2O_8S_2$) in -80 mL distilled water and make up to the 100

mL mark. Dissolve with gentle stirring and heating. Prepare a fresh solution for every digestion.

C. Digestion reagent -0.6 M sulphuric acid

Add 33 mL conc. sulphuric acid (97%) to -800 mL distilled water. Allow to cool and make up to 1 L.

Preparation of standards

Stock solution l00 mg/L

Dissolve precisely 0.8794 g potassium dihydrogen orthophosphate (KH_2PO_4) in ~ 1 500 mL distilled water. Fill up to 2 L with distilled water and mix well. Store the solution in a fridge at 4C and prepare every 3 months.

Working standards

0	mgP/L:	distilled water
5	mgP/L:	dilute 50 mL stock solution to 1000 mL with distilled water
10	mgp/L:	dilute 100 mL stock solution to 1000 mL with distilled water
15	mgP/L:	dilute 150 mL stock solution to 1000 mL with distilled water
25	mgP/L:	dilute 250 mL stock solution to 1000 mL with distilled water
50	mgP/L:	dilute 500 mL stock solution to 1000 mL with distilled water

Add I mL conc. HNO_3 to each standard (preservative).

Analytical procedure

Transfer 20 mL sample, standard and AQC to 50 mL borosilicate tubes. Add 5 mL 0.6 M H_2 SO_4 and 5 mL 7.1 % (w/v) $K_2O_8S_2$) to each tube and mix well. Cover tubes with aluminium foil and digest for 1 hour in a pressure cooker at 100 kPa. Allow tubes to cool to room temperature and mix contents by inverting. Turbid samples are filtered into clean tubes (standards must not be filtered). Transfer 5 mL aliquots of each sample, standard and AQC into 20 mL test tubes. Add 5 mL vanadomolybdate solution to each tube and allow to stand for 30 mins for full colour development before reading absorbance at 470 nm. Use deionised water as a reference.

Calculation of results

A spreadsheet using Excel computer software was used for interpolation of data and a standard curve plotted. Concentration (mgP/L) was calculated from absorbance using the equation for a linear curve:

$y=mx+c$

where, y = concentration (mgp/L)

m = gradient of curve

x = absorbance (nm)

c = y-intercept

Values for m, x and c were determined using a spreadsheet analysis.

APPENDIX 3

SOLUBLE REACTIVE PHOSPHATE DETERMINA TION

Overview

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed 'reactive phosphorus'. Filtration through 0.45 μm membrane filters is a replicable method used to separate suspended and soluble forms of reactive phosphorus. Soluble reactive phosphorus is routinely monitored in wastewater treatment plants due to its availability to the resident micro flora of receiving water bodies.

Reagents

A. Vanadomolybdate solution

As for Appendix 2

Analytical procedure

Samples were immediately filtered through 0.45 μrn membrane filters. Filtered sample (5 mL) was pipetted into a test tube. All samples were done in duplicate to allow for blanks. Deionised water (5 mL) and vanadomolybdate solution (5 mL) were added to each blank and sample, respectively. Contents of the tubes were mixed well and allowed to stand for 30 mins for full colour development. The absorbance of samples and blanks was measured at 470 nm using

deionised water as a reference.

Calculation of results

SRP concentration of wastewater samples (mgP/L) was calculated as follows:

SRP= (Abs of sample) -(Abs of blank) x 110.46

It must be noted that this was a rapid formula for SRP determination proposed by de Haas (1998). Although the formula was initially adapted for this project, standard Curves were also plotted due to discrepancies in the results.

APPENDIX 4

DETERMINATION OF FREE AND SALINE AMMONIA (NH_4^+)

Overview

The free and saline ammonium nitrogen is readily available in wastewater as a plant nutrient that is responsible for eutrophicaction phenomenon Free and saline ammonia may result in wastewater from biodegradation of organic nitrogen Hence it is important to determine both the 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 H_4-N determination was used with SQ 118 Merck photometer.

APPENDIX 5

DETERMINATION OF NITRATE (NO_3^-)

Overview

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 the Method:

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

Method:

Spectroquant analysis method 14542 for 2.0 -80.0 mg/L NO_3-N was used for the determination of nitrate with SQ 118 Merck photometer.

APPENDIX 6

DETERMINATION OF SULPHATES (SO_4^{2-})

Overview

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

Principle of the 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:

SQ 118 Merck, photometer was used for the spectroquant analysis for the determination of sulphates. Method 14564 was employed for the determination of sulphates.

APPENDIX 7

DETERMINATION OF TOTAL SUSPENDED SOLIDS (TSS)

Overview

Solids refer to matter suspended or dissolved in water or wastewater. Solids may affect water or effluent quality adversely in a number of ways. Highly mineralised waters are unsuitable for many industrial applications. Waters high in suspended solids may be esthetically unsatisfactory for such purposes as bathing. Hence solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory limits.

Principle of the Method:

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

Method:

The samples were analysed in duplicate. A 20 mL sample was analysed according to Standard Method 2540 D (Standard Methods, 1989) for total suspended solids dried at 103 -105 C.

Calculation

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

where, A = weight of dried residue + crucible, mg

B = weight of crucible, mg

APPENDIX 8

DETERMINATION OF FATS, OILS AND GREASE (FOG)

Overview

In determination of fats, oils and grease an absolute quantity of a specific substance is not measured. Rather groups of substances with similar physical characteristics are determined qualitatively on the basis of their common solubility in trichlorotrifluoroethane or in diethyl ether. FOG is any material recovered as a substance soluble in trichlorotrifluoroethane or in diethyl ether. It includes other solvent extractable material from acidified samples such as sulfur compounds, certain organic dyes and chlorophyll and not volatilised during the test. This method of FOG analysis is suitable for biological lipids and mineral hydrocarbons and is also suitable for most industrial wastewaters or treated effluents containing these material.

Principle of the Method:

Soluble metallic soaps are hydrolysed by acidification. Any oils and solids or viscous grease present are separated from the liquid samples by filtration. After extraction in the Soxhlet apparatus with trichlorotrifluoroethane or diethyl ether the residue remaining after solvent evaporation is weighed to determine the fats, oils and grease content. Compounds volatilised at or below 103 C will be lost when the filter is dried.

Method:

Diethyl ether was used as a solvent for the determination of FOG content. The analyses were done in duplicate. A 100 mL effluent sample was analysed using Standard Method 5520 D (Standard Methods, 1989).

Calculation

The FOG concentration is calculated as in APPENDIX L 7.

APPENDIX 9

DETERMINATION OF SOLIDS (MIXED LIQUOR SUSPENDED SOLIDS AND VOLATILE SUSPENDED SOLIDS)

Overview

Mixed liquor suspended solids (MLSS) is defined as the total amount of organic and mineral suspended solids contained in the mixed liquor of the activated sludge reactor. The procedure of MLSS determination is relatively simple to perform and offers system operators a rapid indication of sludge biomass concentration. The organic portion of MLSS is represented by mixed liquor volatile suspended solids (also referred to as mixed liquor organic suspended solids), which is comprised of non-microbial organic matter as well as dead and live microorganisms and cellular debris. MLVSS values are usually reported as a percentage of the MLSS

Determination of MLSS

Pipette 100 mL of sample into a centrifuge tube. Centrifuge at 3 000 rpm for 6 min. Discard supernatant and quantitatively scoop sludge pellet into a pre-weighed crucible. Place crucible into a drying oven at 105C and leave overnight. Remove from oven and allow to cool in desiccator. Reweigh crucible. MLSS is determined according to the following calculation:

$$\text{MLSS (g/L)} = \frac{\text{mass of (crucible + sludge) -mass of (crucible)}}{100\ \text{mL}} \times 10$$

Determination of VSS

Place pre-weighed crucible containing sludge (from MLSS determination) into a muffle furnace. Ash at 550C for 1 h. Remove crucible, allow to cool in desiccator and re-weigh. It is common practice to express VSS as a percentage of the MLSS according to the following equation:

$$\text{VSS (\%)} = \frac{\text{mass of (crucible + sludge) - mass of (crucible + ash)}}{\text{mass of (crucible + sludge) -mass of (crucible)}} \times 100$$

APPENDIX 10

DETERMINATION OF SLUDGE VOLUME INDEX (SVI)

Overview

The significance of calculating SVI is to monitor the settling characteristics of the mixed liquor and to alert the system operator if the sludge is displaying bulking characteristics. The test involves settling a sludge sample (SVI) or diluted sludge sample (DSVI) in a measuring cylinder for a specific time period and recording the volume it occupies, in conjunction with a MLSS test.

Procedure

A 1000 mL measuring cylinder is filled with mixed liquor from the outlet of the aeration basin (SBR) and agitated. The cylinder is then inverted three times (using a hand over the top) to homogenise the sludge mixture. The cylinder is then placed on a flat surface and allowed to settle quiescently for 30 mins. After 30 mins of settling, the volume occupied by the sludge in the cylinder is recorded.

Determination of SVI

SVI is defmed by the following equation:

$$SVI\ (mL/g) = V_{30min}/X$$

Where, V_{30min} volume of settled sludge after 30 mins sedimentation

X concentration of activated sludge (g/L)

Q

APPENDIX 11

OPTIMISATION OF FOG BY C-40 FLOCCULENT

Overview

C-40 is a multi-component system used as a oil absorption compound or flocculent in removing water insoluble fats, oils, dyes and organic solvents. It's major constituent is a hydrophobic silica of microfine particle size. Silica, which is inorganic and absorbent reacts neutral in all liquids and is insoluble in strong acids (except in hydrofluoric acid) and weak alkalis.

Bench Test

A series of bench test were run to determine the optimum dosage for C-40 addition for FOG removal. After these series of tests the optimum dosage was achieved at 13.5g/L.

Procedure

The refinery oil effluent samples from Sealake Industries collected in 25 L containers were drained into 100 L tank at CWWR test laboratory. The contents were allowed to acclimatise to room temperature and mixed using a mechanical stirrer. The sample pH was measured and adjusted to pH of ~ 7.0 if necessary. 1350 g of C-40 was added into the tank and mixed vigorously. The tank contents were allowed to stand for 30 minutes to achieve adequate floc settling. The supernatant liquid was then decanted and characterised (see APPENDIX 1 for the results obtained).

APPENDIX 12A

EFFLUENT CHARACTERISATION

Week	CODt	CODs	TP	NH4-N	NO3-N	SO42-	FOG	pH	TSS
1	8523	7122	530	0.66	1.61	285	80110	11.97	5240
2	6991	5023	406	0.57	1.36	210	58620	12.05	4139
3	4007	2997	260	0.42	1.18	82.8	13580	10.88	1336
4	5118	3890	315	0.49	1.27	106	23470	12.16	1866
5	6892	6015	448	0.53	1.12	214	54530	9.95	3827
6	3655	2011	189	0.46	1.34	73.9	11340	11.41	1125
7	5980	4163	432	0.51	1.01	64	49370	10.37	3542
8	7664	6204	509	0.6	1.55	186	69040	9.81	4622
12	3631	1651	112	0.5	1	83.5	11130	12.02	1060
16	7867	6133	-	0.53	1.05	50.5	72430	10.94	4841
20	5211	3297	-	0.47	1.01	47.1	35910	10.23	2590
24	5377	4325	-	0.68	1.51	62.9	38990	9.66	2871
28	4810	3090	-	0.39	0.98	75.7	18720	12.01	1764
32	5165	4360	-	0.72	1.5	55.8	32480	11.55	2148
36	5890	4710	-	0.55	1.32	94.2	46830	11.24	3116
40	8323	7427	-	0.71	1.44	83	76760	11.78	5019
44	7238	6043	-	0.64	1.36	117	63550	12	4376
48	5479	4350	-	0.88	1.63	128	42320	10.99	2992

APPENDIX 12B

Week	FOGunt	FOGeff	FOGrem	%FOGrem
1	80110	7211	72899	91.00
2	58620	4998	53622	91.47
3	13580	1420	12160	89.54
4	23470	1876	21594	92.01
5	54530	5002	49528	90.83
6	11340	1358	9982	88.02
7	49370	4912	44458	90.05
8	69040	6605	62435	90.43
12	11130	1301	9829	88.31
16	72430	5994	66436	91.72
20	35910	3588	32322	90.01
24	38990	3577	35413	90.83
28	18720	1854	16866	90.10
32	32480	3367	29113	89.63
36	46830	4355	42475	90.70
40	76760	7322	69438	90.46
44	63550	6674	56876	89.50
48	42320	4562	37758	89.22

APPENDIX 13A

12 HOURS HRT TREATABILITY RESULTS

Day	Cycle	COD_{inf}	COD_{eff}	$\%COD_{rem}$	TP_{inf}	TP_{eff}	%Tprem
1	1	1498	305	79.64	48.98	21.96	55.17
	2	1495	300	79.93	49.79	21.99	55.83
2	1	1488	298	79.97	45.95	20.75	54.84
	2	1480	293	80.20	49.32	21.68	56.04
3	1	1490	297	80.07	50.01	22.22	55.57
	2	1490	300	79.87	50.06	22.18	55.69
4	1	1484	291	80.39	49.94	22.24	55.47
	2	1493	299	79.97	48.85	21.9	55.17
5	1	1488	316	78.76	49.98	22.19	55.60
	2	1495	319	78.66	49.97	22.14	55.69
6	1	1505	311	79.34	49.04	21.95	55.24
	2	1491	305	79.54	49.06	21.91	55.34
7	1	1478	297	79.91	48.96	21.77	55.54
	2	1469	289	80.33	49.08	21.7	55.79
8	1	1487	288	80.63	48.95	21.69	55.69
	2	1476	294	80.08	48.93	21.75	55.55
9	1	1480	291	80.34	48.84	21.72	55.53
	2	1466	295	79.88	48.79	21.78	55.36
10	1	1474	302	79.51	48.71	21.84	55.16
	2	1481	300	79.74	48.76	21.66	55.58
11	1	1465	290	80.20	46.08	20.45	55.62
	2	1472	303	79.42	46	20.39	55.67
12	1	1483	301	79.70	47.35	20.99	55.67
	2	1498	297	80.17	47.56	21.23	55.36
13	1	1485	292	80.34	48.44	21.84	54.91
	2	1477	295	80.03	48.49	22.09	54.44
14	1	1481	291	80.35	48.58	21.86	55.00
	2	1472	298	79.76	48.55	21.89	54.91
15	1	1464	294	79.92	48.65	21.8	55.19
	2	1467	290	80.23	48.7	21.79	55.26
16	1	1479	299	79.78	48.88	22.08	54.83
	2	1473	308	79.09	48.8	22.35	54.20
17	1	1472	316	78.53	50.03	22.98	54.07
	2	1474	329	77.68	49.76	22.95	53.88
18	1	1483	335	77.41	48.95	23.34	52.32
	2	1489	351	76.43	48.9	23.66	51.62
19	1	1488	373	74.93	48.85	23.95	50.97
	2	1494	395	73.56	48.81	24.44	49.93
20	1	1488	401	73.05	48.7	24.65	49.38
	2	1494	422	71.75	48.78	25.03	48.69
21	1	1479	454	69.30	48.62	25.33	47.90
	2	1482	301	79.69	48.58	21.55	55.64

22	1	1480	296	80.00	48.69	21.59	55.66
	2	1476	292	80.22	48.74	21.63	55.62
23	1	1493	295	80.24	48.8	21.57	55.80
	2	1490	291	80.47	48.74	21.66	55.56
24	1	1494	298	80.05	48.84	21.85	55.26
	2	1500	300	80.00	48.93	22.04	54.96
25	1	1499	305	79.65	49.04	21.84	55.46
	2	1499	301	79.92	49.08	22.09	54.99
26	1	1488	296	80.11	49	22.01	55.08
	2	1499	295	80.32	48.98	21.79	55.51
27	1	1482	295	80.09	48.95	21.83	55.40
	2	1481	289	80.49	48.92	21.91	55.21
28	1	1470	289	80.34	48.9	22.16	54.68
	2	1475	296	79.93	48.88	22.12	54.75
29	1	1478	300	79.70	48.81	22.23	54.46
	2	1480	302	79.59	48.85	22.15	54.66
30	1	1476	297	79.88	48.8	22.03	54.86
	2	1486	292	80.35	48.75	22	54.87
31	1	1482	295	80.09	48.76	21.92	55.05
	2	1479	294	80.12	48.79	21.94	55.03
32	1	1469	290	80.26	48.68	22.04	54.72
	2	1474	296	79.92	48.69	22.11	54.59
33	1	1485	302	79.66	49.93	23.06	53.82
	2	1480	318	78.51	49.9	22.99	53.93
34	1	1466	339	76.88	49.73	23.15	53.45
	2	1470	361	75.44	49.66	23.67	52.34
35	1	1478	377	74.49	48.92	23.85	51.25
	2	1484	394	73.45	48.9	24.08	50.76
36	1	1480	448	69.73	48.76	24.56	49.63
	2	1484	303	79.58	48.75	22.36	54.13
37	1	1490	297	80.07	48.94	22.3	54.43
	2	1488	299	79.91	48.97	22.28	54.50
38	1	1482	295	80.09	48.91	22.22	54.57
	2	1480	291	80.34	48.9	22.18	54.64
39	1	1483	296	80.04	48.84	22.01	54.93
	2	1476	290	80.35	48.81	21.95	55.03
40	1	1481	289	80.49	48.68	21.79	55.24
	2	1484	296	80.05	48.66	21.72	55.36

APPENDIX 13B

8 HOURS HRT TREATABILITY RESULTS

Day	Cycle	COD_{inf}	COD_{eff}	$\%COD_{rem}$	TP_{inf}	TP_{eff}	%Tprem
1	1	1498	225	84.98	48.98	20.1	58.96
	2	1495	219	85.35	49.79	20.45	58.93
	3	1496	218	85.43	44.88	19.03	57.60
2	1	1488	220	85.22	45.95	19.33	57.93
	2	1480	224	84.86	49.32	20.46	58.52
	3	1484	226	84.77	46.43	19.58	57.83
3	1	1490	221	85.17	50.01	20.67	58.67
	2	1490	217	85.44	50.06	20.66	58.73
	3	1493	223	85.06	49.66	20.6	58.52
4	1	1484	228	84.64	49.94	20.69	58.57
	2	1493	231	84.53	48.85	20.63	57.77
	3	1484	236	84.10	49.11	20.72	57.81
5	1	1488	239	83.94	49.98	20.78	58.42
	2	1495	235	84.28	49.97	20.84	58.29
	3	1500	230	84.67	48.78	20.75	57.46
6	1	1505	227	84.92	49.04	20.81	57.57
	2	1491	222	85.11	49.06	20.86	57.48
	3	1500	218	85.47	48.99	20.8	57.54
7	1	1478	215	85.45	48.96	20.75	57.62
	2	1469	212	85.57	49.08	20.88	57.46
	3	1471	219	85.11	49.16	20.96	57.36
8	1	1487	221	85.14	48.95	20.9	57.30
	2	1476	223	84.89	48.93	20.84	57.41
	3	1476	228	84.55	48.9	20.77	57.53
9	1	1480	225	84.80	48.84	20.69	57.64
	2	1466	220	84.99	48.79	20.64	57.70
	3	1470	215	85.37	48.77	20.55	57.86
10	1	1474	214	85.48	48.71	20.41	58.10
	2	1481	218	85.28	48.76	20.38	58.20
	3	1473	221	85.00	48.8	20.44	58.11
11	1	1465	223	84.78	46.08	19.18	58.38
	2	1472	225	84.71	46	19.13	58.41
	3	1476	220	85.09	46.88	19.38	58.66
12	1	1483	219	85.23	47.35	19.88	58.01
	2	1498	218	85.45	47.56	19.9	58.16
	3	1490	223	85.03	47.99	20.15	58.01
13	1	1485	228	84.65	48.44	20.46	57.76
	2	1477	227	84.63	48.49	20.57	57.58
	3	1480	224	84.86	48.53	20.88	56.98
14	1	1481	220	85.15	48.58	21.05	56.67
	2	1472	217	85.26	48.55	21.29	56.15
	3	1468	214	85.42	48.59	21.46	55.83

15	1	1464	219	85.04	48.65	21.87	55.05
	2	1467	225	84.66	48.7	21.99	54.85
	3	1462	228	84.40	48.71	22.08	54.67
16	1	1479	239	83.84	48.88	22.26	54.46
	2	1473	245	83.37	48.8	22.56	53.77
	3	1468	256	82.56	48.96	22.95	53.13
17	1	1472	264	82.07	50.03	23.66	52.71
	2	1474	277	81.21	49.76	23.64	52.49
	3	1466	289	80.29	48.9	23.46	52.02
18	1	1483	298	79.91	48.95	23.48	52.03
	2	1489	309	79.25	48.9	23.51	51.92
	3	1485	316	78.72	48.89	23.57	51.79
19	1	1488	331	77.76	48.85	23.69	51.50
	2	1494	340	77.24	48.81	23.78	51.28
	3	1485	349	76.50	48.77	23.74	51.32
20	1	1488	357	76.01	48.7	23.78	51.17
	2	1494	362	75.77	48.78	23.85	51.11
	3	1485	371	75.02	48.72	23.89	50.96
21	1	1479	380	74.31	48.62	20.11	58.64
	2	1482	220	85.16	48.58	20.08	58.67
	3	1486	217	85.40	48.63	20.14	58.59
22	1	1480	214	85.54	48.69	20.1	58.72
	2	1476	219	85.16	48.74	20.18	58.60
	3	1477	220	85.10	48.75	20.2	58.56
23	1	1493	221	85.20	48.8	20.22	58.57
	2	1490	220	85.23	48.74	20.29	58.37
	3	1485	227	84.71	48.71	20.37	58.18
24	1	1494	229	84.67	48.84	20.45	58.13
	2	1500	228	84.80	48.93	20.58	57.94
	3	1498	224	85.05	48.95	20.63	57.85
25	1	1499	220	85.32	49.04	20.72	57.75
	2	1499	222	85.19	49.08	20.75	57.72
	3	1491	223	85.04	49.08	20.84	57.54
26	1	1488	220	85.22	49	20.65	57.86
	2	1499	216	85.59	48.98	20.65	57.84
	3	1491	213	85.71	48.96	20.6	57.92
27	1	1482	217	85.36	48.95	20.55	58.02
	2	1481	219	85.21	48.92	20.51	58.07
	3	1474	222	84.94	48.9	20.43	58.22
28	1	1470	226	84.63	48.9	20.3	58.49
	2	1475	221	85.02	48.88	20.36	58.35
	3	1478	218	85.25	48.88	20.4	58.27
29	1	1478	215	85.45	48.81	20.48	58.04
	2	1480	220	85.14	48.85	20.39	58.26
	3	1483	226	84.76	48.87	20.33	58.40
30	1	1476	232	84.28	48.8	20.45	58.09
	2	1486	239	83.92	48.75	20.5	57.95

	3	1490	248	83.36	48.72	20.59	57.74
31	1	1482	257	82.66	48.76	20.66	57.63
	2	1479	266	82.01	48.79	20.7	57.57
	3	1488	277	81.38	48.77	20.79	57.37
32	1	1469	286	80.53	48.68	20.99	56.88
	2	1474	299	79.72	48.69	21.06	56.75
	3	1478	311	78.96	50.05	21.56	56.92
33	1	1485	320	78.45	49.93	21.58	56.78
	2	1480	332	77.57	49.9	21.78	56.35
	3	1472	340	76.90	49.84	21.66	56.54
34	1	1466	348	76.26	49.73	21.68	56.40
	2	1470	352	76.05	49.66	21.73	56.24
	3	1460	361	75.27	48.99	21.59	55.93
35	1	1478	368	75.10	48.92	21.62	55.81
	2	1484	373	74.87	48.9	21.74	55.54
	3	1479	379	74.37	48.84	21.89	55.18
36	1	1480	384	74.05	48.76	22.08	54.72
	2	1484	219	85.24	48.75	22.36	54.13
	3	1487	215	85.54	48.79	22.45	53.99
37	1	1490	217	85.44	48.94	20.34	58.44
	2	1488	219	85.28	48.97	20.37	58.40
	3	1486	221	85.13	48.99	20.32	58.52
38	1	1482	225	84.82	48.91	20.26	58.58
	2	1480	228	84.59	48.9	20.3	58.49
	3	1488	224	84.95	48.88	20.25	58.57
39	1	1483	220	85.17	48.84	20.33	58.37
	2	1476	222	84.96	48.81	20.38	58.25
	3	1470	220	85.03	48.75	20.41	58.13
40	1	1481	217	85.35	48.68	20.49	57.91
	2	1484	214	85.58	48.66	20.43	58.01
	3	1489	218	85.36	48.55	20.38	58.02

APPENDIX 13C

6 HOURS HRT TREATABILITY RESULTS

Day	Cycle	COD_{inf}	COD_{eff}	$\%COD_{rem}$	TP_{inf}	TP_{eff}	$\%TP_{rem}$
1	1	1498	386	74.23	48.98	32.15	34.36
	2	1495	380	74.58	49.79	32.11	35.51
	3	1496	366	75.53	44.88	29.05	35.27
	4	1490	339	77.25	47.54	30.66	35.51
2	1	1488	370	75.13	45.95	30.01	34.69
	2	1480	358	75.81	49.32	32.56	33.98
	3	1484	355	76.08	46.43	30.18	35.00
	4	1487	326	78.08	49.92	31.88	36.14
3	1	1490	310	79.19	50.01	32.45	35.11
	2	1490	300	79.87	50.06	32.41	35.26
	3	1493	315	78.90	49.66	32.33	34.90
	4	1488	329	77.89	49.73	32.43	34.79
4	1	1484	338	77.22	49.94	32.42	35.08
	2	1493	342	77.09	48.85	31.98	34.53
	3	1484	332	77.63	49.11	32.02	34.80
	4	1490	328	77.99	49.69	31.88	35.84
5	1	1488	320	78.49	49.98	31.8	36.37
	2	1495	319	78.66	49.97	31.8	36.36
	3	1500	334	77.73	48.78	31.69	35.03
	4	1505	372	75.28	48.92	32	34.59
6	1	1505	366	75.68	49.04	31.88	34.99
	2	1491	372	75.05	49.06	31.86	35.06
	3	1500	365	75.67	48.99	31.82	35.05
	4	1471	354	75.93	48.95	31.77	35.10
7	1	1478	366	75.24	48.96	31.73	35.19
	2	1469	368	74.95	49.08	31.79	35.23
	3	1471	369	74.92	49.16	31.84	35.23
	4	1475	360	75.59	48.99	31.9	34.88
8	1	1487	354	76.19	48.95	31.82	34.99
	2	1476	355	75.95	48.93	31.78	35.05
	3	1476	350	76.29	48.9	31.73	35.11
	4	1477	351	76.24	48.86	31.69	35.14
9	1	1480	367	75.20	48.84	31.74	35.01
	2	1466	369	74.83	48.79	31.66	35.11
	3	1470	360	75.51	48.77	31.61	35.19
	4	1470	350	76.19	48.74	31.55	35.27
10	1	1474	347	76.46	48.71	31.52	35.29
	2	1481	348	76.50	48.76	31.48	35.44
	3	1473	341	76.85	48.8	31.46	35.53
	4	1470	344	76.60	48.75	31.45	35.49
11	1	1465	340	76.79	46.08	29.92	35.07
	2	1472	339	76.97	46	29.84	35.13

	3	1476	339	77.03	46.88	29.89	36.24
	4	1480	336	77.30	46.98	29.95	36.25
12	1	1483	352	76.26	47.35	30.68	35.21
	2	1498	345	76.97	47.56	30.7	35.45
	3	1490	338	77.32	47.99	30.76	35.90
	4	1487	345	76.80	48.36	31.04	35.81
13	1	1485	358	75.89	48.44	31.19	35.61
	2	1477	360	75.63	48.49	31.28	35.49
	3	1480	366	75.27	48.53	31.39	35.32
	4	1477	388	73.73	48.58	31.68	34.79
14	1	1481	394	73.40	48.58	31.79	34.56
	2	1472	400	72.83	48.55	32	34.09
	3	1468	410	72.07	48.59	32.16	33.81
	4	1469	418	71.55	48.65	32.45	33.30
15	1	1464	433	70.42	48.65	32.66	32.87
	2	1467	447	69.53	48.7	32.89	32.46
	3	1462	458	68.67	48.71	33.07	32.11
	4	1470	462	68.57	48.74	33.36	31.56
16	1	1479	469	68.29	48.88	33.65	31.16
	2	1473	465	68.43	48.8	33.74	30.86
	3	1468	470	67.98	48.96	34.06	30.43
	4	1469	493	66.44	48.94	34.17	30.18
17	1	1472	499	66.10	50.03	35.05	29.94
	2	1474	505	65.74	49.76	35.22	29.22
	3	1466	498	66.03	48.9	35.46	27.48
	4	1475	499	66.17	48.99	35.68	27.17
18	1	1483	514	65.34	48.95	35.78	26.91
	2	1489	520	65.08	48.9	35.85	26.69
	3	1485	525	64.65	48.89	35.99	26.39
	4	1480	528	64.32	48.88	36.23	25.88
19	1	1488	536	63.98	48.85	36.44	25.40
	2	1494	539	63.92	48.81	36.66	24.89
	3	1485	547	63.16	48.77	36.98	24.17
	4	1483	588	60.35	48.74	38.25	21.52
20	1	1488	586	60.62	48.7	38.36	21.23
	2	1494	589	60.58	48.78	38.49	21.09
	3	1485	593	60.07	48.72	38.68	20.61
	4	1488	598	59.81	48.67	38.91	20.05
21	1	1479	355	76.00	48.62	31.03	36.18
	2	1482	351	76.32	48.58	31.09	36.00
	3	1486	358	75.91	48.63	31.12	36.01
	4	1484	354	76.15	48.66	31.14	36.00
22	1	1480	350	76.35	48.69	31.18	35.96
	2	1476	348	76.42	48.74	31.25	35.88
	3	1477	344	76.71	48.75	31.23	35.94
	4	1480	346	76.62	48.79	31.26	35.93
23	1	1493	349	76.62	48.8	31.29	35.88

	2	1490	346	76.78	48.74	31.33	35.72
	3	1485	351	76.36	48.71	31.3	35.74
	4	1490	354	76.24	48.79	31.28	35.89
24	1	1494	350	76.57	48.84	31.35	35.81
	2	1500	356	76.27	48.93	31.39	35.85
	3	1498	359	76.03	48.95	31.44	35.77
	4	1501	359	76.08	48.99	31.58	35.54
25	1	1499	366	75.58	49.04	31.69	35.38
	2	1499	362	75.85	49.08	31.82	35.17
	3	1491	358	75.99	49.08	31.81	35.19
	4	1488	355	76.14	49.04	31.85	35.05
26	1	1488	351	76.41	49	31.88	34.94
	2	1499	356	76.25	48.98	31.8	35.08
	3	1491	350	76.53	48.96	31.75	35.15
	4	1489	357	76.02	48.99	31.71	35.27
27	1	1482	358	75.84	48.95	31.65	35.34
	2	1481	364	75.42	48.92	31.62	35.36
	3	1474	362	75.44	48.9	31.6	35.38
	4	1477	356	75.90	48.89	31.57	35.43
28	1	1470	357	75.71	48.9	31.54	35.50
	2	1475	352	76.14	48.88	31.51	35.54
	3	1478	350	76.32	48.88	31.46	35.64
	4	1472	345	76.56	48.84	31.42	35.67
29	1	1478	347	76.52	48.81	31.38	35.71
	2	1480	344	76.76	48.85	31.33	35.86
	3	1483	340	77.07	48.87	31.16	36.24
	4	1478	343	76.79	48.84	31.22	36.08
30	1	1476	348	76.42	48.8	31.27	35.92
	2	1486	353	76.24	48.75	31.32	35.75
	3	1490	356	76.11	48.72	31.38	35.59
	4	1485	367	75.29	48.76	31.43	35.54
31	1	1482	369	75.10	48.76	31.49	35.42
	2	1479	370	74.98	48.79	31.54	35.36
	3	1488	372	75.00	48.77	31.68	35.04
	4	1474	370	74.90	48.73	31.77	34.80
32	1	1469	375	74.47	48.68	31.85	34.57
	2	1474	371	74.83	48.69	31.99	34.30
	3	1478	378	74.42	50.05	33.06	33.95
	4	1485	381	74.34	49.98	33.09	33.79
33	1	1485	385	74.07	49.93	33.18	33.55
	2	1480	393	73.45	49.9	33.29	33.29
	3	1472	399	72.89	49.84	33.38	33.03
	4	1466	410	72.03	49.78	33.51	32.68
34	1	1466	416	71.62	49.73	33.63	32.37
	2	1470	418	71.56	49.66	33.88	31.78
	3	1460	423	71.03	48.99	33.94	30.72
	4	1467	426	70.96	48.95	34.07	30.40

35	1	1478	430	70.91	48.92	34.15	30.19
	2	1484	438	70.49	48.9	34.38	29.69
	3	1479	444	69.98	48.84	34.47	29.42
	4	1474	448	69.61	48.8	34.56	29.18
36	1	1480	456	69.19	48.76	34.69	28.86
	2	1484	456	69.27	48.75	34.75	28.72
	3	1487	461	69.00	48.79	34.88	28.51
	4	1492	466	68.77	48.86	35.16	28.04
37	1	1490	350	76.51	48.94	31.55	35.53
	2	1488	359	75.87	48.97	31.68	35.31
	3	1486	360	75.77	48.99	31.69	35.31
	4	1490	362	75.70	48.93	31.75	35.11
38	1	1482	358	75.84	48.91	31.79	35.00
	2	1480	354	76.08	48.9	31.44	35.71
	3	1488	350	76.48	48.88	31.23	36.11
	4	1486	353	76.24	48.88	31.35	35.86
39	1	1483	359	75.79	48.84	31.4	35.71
	2	1476	356	75.88	48.81	31.46	35.55
	3	1470	358	75.65	48.75	31.49	35.41
	4	1478	359	75.71	48.72	31.52	35.30
40	1	1481	352	76.23	48.68	31.59	35.11
	2	1484	354	76.15	48.66	31.55	35.16
	3	1489	357	76.02	48.55	31.43	35.26
	4	1482	359	75.78	48.51	31.48	35.11

APPENDIX 14A

Days	COD_{INF}	$COD_{sol(inf)}$	TP_{INF}	$P_{sol(inf)}$	FOG_{INF}	COD_{EFF}	$COD_{sol(eff)}$	TP_{EFF}	$P_{sol(eff)}$	FOG_{EFF}	% COD_{rem}	$\%P_{rem}$	$\%FOG_{rem}$	COD/P	COD_{util}	% COD_{util}	$\%COD_{sol}$
4	1495	1365	50.03	38.74	1242	316	366	11.08	8.44	340	78.86	77.85	72.62	35.23	999.00	66.82	91.30
8	1405	1265	49.79	39.98	1200	276	300	11.28	9.05	336	80.36	77.34	72.00	31.64	965.00	68.68	90.04
12	1425	1311	49.55	39.08	1218	292	347	10.32	5.88	332	79.51	79.17	72.74	33.55	964.00	67.65	92.00
16	1410	1280	49.31	38.61	1214	277	310	10.23	7.56	335	80.35	79.25	72.41	33.15	970.00	68.79	90.78
20	1385	1239	48.98	37.55	1188	280	289	10.45	6.91	324	79.78	78.66	72.73	33.00	950.00	68.59	89.46
24	1423	1285	49.32	39.08	1220	275	337	10.36	8.48	341	80.67	78.99	72.05	32.88	948.00	66.62	90.30
28	1340	1215	48.76	38.34	1147	281	266	10.7	7.34	320	79.03	78.06	72.10	31.69	949.00	70.82	90.67
32	1395	1258	49.06	40.3	1205	283	297	11.36	9.17	333	79.71	76.84	72.37	31.22	961.00	68.89	90.18
36	1338	1210	48.72	40.46	1151	264	257	11.21	10.74	316	80.27	76.99	72.55	29.91	953.00	71.23	90.43
40	1325	1207	48.55	40.58	1110	266	254	11.04	11.02	310	79.92	77.26	72.07	29.74	953.00	71.92	91.09
44	1358	1231	48.65	39.07	1163	260	244	10.8	8.62	322	80.85	77.80	72.31	31.51	987.00	72.68	90.65
48	1370	1238	48.82	39.85	1171	244	236	10.44	8.67	314	82.19	78.62	73.19	31.07	1002.00	73.14	90.36
52	1292	1153	48.06	40.11	1108	264	249	11.44	9.21	308	79.57	76.20	72.20	28.75	904.00	69.97	89.24
56	1348	1217	48.85	38.69	1154	267	280	10.6	8.08	325	80.19	78.30	71.84	31.46	937.00	69.51	90.28
60	1407	1277	49.11	38.93	1211	296	304	10.3	8.46	329	78.96	79.03	72.83	32.80	973.00	69.15	90.76
64	1358	1228	48.71	39.77	1163	270	281	9.98	8.63	306	80.12	79.51	73.69	30.88	947.00	69.73	90.43
68	1390	1248	49.01	40.38	1195	265	294	10.99	10.49	328	80.94	77.58	72.55	30.91	954.00	68.63	89.78
72	1376	1234	48.84	40.35	1168	274	288	11.79	9.32	320	80.09	75.86	72.60	30.58	946.00	68.75	89.68
76	1371	1230	48.78	38.65	1176	271	282	10.92	7.83	319	80.23	77.61	72.87	31.82	948.00	69.15	89.72
80	1480	1340	49.94	40.69	1247	300	358	11.05	8.39	341	79.73	77.87	72.65	32.93	982.00	66.35	90.54
84	1376	1240	49.04	39.89	1173	276	298	11.05	8.48	318	79.94	77.47	72.89	31.09	942.00	68.46	90.12
88	1371	1235	48.99	39.99	1164	268	285	10.78	8.88	312	80.45	78.00	73.20	30.88	950.00	69.29	90.08
102	1480	1336	49.89	40.78	1244	266	273	10.71	9.05	330	82.03	78.53	73.47	32.76	1063.00	71.82	90.27
106	1477	1329	49.85	40.55	1240	289	276	10.82	10.11	326	80.43	78.29	73.71	32.77	1053.00	71.29	89.98
110	1486	1344	49.97	41.36	1256	293	288	9.94	9.47	335	80.28	80.11	73.33	32.50	1056.00	71.06	90.44
114	1475	1328	49.8	42.55	1243	298	293	10.46	9.49	339	79.80	79.00	72.73	31.21	1035.00	70.17	90.03
118	1380	1234	48.25	39.35	1182	270	289	10.51	9.13	324	80.43	78.22	72.59	31.36	945.00	68.48	89.42

122	1376	1232	48.02	39.59	1178	275	270	10.45	9.01	315	80.01	78.24	73.26	31.12	962.00	69.91	89.53
126	1371	1225	48.78	39.88	1174	271	266	10.76	9.36	329	80.23	77.94	71.98	30.72	959.00	69.95	89.35
130	1372	1228	49.05	39.94	1179	269	281	10.82	9.24	323	80.39	77.94	72.60	30.75	947.00	69.02	89.50
134	1456	1309	49.74	41.25	1230	289	287	10.99	9.09	321	80.15	77.91	73.90	31.73	1022.00	70.19	89.90
138	1462	1318	49.76	40.86	1236	295	275	10.93	8.85	312	79.82	78.03	74.76	32.26	1043.00	71.34	90.15
142	1457	1312	48.34	41.33	1232	291	282	10.89	8.93	318	80.03	77.47	74.19	31.74	1030.00	70.69	90.05
146	1459	1329	49.79	41.25	1230	282	277	10.56	9.08	334	80.67	78.79	72.85	32.22	1052.00	72.10	91.09
150	1399	1277	49.08	41.12	1198	287	274	10.76	9.22	330	79.49	78.08	72.45	31.06	1003.00	71.69	91.28
154	1402	1289	49.14	40.72	1206	280	268	10.65	9.16	335	80.03	78.33	72.22	31.66	1021.00	72.82	91.94
158	1416	1236	49.27	39.94	1218	274	259	10.98	9.32	338	80.65	77.71	72.25	30.95	977.00	69.00	87.29
162	1377	1248	48.87	39.91	1180	270	263	10.48	9.33	331	80.39	78.56	71.95	31.27	985.00	71.53	90.63

APPENDIX 14B

Day	P_{inf}	Puptake	Prelease	Prel/Pup	P_{eff}	Prem	%Prem
4	38.74	49.76	43.11	1.1543	8.33	30.41	78.50
8	39.98	49.14	42.70	1.1508	8.43	31.55	78.91
12	39.08	50.43	43.72	1.1535	8.03	31.05	79.45
16	38.61	50.62	43.87	1.1539	7.89	30.72	79.56
20	37.55	50.88	44.04	1.1553	7.67	29.88	79.57
24	39.08	50.66	43.88	1.1545	8.07	31.01	79.35
28	38.34	50.67	43.97	1.1524	7.99	30.35	79.16
32	40.3	48.99	42.61	1.1497	8.47	31.83	78.98
36	40.46	49.06	42.55	1.1530	8.54	31.92	78.89
40	40.58	48.93	42.45	1.1527	8.49	32.09	79.08
44	39.07	50.01	43.41	1.1520	8.22	30.85	78.96
48	39.85	49.85	43.31	1.1510	8.44	31.41	78.82
52	40.11	48.72	42.22	1.1540	8.41	31.7	79.03
56	38.69	49.43	42.84	1.1538	8.15	30.54	78.94
60	38.93	49.89	43.17	1.1557	8.21	30.72	78.91
64	39.77	49.98	43.33	1.1535	8.43	31.34	78.80
68	40.38	48.74	42.33	1.1514	8.55	31.83	78.83
72	40.35	49.73	43.05	1.1552	8.59	31.76	78.71
76	38.65	49.89	43.21	1.1546	8.21	30.44	78.76
80	40.69	50.87	43.87	1.1596	8.64	32.05	78.77
84	39.89	50.31	43.44	1.1581	8.42	31.47	78.89
88	39.99	49.59	43.07	1.1514	8.42	31.57	78.94
102	40.78	49.64	43.06	1.1528	8.58	32.2	78.96