BIOAUGMENTATION OF ACTIVATED SLUDGE FOR ENHANCED PHOSPHORUS REMOVAL

by

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Submitted in partial fulfillment of the requirements for the degree

M Sc. MICROBIOLOGY

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in the

Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria
South Africa

JUNE 2001

DECLARATION

I, the undersigned, certify the thesis hereby submitted to the University of Pretoria for the degree of M Sc. and the study contained herein is my own original work and has not previously been submitted at another university for any degree.

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Date: 28 June 2001

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DEPARTMENT	:	Microbiology and Plant Pathology
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SUMMARY

Activated sludge systems are designed for the biological removal of phosphorus in wastewater. However, the process is not yet clearly understood. Presently, there is little knowledge of the microbiological metabolic details of biological phosphorus removal. It has been reported that systems often fail and one of the reasons for failure has been suggested as biomass depletion. It has also been reported that an increase in biomass resulted in an increase in phosphorus removal in activated sludge. This suggested that bioaugmentation may offer a solution to system failure and was hence investigated.

The effect of bioaugmentation on phosphorus removal was determined by using different concentrations of a commercially available bioaugmentation product in mixed liquor. There was an initial relationship between phosphate uptake and biomass, which was negatively affected by the phosphate concentration of the bioaugmentation product. At concentrations exceeding 80 mg.l^{-1} of the bioaugmentation product, no phosphorus was removed, instead the phosphate concentration increased as a result of the phosphate content of the bioaugmentation product. The bioaugmentation product used contained a high phosphate concentration, making it unsuitable for bioaugmentation.

The possibility of culturing a bioaugmentation product in a separate fermentation unit for addition to activated sludge was therefore determined by comparing the growth of the product and anaerobic sludge in sterile anaerobic mixed liquor medium, anaerobic mixed liquor with added nutrients (sodium acetate, magnesium sulphate and potassium nitrate) and Nutrient broth. No growth of the microorganisms occurred in sterile mixed liquor medium, even when nutrients were added. Nutrient broth supported the growth of the microorganisms but it cannot be used since it is very expensive.

For determining the phosphate removal capacity of a system based on biomass, aerobic, anaerobic and return sludges were used in batch experiments as inocula in sterile mixed liquor. The results indicated that there was a relationship between phosphate removal and biomass when aerobic sludge was used. No difference could be detected in phosphate removal by different quantities of anaerobic and return sludge mass from the same system, hence, the simulation of the actual MLSS concentration in a specific plant. The results of the simulation experiments varied. In the first experiment, no difference could be detected between the phosphate removal of aerobic and anaerobic sludge, although the use of return sludge resulted in a higher phosphate removal. In the second experiment, the anaerobic and the return sludges indicated the same trend in phosphate removal and were more effective than aerobic sludge. The return sludge mass performed consistently better at phosphorus removal than the aerobic and anaerobic sludges.

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OPSOMMING

Geaktiveerde slyk stelsels word ontwerp vir die biologiese verwydering van fosfaat vanuit afvalwater. Die proses word egter nog nie volkome verstaan nie. Daar is huidiglik beperkte kennis beskikbaar oor die mikrobiologie van fosfaat verwydering. Dit is al aangeteken dat sisteme gereeld misluk en een van die redes hiervoor kan die uitputting van biomassa wees. Daar is ook al vermeld dat die toename in biomassa 'n toename in fosfaat verwydering in geaktiveerde slyk tot gevolg het. Dit het naag laat ontstaan of biomassa aanvulling dalk 'n oplossing kan bied vir sisteem mislukking en was vervolgens ondersoek.

Die effek van biomassa aanvulling op fofaat verwydering is bepaal deur verskillende konsentrasies van 'n kommersiel beskikbare biomassa aanvulling produk te gebruik. Die oorspronklike verhouding tussen fosfaatopname en biomassa is negatief beïnvloed deur die fosfaat konsentrasie in die biomassa aanvulling produk. By konsentrasies hoër as 80 mg.l^{-1} van die biomassa aanvulling produk is geen fosfaat verwyder nie en die fosfaat konsentrasie het toegeneem as gevolg van die fosfaat inhoud van die biomassa aanvulling produk. Die biomassa aanvulling produk het 'n hoë fosfaat konsentrasie gehad wat dit ongeskik vir praktiese gebruik het.

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Die moontlikheid om 'n biomassa in 'n aparte fermentasie eenheid te kweek vir byvoeging in geaktiveerde slyk is bepaal deur 'n vergelyking te tref tussen die groei van die produk en anaërobiese slyk in steriele anaërobe slyk, anaërobiese slyk met bygevoegde nutriënte (natriumasetaat, magnesiumsulfaat en kaliumnitraat) en Voedingsop. Geen groei van mikroörganismes het voorgekom in die steriele slyk medium nie, ook nie met die bygevoeging van nutriënte nie. Alhoewel Voedingsop die groei van die mikroörganismes ondersteun het, is dit te duur om te gebruik.

Om die fosfaat verwyderings kapasiteit van 'n sisteem te bepaal is aërobiese, anaërobiese en retoer slyk gebruik in lot eksperimente as inokulums in slyk. Die resultate het aangedui dat daar 'n verwantskap was tussen fosfaat verwydering en biomassa wanneer aërobiese slyk gebruik was. Geen verskil kon waargeneem word in fosfaatverwydering by verskillende hoeveelhede anaërobiese en retoerslyk massa van dieselfde sisteem nie. Vervolgens is die werklike MLSS konsentrasie in 'n spesefieke aanleg nageboots. In die eerste eksperiment kon geen verskil waargeneem word tussen die fosfaatverwydering van aërobiese en anaërobiese slyk nie, terwyl die gebruik van retoerslyk 'n hoër vlak van fosfaat verwydering tot gevolg gehad het. In die tweede eksperiment het die anaërobiese en retoer slyk dieselfde tendens getoon in fosfaatverwydering en was meer effektief as aërobiese slyk. Die retoerslyk massa het konstant better fosfaat verwyder as die aërobiese en anaërobiese slyk.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ATP:	Adenosine triphosphate
BOD:	Biological Oxygen Demand
cfu:	Colony forming units
COD:	Chemical Oxygen Demand
DNA:	Deoxyribonucleic acid
EBPR:	Enhanced biological phosphate removal
FITC:	Fluorescein isothiocyanate
g:	Gram
$g.g^{-1}$:	Gram per gram
$g.l^{-1}$:	Gram per litre
H_2S:	Hydrogen sulfide
H_2SO_4	Sulphuric acid
h:	Hour
kg:	Kilogram
KH_2PO_4:	Potassium dihydrogen phosphate
KNO_3:	Potassium nitrate
l:	Litre
$MgSO_4.7H_2O$:	Magnesium sulphate heptahydrate
mg:	Milligram
$mg.g^{-1}$:	Milligram per gram
$mg.l^{-1}$:	Milligram per litre
min:	Minutes
ml:	Millilitre
MLSS:	Mixed liquor suspended solids
MLVSS:	Mixed Liquor Volatile Suspended Solids
MPN:	Most Probable Number
N:	Nitrogen
$NaC_2H_3O_2$:	Sodium acetate
ND:	Not Determined

NH_4:	Ammonium
nm:	Nanometer
NO_3^-:	Nitrate
P:	Phosphorus
ΔP:	Change in phosphate concentration
pH:	Hydrogen ion concentration
PO_4^{3-}:	Phosphate
Poly-P:	Polyphosphate
RNA:	Ribonucleic acid
rpm:	Revolutions per minute
SO_4^{2-}:	Sulphate
Tra+:	Transfer positive
TPC:	Total plate counts
VSS:	Volatile suspended solids

CHAPTER 1

INTRODUCTION

South Africa's water supplies are limited and droughts are a constant threat, making it essential to protect the aquatic environment from pollution. Nutrient removal from wastewater is thus important to prevent eutrophication (Bosch and Cloete, 1993). The removal of phosphorus from sewage works effluent is considered as one of the methods for eutrophication control of lakes and impoundments (Buchan, 1980; Bosch and Cloete, 1993). Phosphate from wastewater can be removed by chemical or biological methods. Chemical removal is expensive since it involves the addition of lime, aluminium and ferric chloride and it goes along with the cost of the mentioned chemicals and accumulation of large quantities of chemical waste sludge (Momba, 1995). Hence, biological phosphate removal by activated sludge process has gained support. According to the Water Act (Act 54 of 1956), the discharge of orthophosphate should be limited to 1.0 mg phosphate per liter (Lilley *et al.*, 1997).

Biological phosphate removal processes produce an effluent of high quality in order to meet the required standard at the lowest possible cost. Unfortunately, many of these systems do fail necessitating chemical addition. One of the most important reasons for systems failure is the lack of knowledge of the role of microorganisms in these systems. Research on phosphate removal by biological methods became more intensified and the process of enhanced phosphate removal by activated sludge systems, although well documented, is not yet clearly defined or understood (Momba, 1995).

Activated sludge systems are not dominated by one or a few specific bacterial species, but a combination of different bacterial species which co-exists and function together in a complex community (Ehlers, 1997). Hence, no single species of bacteria is capable of removing all the phosphate from wastewater, suggesting that a diverse bacterial community is responsible for the process. Momba and Cloete (1996) indicated that an increase in biomass resulted in an increase in phosphate removal. This raised the question whether the wastewater treatment system could be bioaugmented in order to increase biomass.

Bioaugmentation is the practice of assisting the bacterial population by the addition of specialized cultures developed to provide increased rates of organic reduction or capabilities of degrading compounds previously considered nonbiodegradable (Oellerman and Pearce, 1995). The object is not to replace the existing biomass, but augments it's ability to respond to certain situations or it's ability to degrade components of the waste-stream, resulting in improved treatment (www.sybronchemicals.com/biochem/waste.html). The system performance may be enhanced with both biomasses present (Chong *et al.*, 1997).

The process of bioaugmentation is said to be beneficial in many cases, including the protection of activated sludge systems against perturbations resulting from transient or continuous overloading and the increased degradability of previously hard-to-treat organics. The possibility of operation at higher rates during cold weather and in periods of reduction in sludge production, are additional benefits of bioaugmentation (Chong *et al.*, 1997). Inconsistent results, however, were found when different tests of bioaugmentation were conducted under different conditions or situations. Other studies with unsatisfactory results have been reported (Koe and Ang, 1992). It was therefore concluded that no significant improvement could be achieved with bioaugmentation (De Haas, 1999).

The objectives of the study were therefore, to determine the effect of bioaugmentation on phosphorus removal in activated sludge by adding commercially available bioaugmentation product in order to increase biomass and to determine the relationship between anaerobic, aerobic and return sludge and phosphate removal in activated sludge.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Eutrophication of South Africa's natural waters is greatly accelerated by human activities which result in the discharge of the nutrients such as nitrogen (N) and phosphorus (P) (WRC, 1997). Gross eutrophication is marked by large visible blooms of algae, which makes water treatment difficult. Many algae have the ability to fix nitrogen into the water and therefore, phosphorus is the element that should be considered to minimise eutrophication. Due to the excessive phosphorus concentrations found in wastewaters, resulting from industrial effluents and domestic detergents, efficient phosphorus removal is essential (Bosch and Cloete, 1993). This goal can be achieved by either chemical and/or biological means. Biological phosphorus removal has gained support worldwide. Activated sludge plants have been developed for biological phosphorus removal, but the prevention of eutrophication however remains a problem due to inadequate biological phosphorus removal (Bosch and Cloete, 1993).

Phosphate is a major component of the nucleic acid and no organism can reproduce without it. All energy consumption and production by living organisms require phosphate (Momba, 1995). Its removal from wastewater should eliminate eutrophication. The use of microorganisms in the treatment of sewage and wastewater is one of the possible methods to solve the problem. In wastewater treatment system, bacteria and other microorganisms use the soluble organic matter in the waste stream as a food source. The bacteria use oxygen and carbon dioxide in degrading organic matter. Microorganisms interact to transform organic matter into new biomass, carbon dioxide and water. Collectively, these microorganisms are called biomass. The biomass is the "workforce" of the wastewater treatment system (www.bioaugmentation.com/article.html).

The total biomass is more important in terms of phosphate removal from wastewater than individual species or specific populations (Momba, 1995). Hence, no single species of bacteria is capable of

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removing all the phosphate from wastewater, suggesting that a diverse bacterial community is responsible for the process. This has led to the process of bioaugmentation of wastewater in order to increase biomass. Bioaugmentation involves the use of specially selected and adapted microorganisms to enhance the biodegradation of specific toxic, hazardous and recalcitrant compounds which are difficult to remove from the environment by conventional treatment processes (Oellerman and Pearce, 1995). It also infers the stimulation of microorganisms indigenous to the contaminated environment and wastewater to enhance and improve the treatment performance. Successful application of bioaugmentation techniques requires the identification and isolation of useful microorganisms and the survival of those organisms after release into the environment (Walter, 1997).

2.2 The activated sludge process

The activated sludge process is the most widely used biological process for the treatment of organic and industrial wastewater. It refers to a slurry of microorganisms that remove organics from wastewater and these organisms are themselves removed by sedimentation under aerobic conditions (Ehlers, 1997).

The basic principle of the process is that wastewater is brought into contact with a mixed population in the form of flocculent suspension in an aerated and agitated system. Suspended and colloidal material is removed from the wastewater by adsorption and agglomeration on to the microbial flocs (Wrinkler, 1981). The materials and the dissolved nutrients are then broken down slowly by microbial metabolism (Gray, 1989). Part of the nutrient material is oxidised to simple substances such as carbon dioxide and part is converted into new microbial cell material. When the desired degree of treatment is achieved, the sludge is separated from the treated wastewater by settling (Gray, 1989). Part of the settled sludge is drawn off as waste and the rest is recycled to the aeration basin in order to maintain a high concentration of bacteria and serve as microbial inoculum (Momba, 1995).

When the aeration is started, the nutrient to microorganisms ratio is large and the nutrients are in abundance and the initial growth rate is exponential. During this growth phase the organic matter in the waste effluent is removed at its maximum rate with optimum conversion of organic wastes into new bacterial cells (Cloete, 1984). At this stage the energy level is high enough to keep all the microorganisms completely dispersed. Therefore, it is impossible to get activated sludge to form as long as the microorganisms remain in the exponential growth phase (Cloete, 1984).

The activated sludge process has undergone various modifications able to meet most wastewater treatment needs. These changes relate to the flow regime in the reactor, the size, number and configuration of the reactors, recycled flow, influent flow and others incorporated either emphatically or present inadvertently or unavoidable (Momba, 1995).

The activated sludge process appears similar in principle to large scale industrial fermentation process, in that a dense microbial population is maintained in suspension in a nutrient medium and has to be provided with an adequate supply of dissolved oxygen (Wrinkler, 1981). The concentration of nutrients in wastewater is low as compared to that used in fermentation growth media.

A number of activated sludge systems have been developed (e.g., Bardenpho, UCT, Biodenipho), only the Phoredox system will be discussed

2.3 The Phoredox activated sludge system

The basic process for the simultaneous biological removal of phosphate and nitrogen was proposed by Barnard in 1976 and is known as the Phoredox (phosphorus reduction oxidation) activated sludge process in South Africa and Bardenpho in the United States (Ehlers, 1997). It is a five staged process designed to remove nitrogen and phosphorus by biological means. The system consists of a sequence of primary anaerobic, primary anoxic, primary aerated, secondary anoxic and an aerated basin followed by a clarifier. In this system, sludge is returned from the clarifier to re-enter the anaerobic zone with the influent and the mixed liquor is returned from the aerobic zone to the

primary anoxic zone and so on (Toerien *et al.*, 1991). The application of this 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 between 80 - 90 % of the nitrogen (Momba, 1995). The description of different stages of the process is necessary to understand the process.

The primary anaerobic zone is the zone where both the dissolved oxygen and nitrates or nitrites are absent (Barnard, 1975; Buchan, 1984). Sludge from the clarifier flows together with the influent wastewater into this zone. This zone is essential for phosphate removal, as the bacteria in the activated sludge passing this zone are preconditioned to take up excess phosphate under aerobic conditions (Cloete and Muyima, 1997). The release of some of the quantity of phosphate from the biomass in the solution indicates that the bacteria have been suitably conditioned (Pitman, 1984).

The presence of nitrate in an anaerobic zone is a handicap to the phosphate removing potential of the activated sludge system (Momba, 1995). High concentrations of nitrate present in the anaerobic zone resulted in poor phosphate removal during aerobiosis. In the presence of nitrate, the redox potential is too high to produce lower fatty acids for the release of phosphate (Cloete and Muyima, 1997). However, the use of unsettled influent and the presence of sludge from the treatment in the primary clarifiers, probably producing lower fatty acids, had a positive effect on the phosphate removal (Mulder and Rensink, 1987). Nitrates and dissolved oxygen discharge into the zone must be zero or as near to zero as possible because their excessive amounts cause phosphate release to cease (Toerien *et al.*, 1991).

The addition of acetate to the medium and by lowering of pH under anaerobic conditions resulted in phosphate release (Fuhs and Chen, 1975; Barnard, 1976). Phosphate release by the addition of acetate has also been observed by Comeau *et al.* (1986) and Murphy and Lotter (1986). It was indicated that a lower nutrient environment resulted in less phosphate removal from the mixed liquor medium while a higher nutrient concentration lead to a greater phosphate removal (Bosch, 1992).

The principal function of the anaerobic zone is to establish a facultatively anaerobic microbial community as indicated by the Embden Meyerhof fermentation pattern (Fuhs and Chen, 1975). During anaerobiosis this bacterial community produce compounds such as ethanol, acetate and succinate, which serves as carbon sources for phosphate-accumulating bacteria (Cloete and Muyima, 1997).

The effluent from the anaerobic zone and the mixed liquor recycled from the aerobic zone flows into the primary anoxic zone. Anoxic refers to the presence of nitrates and the absence of dissolved oxygen, and this leads to the enrichment of denitrifying bacteria (Buchan, 1984; Pitman, 1984). The purpose of this stage is the denitrification of nitrates in recycled mixed liquor from the primary aeration stage. Denitrification will commence only after the dissolved oxygen concentration has been reduced to such a level that denitrification is induced (Cloete, 1984). The process will then continue until the available nitrate has been depleted or the mixed liquor leaves this stage and enters the primary aeration stage (Cloete, 1984). Soluble and colloidal biodegradable matter are also removed in this zone.

The mixed liquor from the anoxic zone flows into the aerobic zone whereby nitrification and phosphate uptake take place (Keay, 1984). This is the principal site for the oxidation of biodegradable nitrogen and carbonaceous material (Cloete, 1984). It oxidises the organic material in the sewage, oxidises ammonia to nitrite and then to nitrate and provides an environment in which the biomass can take up phosphate released in the anaerobic zone, plus all the phosphate which enters the process in the feed sewage (Cloete and Muyima, 1997). The microorganisms responsible for the oxidation of ammonia to nitrite belong to the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus* and *Nitrosolobus* species, whereas nitrite is oxidised to nitrate by *Nitrobacter*, *Nitrosospira* and *Nitrococcus* species (Buchan, 1984).

The efficiency of phosphate removal appeared to be dependent on the intensity of aeration. The aeration rate should ensure the oxidation of the carbon compounds and ammonia and to suppress the growth of filamentous microorganisms that produce poorly settling sludge (Ehlers, 1997). Higher aeration rates resulted in maximum phosphate uptake within 2 h (Momba, 1995). The mixed liquor

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supplied with pure oxygen took up orthophosphate than did the mixed liquor supplied with an equal quantity of oxygen in air. Small changes in aeration rate showed differences in orthophosphate uptake of the sludge organisms (Momba, 1995).

The mixed liquor flows from the primary aerobic zone to the secondary anoxic zone for further denitrification of nitrates in the system. Denitrification rate in this zone is slow and therefore the quantity of nitrate removed is small (Cloete, 1984). The retention time in the secondary anoxic stage is relatively long due to the lower chemical oxygen demand (COD) (Cloete and Muyima, 1997). There is no change in the phosphorus concentration in this stage. Anaerobic conditions should not develop since it will lead to the release of accumulated phosphorus.

The mixed liquor continues its circuit from the secondary anoxic zone to the secondary aerobic zone for further removal of any residual organic material, ammonia and any phosphate which might be released in the secondary anoxic zone (Bosch and Cloete, 1993). The function of this stage is to ensure that anaerobic conditions do not occur after the secondary anoxic stage. This stage also prevents anaerobic conditions from developing in the clarifier which will result in phosphorus release (Buchan, 1984). The secondary aerobic zone is required to increase the dissolved oxygen to a level between 2-4 mg.l^{-1} in the mixed liquor before it enters the clarifier (Barnard, 1976) and to refine the final effluent by the removal of additional phosphate and the oxidation of residual ammonia (Cloete and Muyima, 1997). Mixed liquor must be aerated for a period of at least one hour before passing into the clarifier (Keay, 1984; Pitman, 1984). Aeration should be satisfactory to promote phosphate uptake and maintain good aerobic conditions. Excess aeration should be prevented, as it will encourage the conversion of organically bound nitrogen to nitrate and also cause the slow aerobic release of phosphate from the solid (Keay, 1984; Pitman, 1984).

From the secondary aerobic zone, the mixed liquor flows into the clarifier. In the clarifier, the flocs of microorganisms are allowed to settle out and the effluent is removed form the system. Part of the settled activated sludge is drawn off as waste and returned via the clarifier underflow to the influent sewage and serves as inoculum of microorganisms, the rest is treated further for use as fertilizer (Bosch and Cloete, 1993). The phosphate concentration of the effluent must be 1.0 mg phosphate

per liter, if not, the residual phosphate in the effluent may be precipitated out chemically before discharge into rivers or dams. The aim of the clarifier is to produce a clear effluent free of suspended solids and a thickened sludge for recycling to the inlet of the process. The quality of the underflow sludge should be such that nitrate is not recycled to the anaerobic zone (Ekama *et al.*, 1984).

2.4 The role of biomass in the activated sludge system

Activated sludge consists of microbial communities present in discrete flocs and individual bacteria that are suspended in the wastewater and mixed together by the aeration system. The main biological groups present are bacteria, fungi, protozoa, algae and filamentous microorganisms (Gray, 1989). The role of fungi and algae are not considered so important, whereas protozoa, filamentous organisms and bacteria actively participate in biological treatment of wastewater by the activated sludge system (Cloete and Muyima, 1997).

Protozoa make a significant contribution to the activated sludge process and their presence is regarded as a sign of a healthy sludge (Wrinkler, 1981). Pike and Curds (1971) reported that protozoa-free sludge produced very poor effluent containing large numbers of dispersed bacteria and improved shortly after inoculation with a protozoal culture. Some protozoa are attached to the flocs and feed on dispersed bacteria, others swim freely in the interstitial liquid of the flocs and also consume dispersed bacteria. The protozoa are responsible for "grooming" the zoogloeal mass by grazing on it and are consumed by other organisms present in the system (Ehlers, 1997). In this way, a food chain is created and at each stag of the food chain a fraction of the original material is removed from the system as carbon dioxide (Momba, 1995). There are several classes of protozoa found in activated sludge, they are Sarcodina (Amoeba), Flagellates (Euglena) and Ciliates (Paramecium or Vorticella). The ciliates play an important role in activated sludge systems (Momba, 1995). Activated sludge containing ciliates usually exhibits good settling, low turbidity and floc with crisp, clean edges.

The role of the filamentous organisms is important as they promote the formation of bacterial flocs. They can contribute to the proliferation of large, firm, activated sludge flocs exhibiting good settling properties (Momba, 1995). The major biological problems linked to filamentous populations are bulking (due to the proliferation of these organisms) and foaming (absence of these organisms). The filamentous microorganisms most frequently found at present are: *Microthrix parvicella, Halicsomenobacter, Nocardia amarae, Norcadia pinensis, Sphaerotillus natans, Nostocoida limicolla* and *Rhodococcus* spp (Momba, 1995)

Like all biological treatment processes, the activated sludge system relies on heterotrophic bacterial community to carry out the basic oxidation of the substrate present (Gray, 1989) The heterotrophic bacteria form the basis of the flocs that form the basic ecological unit of the activated sludge process. However, the lack of suitable media which would support the growth of all viable nutritional types of bacteria in activated sludge has been a limiting factor in the identification of the bacterial community in activated sludge systems (Momba, 1995). Despite this handicap, bacterial studies have been done in the system. The bacterial population in activated sludge systems has been studied by many researchers and the organisms investigated include species of *Acinetobacter, Moraxella, Flavobacterium, Pseudomonas, Vibrio, Achromobacter, Alcaligenes, Enterobacter, Serratia, Proteus, Aeromonas, Proteobacter, Xanthobacter, Aerobacter, Klebsiella, Bordetella, Citrobacter, Shigella, Pasteurella, Yersinia* spp, *Escherichia intermedium* and *Bacillus cereus* (Momba, 1995). Activated sludge is therefore a diverse ecosystem which consists of different bacterial species which all function together to maintain a stable bacterial community (Ehlers, 1997).

2.5 Failure of enhanced biological phosphate removal (EBPR) systems

The successful operation of nutrient removing activated sludge plants depends on good operation, good design and the maintenance of the correct biomass load. Biological phosphate removal plants in South Africa have not always given reliable and satisfactory performance (Osborn *et al.*, 1986). Enhanced biological phosphorus removal fail either as a result of toxic shock due to the accumulation of H_2S or the nitrate feedback into the anaerobic zone (Bitton, 1994). Operational

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optimisation of the enhanced biological phosphorus removal process is dependent on meeting the oxygen demand in the aerobic zone and providing the correct substrate in the anaerobic zone. Some organisms can compete with poly-P bacteria in anaerobic-aerobic activated sludge systems, leading to systems failure (Mino *et al.*, 1994).

Failure of the EBPR process has attracted various methods to remedy this problem, for instance augmenting of the activated sludge by addition of readily biodegradable COD to promote the growth of phosphate removing bacteria has been applied (Osborn *et al.*, 1986). Bioaugmentation has also been recommended as an approach to redress system failure (Bitton, 1994).

The availability and use of bioaugmentation products in wastewater treatment system has increased significantly in recent years (De Haas, 1999). The effectiveness of adding live cultures of microorganisms produced commercially to systems, which naturally develop mixed and complex populations of many different microorganisms, may be questioned.

Biosupplements have been marketed and used in wastewater treatment processes for a number of years with varying degrees of success (De Haas, 1999). Results from the commercial application of such biosupplements, particularly in wastewater treatment systems facing operational problems, have tended to be positive (Koe and Ang, 1992). However, laboratory research investigations have contradicted these results and many researchers have concluded that no significant improvement in process performance can be achieved with biosupplementation (De Haas, 1999).

2.6 Bioaugmentation

For many years, chemicals like polyelectrolytes, lime and metallic hydroxides have been used in wastewater treatment plants to improve settle ability. Unfortunately, these chemicals are very expensive and become part of the sludge and can through additional bound with water, contribute to volume increases in the sludge (Grubbs, 1979). The technology of bioaugmentation has provided an alternative that is not only cost effective, but which does not increase the sludge volume and it revolves specific problems for municipal waste water treatment (Oellermann and Pearce, 1995).

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Bioaugmentation has been practised since the early 1960s. Because of frequent misapplication of additives or poor documentation of results, the technology has been regarded as less than scientific (www.bioaugmentation.com/article.html).

Bioaugmentation is the practice of enhancing the performance of indigenous bacterial populations of wastewater treatment systems through the addition of bacterial cultures with specific degradative abilities (www.sybronchemicals.com/biochem/waste). It also involves the use of genetically manipulated or specially selected and adapted microorganisms for the biodegradation of specific toxic, hazardous and often recalcitrant compounds which are very difficult to be removed from the environment by conventional treatment processes (Oellermann and Pearce, 1995). Bioaugmentation does not replace an existing bacterial population, but augments its' ability to respond to certain situations or its' ability to degrade components of the wastestream, resulting in improved treatment (www.sybronchemicals.com/biochem/waste).

Bioaugmentation has been applied in brewing, pharmaceutical and dairy industries to optimise the performance of fermenters (www.bioaugmentation.com/article.html). Its use in the wastewater treatment industry has developed over the past twenty years and is now an accepted practice throughout the country as well as the world (www.goodnet.com/dmpbio/how.html).

In the treatment of wastewater, microorganisms (mainly bacteria) use the soluble organic matter in the waste stream as a food source (www.bioaugmentation.com/article.html). The bacteria consume the organic compounds and convert them into carbon dioxide, water and energy to produce new cells. Ultimately, the soluble pollutants are converted into insoluble biomass, which can be removed mechanically from the waste stream and sent to disposal (www.bioaugmentation.com/article.html).

The technology of bioaugmentation may also be applied in the abattoir and tourist industry for the effective and inexpensive treatment of fats and oils (Oellermann and Pearce, 1995). Municipal treatment works could benefit by boosting their capacity to treat toxic and hazardous wastes, which are discharged to disrupt their systems.

All municipal sewer treatment facilities use the bioaugmentation products to remove organic waste from wastewater. When using bacterial digestion, the system benefits very much;

- bacterial oxidation of the liquid phase will be faster and more complete
- digesters in the plant will operate evenly and uniformly
- sludge volume will be reduced as solids are digested
- plant capacity will be effectively increased because more waste can be processed in less time
- the entire system will be able to absorb the shock of toxic influent
- Effluent water quality standards will be met more consistency, helping to maintain a cleaner environment and safer drinking water.

2.6.1 Applications of bioaugmentation

- **Increased BOD removal**

BOD is an indirect measurement of the amount of organic matter in water (Bitton, 1994). It shows how much oxygen is required by bacteria if they were to digest all the organic material in the water. When the BOD of water is high, it means that the water contains a lot of organic matter in it and that bacteria would demand or use a lot of oxygen to digest that amount of organic material. By increasing the microbiological numbers and diversity via bioaugmentation, the removal of BOD may be achieved. The strains of the microorganisms that are selected may be used to increase the BOD removal in wastewater treatment plants.

- **Reduction of sludge volume**

In an aerobic waste treatment, there is a serious problem of the production of large amounts of sludge and thus reduction of sludge volume is desirable. The reduction is the result of increased organic removal following addition of a mixed culture of selected microorganisms (Bitton, 1994).

- **Use of mixed cultures in sludge digestion**

The use of mixed cultures in anaerobic digesters has led to significant savings in energy requirements. In anaerobic digesters, bioaugmentation has resulted in enhanced methane production.

- **Biotreatment of hydrocarbon wastes**

The removal of hydrocarbon wastes may be achieved by microbiological bioaugmentation. Commercial bacterial formulations have been used to treat such wastes. For example, the addition of cultures of mutant bacteria improved the effluent quality of a petrochemical wastewater treatment plant (Bitton, 1994).

- **Biotreatment of hazardous wastes**

Bioaugmentation involves the use of added microorganisms for treating toxic and hazardous wastes. Exposure of wastewater microbial communities to toxic xenobiotics results in the selection of resistant microorganisms that have the appropriate enzymes to use the xenobiotics as the sole source of carbon and energy. Bioaugmentation needs an acclimation period prior to onset of biodegradation and short survival or lack of growth of microbial inocula in the seeded bioreactors (Bitton, 1994). Some of the commercial products are negative or inconclusive when are evaluated.

- **Improved solids settling**

An important step in biological waste treatment is solids removal, usually through settling in a lagoon or clarifier. Bacteria form a natural biopolymer that aids in settling. Toxic shocks and system changes can result in a bacterial population with little biopolymer and poor settling characteristics. The traditional approach of adding organic polymers or inorganic coagulants as settling aids can be effective but expensive. By inoculating the system with organisms known to be

both resistant to the toxicity and excellent floc formers, polymer demand can be greatly reduced or eliminated (www.bioaugmentation.com/article.html).

2.6.2 Technology implementation

The application of bioaugmentation in wastewater treatment was originally the result to solve urgent operational problems such as shock loads in treatment plants or to make a remedial response to spilling emergencies (Oellermann and Pearce, 1995). The addition of the bacterial cultures either assists the operation to return to normal or helps the danger of spilling pollutants. These applications have stimulated the application of bioaugmentation to municipal treatment works.

Bioaugmentation is being used in South Africa with varied success (Oellermann and Pearce, 1995). A number of commercial biosupplements do not work as claimed and reliable manufactures and suppliers of quality biosupplement products for the local market must be identified. The implementation of bioaugmentation technology must minimise risk to the end-user.

Microorganisms are rarely found in pure cultures in nature. Microbial communities in the natural environment represent a complex ecosystem. The application of biosupplements must ensure that the added cultures are present in sufficient numbers at all times to be able to degrade the desired pollutants in the presence of the large numbers of indigenous microorganisms required for the biodegradation of the competing nutrients and more easily degradable pollutants (Oellerman and Pearce, 1995).

Industry should be involved with the research for the specific applications of biosupplements in the biodegradation of toxic and hazardous substances. The research should concentrate on the interactions between microorganisms existing and developing in complex communities. The performance of the treatment works would be affected by the influence of available nutrients. Research into obtaining a better understanding of such complex micro-systems would enhance our understanding of the biology of wastewater works, especially in cases where treatment was necessary at the source of the toxic or hazardous wastewater (Oellerman and Pearce, 1995).

2.7 Bioaugmentation in activated sludge: current features and future perspectives

Bioaugmentation of activated sludge systems with specialised bacterial strains could be a powerful tool to improve several aspects in wastewater treatment processes, such as improved flocculation and degradation of recalcitrant compounds (Van Limbergen *et al.*, 1998). The addition of specialised strains to activated sludge to enhance the removal of pollutants present in the influent is not yet applied. This is due to the fact that bioaugmentation of activated sludge is less predictable and controllable than the direct physical or chemical destruction of pollutants (Van Limbergen *et al.*, 1998).

Natural bacterial strains can be used, but the construction of new genetically modified organisms with the potential for enhanced breakdown of organic compounds or specialised in the degradation of different chemical compounds can also be promising. Transfer of plasmids between bacteria has played an important role in the adaptation of bacteria to the presence of recalcitrant compounds (Sayler *et al.*, 1990).

Bioaugmentation with plasmid-encoded metabolic pathways could be an interesting alternative to the inoculation of strains with chromosomal pathways. The plasmids could be easily exchanged between the bacterial species of the sludge and thus provide the microbial community with these useful genes, thereby improving biodegradation (Van Limbergen *et al.*, 1998).

The activated sludge process is operated as a continuous bioreactor with feedback of the biocatalyst. This biocatalyst ensures rapid oxidation of pollutants present in the influent and also stabilises the system against variations in influent composition. Process conditions are regulated and cell growth is minimised in order to obtain flocculation and a highly clarified effluent (McClure *et al.*, 1990). The formation of well settling activated sludge flocs is based on the ability of the microbial community to aggregate.

2.7.1 Bioaugmentation in activated sludge and other wastewater treating bioreactors

The introduction of degradative bacteria and mobile catabolic genes into activated sludge in order to enhance biodegradation of xenobiotics was evaluated (Van Limbergen *et al.*, 1998). It was indicated that the strains of *Pseudomonas putida* (phenol degrading) which was inoculated into a sequencing batch activated reactor removed phenol from 95% - 100% in 40 days, while in the unaugmented control reactor, phenol removal was initially 100% but decreased to 40%. The addition of the strain provided stability in phenol degradation.

McClure *et al.*, (1991) showed that the transfer of genes into indigenous bacteria could result in enhanced biodegradation. He also indicated that the use of strains that are well adapted to the activated sludge conditions could be very important. The studies indicated that the introduction and dissemination of catabolic plasmids in natural or reactor communities could be a promising bioaugmentation strategy (Van Limbergen *et al.*, 1998).

2.7.2 Flocculation in activated sludge processes and its influence on bioaugmentation

Aerobic wastewater treatment relies on the ability of microorganisms to aggregate, allowing a separation of the formed microbial biomass and the effluent in the final settling tank (Van Limbergen *et al.*, 1998). Aerobic wastewater treatment plants that do not face major shocks or toxic pulses do not attain the discharge standards (Berthouex and Fan, 1986). Hence, even under normal operation conditions, activated sludge microbial communities are quite unstable (Van Limbergen *et al.*, 1998). The structure of sludge flocs is very porous and the bacteria inside undergo the same nutrient flow as suspended bacteria. This means that flocs cannot be seen as dense, solid particles of microorganisms.

Techniques have been developed to stimulate the formation of a good settling sludge. Before the start of bioaugmentation of activated sludge, a good settling sludge should be available (Van Limbergen *et al.*, 1998). The inoculated strains should be integrated in the flocs and they should not negatively influence the sedimentation process.

The identification and characterisation of the genes and plasmids involved in the flocculation-promoting processes can help to construct bacteria that incorporate more efficiently into activated sludge flocs (Van Limbergen *et al.*, 1998). The construction of these bacteria is not only important for a good flocculation, but can also improve bioaugmentation. When the inoculated microorganisms are incorporated into the activated sludge, they can stay in the unit for a longer time, which thus helps to maintain the degradative capacity in the sludge system.

2.7.3 Inoculation of strains in activated sludge

The nature of xenobiotics, the physicochemical conditions and the metabolic potential of the microbiota determines the efficiency, efficacy and cost of bioaugmentation (Van Limbergen *et al.*, 1998). Mineralization in a natural environment is preceded by an acclimation period (time between the inoculation and the onset of degradation of the pollutant). The acclimation period is a result of the time needed for enzymes to be induced or for mutation or genetic exchange to occur.

Research has been conducted whereby the strains have been inoculated into the activated sludge bacteria. It was described that *Pseudomonas putida*, containing the recombinant plasmid survived for more than 8 weeks in the activated sludge unit but did not degrade the target substrate (McClure *et al.*, 1991). The reason for not degrading the target substrate could be the preferential use of alternative substrates. The introduced strain may face intense competition, predation or parasitism in sewage. The conversion of pollutants by several members of an indigenous microbial community can also have negative effects (Van Limbergen *et al.*, 1998).

The genetic optimisation of microorganisms, able to degrade target compounds and the design of novel pathways for the catabolism of recalcitrant xenobiotics, are promising strategies to enhance bioremediation processes (Van Limbergen *et al.*, 1998).

2.7.4 Plasmids and their conjugative transfer in activated sludge

Plasmids and their ability to transfer between different bacteria seem to play an important role in the adaptation of bacteria to xenobiotics through the acquisition of new genetic traits (Mergeay *et al.*, 1990). Plasmids genes can be disseminated into bacteria through conjugation, transformation and transduction. Transformation and transduction provide limited opportunities for genetic exchange (Hirsch, 1990). Plasmid transfer via conjugation play an important role in the environment and has been studied more intensively (Van Limbergen *et al.*, 1998). Conjugation is limited by plasmid incompatibility and modification systems (Frank *et al.*, 1996).

Recombinant plasmids lacking mobilisation and transfer genes have a low probability of dissemination. However, transfer of genes located on these plasmids after recombination with or transposition into Tra+ plasmids is possible and has been demonstrated (Top *et al.*, 1990). Cases of transfer of catabolic plasmids in activated sludge plants clearly indicate that genes from inoculated strains can be disseminated into activated sludge bacteria (Van Limbergen *et al.*, 1998).

Bioaugmentation of activated sludge systems with specialised bacterial strains could be a powerful tool to improve several aspects in wastewater treatment processes, such as improved flocculation and degradation of recalcitrant compounds (Van Limbergen *et al.*, 1998). The construction of new genetically modified organisms with the potential for enhanced breakdown of organic compounds can be a promising method for bioaugmentation of activated sludge (McClure *et al.*, 1990 and 1991).

The introduction and dissemination of catabolic plasmids in natural communities could be a promising bioaugmentation strategy (Van Limbergen *et al.*, 1998). The identification and characteristics of the genes and plasmids involved in the flocculation promoting processes can help to construct bacteria that incorporate more efficiently into activated sludge flocs. The construction of these bacteria could improve bioaugmentation in activated sludge (Van Limbergen *et al.*, 1998).

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2.8 Determination of microbial biomass

Biomass is an important ecological parameter because it measures the energy resources being stored in a particular segment of the biological community as well as the transfer of energy between trophic levels within an ecosystem. It is defined as the dry weight, volume or other quantitative estimation of organisms, the total mass of living organisms in an ecosystem (Atlas and Bartha, 1993). Thus, the various types of organisms in activated sludge system constitute the biomass of that particular system. Biomass can be expressed in units of weight (grams) that can be converted to units of energy (calories).

Current research in wastewater treatment has been directed towards mathematical modelling of basic design and operational procedures (Jorgersen *et al.*, 1992). One important parameter in such models is the amount of viable biomass. For this reason, attempts have been made to find methods to determine the biomass in wastewater and activated sludge. The choice of the methods is a crucial decision to make. Scientists are interested in methods for investigating specific microbially mediated processes (Momba, 1995). An applicable method must be rapid, reasonably simple to use and accurately assess the number of bacteria present. Ideally, it should distinguish living organisms from the dead. These requirements to estimate accuracy and reliably total number of living bacteria in environmental samples have not yet been met (Roszak and Colwell, 1987).

Methods used to enumerate microbial populations fall into two categories, direct and indirect counting methods. Direct counting methods include light microscopy, epifluorescence microscopy, fluorescent antibody techniques, electron microscopy and confocal scanning light microscopy. The microscope methods require skilled personnel and often present difficulties in distinguishing between living and non-living cells (Momba, 1995). Since the direct counting methods fail to differentiate between living and dead cells, it may result in overestimation of the viable cells present. Alternatively, viable counting methods can be used to differentiate between living and dead cells, by assessing their ability to grow either in liquid media or on solid media (Herbert, 1990). Since there is no universal growth medium on which all microorganisms will grow, it is therefore inevitable that viable counting methods substantially underestimate the true microbial

populations present (Herbert, 1990). Viable count data for natural microbial populations should therefore be treated with considerable caution except when other supporting information such as total count data and estimates of biomass are available or when specific physiological groups are being enumerated (Herbert, 1990).

The viable count is based on the assumption that a viable bacterial cell is capable of multiplying to form two progeny under conditions that are optimal for the cell concerned (Momba, 1995). The viable count procedures include the plate count technique and the most probable number (MPN) technique. Plate count techniques were and in many situations remained the method that is used to obtain a total viable count (Roszak and Colwell, 1987). The problem with this method is normally an underestimation of the biomass due to selectivity of the media (Jorgensen *et al.*, 1992).

Indirect counting methods in contrast do not require visual or cultural examination of the microorganisms. They rely upon the presence of a specific chemical component that is only present in the living cells and which is rapidly degraded upon death of the organism (Herbert, 1990). A crucial aspect of indirect methods is that the chemical component to be determined should have a known constant ratio to the cell biomass and not be influenced by factors such as growth rate and nutrient status of the cells (Herbert, 1990). Methods include respirometry, ATP content and different enzyme assays. Attempts have been made to relate activity to biomass. It was found that there is a linear relationship between oxygen utilization rate and ATP content suggesting that ATP reflected viable biomass.

2.8.1 Direct counting procedures

2.8.1.1 Light microscopy

Microorganisms can be counted by direct microscopic observations. Direct count procedures yield the highest estimates of numbers of microorganisms. There are several drawbacks to direct observational methods; dead and live cells are counted, different types of bacteria cannot be

distinguished on the basis of morphology and the sample cannot be used for further experimentation (Atlas and Bartha, 1993).

2.8.1.2 Epifluorescense microscopy

Epifluorescence microscopy with stains such as acridine orange (AODC), 4', 6-diamidino-2-phenyl-indole (DAPI) and fluorescein isothiocyanate (FITC) are widely used for direct counting of bacteria (Atlas and Bartha, 1993). DAPI has been found to be superior to acridine orange for visualizing small bacterial cells (Wolfaardt *et al.*, 1991). Counts obtained by direct epifluorescence microscopy are typically two orders of magnitude higher than counts obtained by cultural techniques (Atlas and Bartha, 1993). Many small and unusually shaped bacteria are observable and countable with epifluorescence microscopy. The value of the direct count epifluorescence microscopy approach to enumeration is that it is applicable to a variety of habitats without the bias inherent in viable plate count procedures (Wolfaardt *et al.*, 1991; Atlas and Bartha, 1993). It allows the estimation of numbers of microorganisms in marine, freshwater and soil habitats despite the great differences in population sizes and physiological types that occur in these various habitats.

2.8.1.3 Fluorescent antibody techniques

The numbers of specific types of microorganisms can be estimated using fluorescent antibody techniques. This method is extremely specific for individual microbial species and permits autecological studies, that is, studies of individual microorganisms in their natural environments (Atlas and Bartha, 1993). Fluorescent antibody techniques have been applied to studies on selected microbial species in their natural habitats, including ecologically important organisms, monoclonal antibodies have been used to specifically detect methanogens in environmental samples and such specific fluorescent dyes are applicable to enumerate many other specific populations (Atlas and Bartha, 1993).

2.8.1.4 Electron microscopy

Direct counting of microorganisms can also be done by using electron microscopy. Preparation time and costs involved make this method impractical to use on a routine basis.

2.8.1.5 Confocal scanning light microscopy

Confocal scanning light microscopy is a new type of microscopy that has generated a considerable excitement. It gives higher resolution and thinner non-invasive optical sections or planar views, than those obtained by classical bright-field or dark-field microscopy and increased contrast is another major advantage (Rochow and Tucker, 1994). In an image-processing system, a hundred or more very thin optical sections can be stored and combined into a composite three-dimensional image. These image views can resemble those from Scanning Electron Microscopy (SEM), but the specimen need not be in a vacuum (Rochow and Tucker, 1994).

2.8.2 Viable counting procedures

There are two approaches to viable count procedures: the plate count technique and the most probable number (MPN). All viable count procedures require separation of microorganisms into individual reproductive units.

2.8.2.1 Plate counts methods

The agar plate count method has been severely criticised. The problem lies in the misuse of the method and the misinterpretation of the results (Atlas and Bartha, 1993). Plate count methods employ different media and incubation conditions. Dilutions of samples can be spread on the top of the agar (spread method) or the sample suspension can be mixed with the agar just before the plates are poured (pour plate method). One must consider whether the microorganisms can survive the plating procedure. Some microbes are killed upon exposure to air in the spread plate method, whereas others cannot tolerate the temperature needed to maintain melted agar in the pour plate

method (Atlas and Bartha, 1993). The plates are incubated under specific conditions for a period of time to allow the bacteria to multiply and form macroscopic colonies, after which the colonies are counted. Plates with too many colonies cannot be counted accurately because one colony may represent more than one original bacterium. Also, plates with too few colonies must be discarded from the counting procedure for statistical reasons.

2.8.2.2 Most probable numbers (MPN)

The most probable number is an alternative to plate count methods for determination of viable organisms. It uses statistical analyses and successive dilution of the sample to reach a point of extinction. Replicate dilutions are scored as positive or negative, and the pattern of positive and negative scores is used in connection with appropriate statistical tables to obtain the most probable number of viable microorganisms (Atlas and Bartha, 1993). This method has the advantage of permitting the use of liquid culture, avoiding the need to add a solidifying agent such as agar, with its possible contaminants, but it is more laborious and less precise than the plate count (Atlas and Bartha, 1993).

2.8.3 Biochemical assays for estimation of bacterial numbers

Techniques for estimation of the biomass activity consist of biochemical tests to measure either certain specific enzymes or products of the bacterial metabolism. A number of these methods are discussed below.

2.8.3.1 Adenosine triphosphate (ATP)

ATP measurement is the most commonly used method of biomass estimation and was originally proposed by Holm-Hansen and Booth (1966). It is easy to assay, is found as a relatively constant proportion of all living cells and is not present in dead cells (Herbert, 1990). ATP disappears within two hours after cell death and the amount is generally constant (Johnson and Stafford, 1984). ATP has the advantage of being a non-conservative constituent of the living cell, which is directly

related to the energy-growth process. In addition, its concentration remains relatively constant and independent of growth rate in living cells (Nelson and Lawrence, 1980). ATP can be detected by the luciferin-luciferase assay in which reduced luciferin reacts with oxygen to form oxidized luciferin in the presence of the luciferase enzyme (Herbert, 1990; Atlas and Bartha, 1993) magnesium ions and ATP (Atlas and Bartha, 1993). Light is emitted in this reaction in an amount directly proportional to the ATP concentration (Johnson and Stafford, 1984; Atlas and Bartha, 1993). There are some difficulties in the accuracy of estimating microbial biomass based on ATP measurements. Some microbes alter their ATP concentration radically when nutritional or physiological conditions change (Atlas and Bartha, 1993). Also, in some ecosystems, such as soil, sediments and nearshore aquatic areas, ATP may be absorbed on particles.

2.8.3.2 Chlorophyll measurements

Chlorophyll a has been extensively used to estimate biomass in planktonic and benthic microbial communities (Herbert, 1990). Since only algae and cyanobacteria contain chlorophyll a this provides a convenient indicator of oxygenic photosynthesis. Estimation of the biomass of photosynthetic microbes based on chlorophyll determinations has been found to correlate well with such estimates based upon ATP determinations (Atlas and Bartha, 1993).

2.8.3.3 DNA (deoxyribonucleic acid) concentration

DNA may be used to provide information on the total biomass present in particular environments since it is universally present in all living cells (Herbert, 1990). This method cannot differentiate bacterial biomass from algal biomass and therefore can only be used as a general biomass indicator in conjunction with other methods. DNA is synthesized in growing cells at a rate proportional to biomass therefore, the rate of DNA synthesis reflects the growth rate of microbes.

2.8.3.4 Cell wall components

Most bacteria contain muramic acid in their cell walls and the specific relationship between murein and bacteria makes quantitation of this cell wall component useful for estimating bacterial biomass (Atlas and Bartha, 1993). There is a gradient of concentrations of muramic acid in Gram-negative and Gram-positive bacteria. To accurately use this method, it is necessary to estimate the proportions of Gram-negative and Gram-positive in the sample; erroneous estimates of these proportions will yield inaccurate estimate of biomass (Atlas and Bartha, 1993).

2.8.4 Spectrophotometry

Light scattering is the most widely used and at least complex method for estimating total microbiological material in a liquid medium. When light passes through matter, it is scattered apart from its original path by inhomogeneities present. If these inhomogeneities of interest are particles considerably larger than small molecules, scattering becomes relatively intense. Light scattering is dependent upon the concentration, size and shape of the particles, the relative refractive indices of particle and medium and the wavelength of the incident light (Mailette, 1969). Bacteria scatter light primarily in the forward direction and the amount of scattering is proportional to the mass of cells present. Scattering is measured by passing a beam of light through the culture in an instrument containing a photocell that registers the amount of light that is scattered at a 900 angle (turbidity meter) or the amount of light that passes through without being scattered (photometer) (Sebata, 1998). The absorbency measurement routinely employed in microbiology is more related to total bacterial mass than to bacterial numbers (Mailette, 1969). Most spectrophotometers have wavelengths of between 350 and 800 nm and this flexibility is important as different substances absorb light at different wavelengths (bacteria for example absorb most of the light at 540-550 nm) (Sebata, 1998). Spectrophotometers are convenient in this respect and have become the most widely used class of instruments in microbiological turbidimetry. However, several problems may arise, including light absorption, the need for calibration curves, collection of light in low scattering angles and the unwanted changes in the biological material may introduce large uncertainties (Mailette, 1969).

2.8.5 Mixed liquor suspended solids

One parameter which is used in activated sludge to measure biomass is the mixed liquor suspended material. The mixed liquor organic suspended solids are made of three components: heterotrophic active biomass; endogenous residue and inert material. In the nitrifying aerobic and anoxic/aerobic activated sludge systems, a fourth mixed liquor organic suspended solids components included: autotrophic active biomass. The heterotrophic active biomass arises from synthesis of living heterotrophic organisms on biodegradable organic substrates and is "lost" via endogenous respiration/death processes; in the activated sludge system this mixed liquor component performs the biodegradation process of COD removal and denitrification (Ubisi *et al.*, 1997).

The autotrophic active biomass arises from synthesis of autotrophic organisms in the nitrification of ammonia to nitrate under aerobic conditions and is "lost" via endogenous respiration/death processes. The non-biodegradable portion of the heterotrophic and autotrophic active biomass that are lost in the endogenous respiration/death process. The inert material arises from the influent wastewater non-biodegradable particulate organics which, on entry into the bioreactor, are enmeshed in the mixed liquor organic suspended solids. All four mixed liquor suspended solids settle out in the secondary settling tank and are returned to the bioreactor via underflow recycle; these components leave the activated sludge system via the waste flow (Ubisi *et al.*, 1997).

Historically the mixed liquor organic suspended solids have been measured as a lump parameter, via the VSS test (Standard Methods, 1985), or more recently, the COD test. The problem in measurement of this parameter has been the lack of suitable experimental techniques. In the literature, principally microbiological techniques have been proposed; for example, pour plate or other culturing techniques (e.g Gaudy and Gaudy, 1980), ATP analysis (Nelson and Lawrence, 1980; Osborn *et al.*, 1986), DNA analysis (Liebeskind and Dohmann, 1994) using fluorescent probes for ribosomal RNA (Wagner *et al.*, 1994) and sequencing of ribosomal sequencing of ribosomal DNA (Blackall, 1994). However, these techniques have not yet been adequately integrated with the design and kinetic modelling theory; the culturing techniques have been widely criticised for their unreliability (Cloete and Steyn, 1988); the RNA and two DNA methods are still

in their infancy; and the last-named four methods require sophisticated equipment and experimental techniques that are not widely available (Ubisi *et al.*, 1997).

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CHAPTER 3

BIOAUGMENTATION PRODUCTS FOR THE OPTIMIZATION OF PHOSPHORUS REMOVAL IN ACTIVATED SLUDGE

3.1 Abstract

Biological phosphorus removal during the activated sludge wastewater treatment process is an acknowledged phenomenon having gained worldwide support. However, the phenomenon of enhanced phosphate removal by activated sludge systems is not yet fully understood. Research has indicated that an increase in biomass resulted in an increase in phosphorus removal in activated sludge. The aims of the study were to determine the effect of bioaugmentation on phosphorus removal in activated sludge by adding a commercial bioaugmentation product in order to increase biomass and to determine the possibility of culturing a bioaugmentation product in a separate fermentation unit for addition to activated sludge. Two bioaugmentation products were used. Different concentrations of bioaugmentation product A were used as inocula in sterile anaerobic mixed liquor medium. Our results indicated that 18.86 mg.l^{-1} of phosphate was removed per gram of the bioaugmentation product A added when using 10 g of inoculum in 1000 ml of the mixed liquor. At concentrations exceeding 80 g per 1000 ml of mixed liquor, no phosphorus was removed, instead the phosphate concentration increased as a result of the phosphorus content of the bioaugmentation product. Bioaugmentation product A had a high phosphate content, making it unsuitable for bioaugmentation. For determining the possibility of culturing a bioaugmentation product in a separate fermentation unit for addition to activated sludge, the growth of bioaugmentation product B and anaerobic sludge was compared. Different concentrations (2.5 ml and 10 ml) of the bioaugmentation product and anaerobic sludge were inoculated into sterile anaerobic mixed liquor, Nutrient broth and sterile anaerobic mixed liquor with added nutrients (sodium acetate, magnesium sulphate and potassium nitrate), respectively. The experiments were conducted under aerobic and anaerobic conditions at room temperature. No growth was observed in the sterile mixed liquor. Nevertheless growth did occur in Nutrient broth. Nutrient broth had enough nutrients to support the growth of microorganisms but it was

too expensive to use. It was concluded that the products tested would be impractical and too expensive to use for bioagmentation in activated sludge.

3.2 Introduction

The increase in the amount of effluent being disposed to natural water bodies is due to rapid industrialization. Major contaminants found in wastewater include biodegradable, volatile and recalcitrant organic compounds, toxic metals, suspended solids, nutrient (nitrogen and phosphorus), microbial pathogens and parasites (Bitton, 1994). The discharge of nitrogen (as nitrates) and phosphorus (as phosphates) to natural waters causes eutrophication (Lilley *et al.*, 1997). This is marked by large visible blooms of algae, which makes water treatment difficult. Since nitrogen (N) can be fixed by algae, phosphorus (P) is the element that should be considered to minimize eutrophication (Lilley *et al.*, 1997). Phosphorus removal from wastewater is therefore seen as one of the best methods for eutrophication control (Toerien, *et al.*, 1990). According to the Water Act (Act 54 of 1956) the concentration of orthophosphate in purified wastewater is limited to 1 mg.l^{-1}.

Phosphate can be removed from wastewater by chemical or biological methods. Chemical methods are expensive, since they involve the addition of lime, aluminium and ferric chloride. Unfortunately these chemicals become part of the sludge and through additional binding with water, contribute to significant volume increases of the sludge (Grubbs, 1979). Hence, biological phosphate removal has gained support. The activated sludge process was therefore designed and operated for excess biological phosphate removal (Cloete and Bosch, 1993). However, many activated sludge systems fail necessitating chemical addition.

Research has indicated that there were no differences amongst the bacterial community structures of different activated sludge zones or amongst different activated sludge systems (Ehlers 1997; Momba 1995). Hence, phosphorus removal cannot be attributed to the activity of a single population, but rather to the combined activity of all the populations in the microbial community. Momba and Cloete (1996) indicated that an increase in biomass resulted in an increase in phosphorus removal. This raised the question whether a wastewater system treatment

could be bioaugmented in order to increase biomass.

Bioaugmentation involves the use of specially selected and adapted microorganisms for the biodegradation of wastewater and often recalcitrant compounds (Oellermann and Pearce, 1995). The object is not to replace the existing biomass, but to supplement it for improved efficiency. Its use could enhance the degradative potential of indigenous microbial populations to avoid predation, nutrient competition and biomass inactivation.

The application of bioaugmentation in wastewater treatment was originally the result of efforts to solve operational problems, such as shock loads in treatment plants (Oellerman and Pearce, 1995). According to De Haas (1999), results from the commercial application of bioaugmentation products, particularly in wastewater treatment systems facing operational problems, have tended to be positive. However, laboratory research investigations have contradicted these results. A number of bioaugmentation products do not perform as claimed by the suppliers (Oellermann and Pearce, 1995). Cases were reported with little or no advantage of bioaugmentation on the improvement of treatment works (Yu and Hung, 1992) and it was concluded that no significant improvement in process performance could be achieved with bioaugmentation (De Haas, 1999). Our hypothesis was that, phosphate uptake could be improved by the addition of commercially available bioaugmentation products to activated sludge mixed liquor.

The objectives of the study were therefore, to determine the effect of bioaugmentation on phosphorus removal in activated sludge by adding a commercially available bioaugmentation product in order to increase biomass and to determine the possibility of culturing a bioaugmentation product in a separate fermentation unit for addition to activated sludge. The hypothesis of culturing a bioaugmentation product in a separate fermentation unit, was to use an inexpensive substrate to grow biomass in a separate tank as a reserve of biomass which could then serve as inoculum to increase the already existing biomass in the system, as and when necessary.

3.3 Materials and Methods

Rationale of experiments 1 and 2

The purpose of these experiments was to determine the relationship between biomass and phosphate removal and not to determine whether bioaugmentation will be of any practical value.

3.3.1 Bioaugmentation products

Commercially available bioaugmentation products were obtained from SA Biotech and Amitek.

3.3.2 Total plate count

One ml of each bioaugmentation product was serially diluted in sterile Ringer's solution. The Ringer's solution was sterilized by autoclaving at 121^0C for 15 min. The bioaugmentation products were then plated on Nutrient agar plates and incubated at room temperature ($\pm 21^0C$) for 48 h, respectively.

3.3.3 Chemical analysis of the bioaugmentation products

The phosphate (PO_4^{3-}) concentration of the bioaugmentation products was determined on samples filtered through Whatman no. 1 filter papers. The sulphate (SO_4^{2-}), nitrate (NO_3^-) and Chemical Oxygen Demand (COD) concentrations in the bioaugmentation product were determined on unfiltered samples. All the chemical analyses were determined using relevant test kits and an SQ 118 photometer (Merck).

3.3.4 Evaluation of bioaugmentation product A

3.3.4.1 Preparation of the mixed liquor medium

Grab mixed liquor samples (15 l) were collected from the anaerobic zone of the Daspoort wastewater care plant, Pretoria, South Africa. The samples were filtered using Whatman no. 1 filter papers and then the supernatant was autoclaved at $121\ ^0C$ for 1h. After autoclaving, the pH was adjusted to between 6 and 7 with concentrated H_2SO_4. The phosphate concentration of the supernatant was determined and adjusted to 32 mg.l^{-1} with KH_2PO_4.

3.3.4.2 Experimental setup

Sterile anaerobic mixed liquor (500 ml) was suspended in 1 l Erlenmeyer flasks. Each flask was inoculated with different quantities (1 g, 3 g, 4 g, 5 g, 10 g, 20 g, 40 g, 50 g, 80 g and 100 g per 500 ml of mixed liquor) of bioaugmentation product. A control, which was not inoculated, was included. The flasks were stirred using a 6 plate magnetic stirrer (Instrulab) and aerated using aquarium air pumps. Experiments were performed in triplicates at room temperature (22 to 25^0 C). The same experiment was repeated with different concentrations (1 g, 3 g, 4 g and 5 g) of sterile bioaugmentation product inoculated into sterile anaerobic mixed liquor, respectively. Bioaugmentation product was sterilized by autoclaving at 121^0C for 15 min.

3.3.4.3 Phosphate analysis

The phosphate content of the mixed liquor medium was analyzed hourly by taking 10 ml samples from the flasks with a micro-pipette. Samples were filtered through Whatman no.1 filter papers. Phosphate concentration was determined using the P (VM) 14842 test kit and SQ 118 photometer (Merck).

3.3.5 Evaluation of bioaugmentation product B

3.3.5.1 Determination of the growth curve

The following experiments were conducted in order to determine the possibility of growth of the bioaugmentation product in sterile anaerobic mixed liquor medium.

3.3.5.1.1 Experiment 1

Different quantities of bioaugmentation product (2.5 ml and 10 ml) and anaerobic sludge (2.5 ml and 10 ml) were inoculated in side-armed flasks containing sterile anaerobic mixed liquor medium (250 ml and 90 ml) and Nutrient broth (90 ml), respectively. A control, which was not inoculated, was also included. One flask from each set was incubated aerobically and the other anaerobically. The aerobic conditions were created by shaking the flasks on a rotary shaker incubator at 130 rpm. The anaerobic conditions were created by putting the flasks on the bench, without shaking. The flasks were incubated at room temperature ($\pm30^0$C). The experiments were conducted in duplicate. The absorbency was measured every 30 min at the wavelength of

590 nm using a spectrophotometer.

3.3.5.1.2 Experiment 2

Sterile anaerobic mixed liquor (90 ml) was suspended in each of the 5 side-armed flasks. The solutions to be added in the sterile mixed were prepared separately. For the first solution, 1.25 g Na-acetate was added into 250 ml mixed liquor, in a Schott bottle. The second solution contained 2.5 g sodium-acetate in 250 ml mixed liquor. The third solution contained 1.25 g Na-acetate, 0.125 g $MgSO_4.7H_2O$ and 0.045 g KNO_3 in 250 ml mixed liquor and the fourth solution was prepared by adding 2.5 g Na-acetate, 0.25 g $MgSO_4.7H_2O$ and 0.09 g KNO_3 g into 250 ml mixed liquor. All solutions were prepared in 500 ml Schott bottles respectively and sterilized by autoclaving at 121^0C for 15 min. The bioaugmentation product (10 ml) was inoculated into each of the 4 side-armed flasks and the last side-armed flask was not inoculated and served as a control. The experiment was done in duplicate. The flasks were shaken on a rotary shake incubator at the speed of 130 rpm at room temperature. Other flasks were incubated anaerobically. The absorbency was measured every 30 min at a wavelength of 590 nm using a spectrophotometer.

3.4 Results

3.4.1 Chemical and microbiological analyses

Table 3.1: Chemical and microbiological analyses of the bioaugmentation products.

Bioaugmentation product	COD (mg.l^{-1})	Phosphate (mg.l^{-1})	Sulphate (mg.l^{-1})	Nitrate (mg.l^{-1})	TPC (cfu.ml^{-1})
A	14 542	107.6	39	50.7	1.22×10^6
B	*ND	87.5	ND	43	9.0×10^6

*ND - Not Determined

The high COD, phosphate, sulphate and nitrate concentrations served as nutrients for sustaining the microorganisms in the product.

3.4.2 Evaluation of bioaugmentation product A

When using 2 g of the bioaugmentation product per 1 000 ml of the mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 39.40 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 37.20 mg.l^{-1} after 9 h (Figure 3.1). The amount of phosphate removed per gram of bioaugmentation product added was 2.20 mg.l^{-1} (Table 3.2).
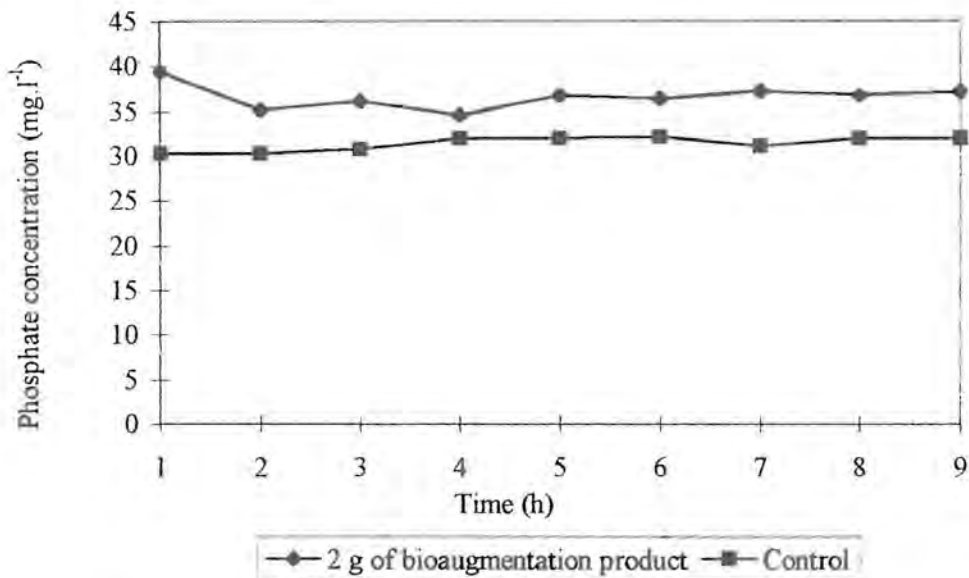

Figure 3.1: **Phosphate removal when 2 g of bioaugmentation product per 1 000 ml of mixed liquor was used.**

When using 6 g of the bioaugmentation product per 1 000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 82.40 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 56.50 mg.l^{-1} after 9 h (Figure 3.2). The amount of phosphate removed per gram of bioaugmentation product added was 8.60 mg.l^{-1} (Table 3.2).

When using 8 g of the bioaugmentation product per 1 000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 111.50 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 39.90 mg.l^{-1} after

9 h (Figure 3.2). The amount of phosphate removed per gram of bioaugmentation product added was 17.90 mg.l^{-1} (Table 3.2).

Table 2: **Phosphate removed per gram of the bioaugmentation product added.**

Bioaugmentation product added per 1 000 ml of mixed liquor	Phosphate (P) concentration (mg.l^{-1}) T = 0 h	Phosphate (P) concentration (mg.l^{-1}) T = 9 h	Change in phosphate concentration, ΔP^* (mg.l^{-1})	Phosphate removed per gram of bioaugmentation product added*** (mg.l^{-1})
Control	30.00	30.30	0.00	-
2 g.l^{-1}	39.40	37.20	2.20	2.20
6 g.l^{-1}	82.40	56.50	25.90	8.60
8 g.l^{-1}	111.50	39.90	71.60	17.90
10 g.l^{-1}	156.10	61.80	94.30	18.86
20 g.l^{-1}	183.34	49.42	133.92	13.40
40 g.l^{-1}	283.17	95.72	187.45	9.370
80 g.l^{-1}	186.65	338.00	-151.35**	-3.78**
100 g.l^{-1}	306.92	751.50	-444.58**	-8.89**
160 g.l^{-1}	949.90	1129.90	-180.00**	-2.25**
200 g.l^{-1}	946.60	834.15	112.45	1.124

*ΔP = (P concentration)$_{T9}$ – (P concentration)$_{T0}$, **Phosphate release

***ΔP/gram(s) of the bioaugmentation product added

When using 10 g of the bioaugmentation product per 1000 ml mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 156.10 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 61.80 mg.l^{-1} after 9 h (Figure 3.2). The amount of phosphate removed per gram of the bioaugmentation product added was 18.86 mg.l^{-1} (Table 3.2).
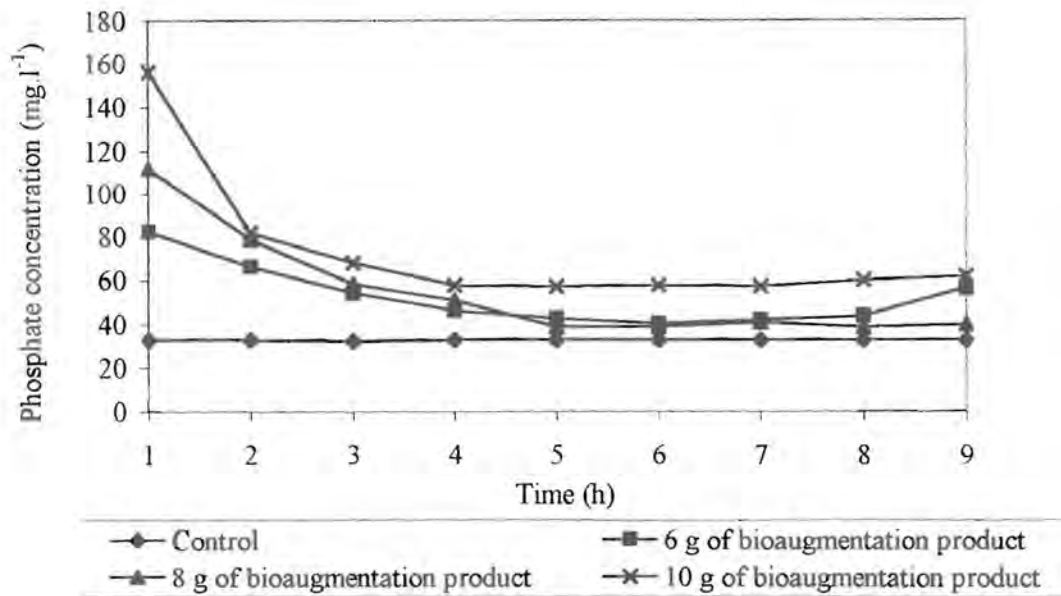
Figure 3.2: **Phosphate removal when 6 g, 8 g and 10 g of bioaugmentation product per 1000 ml of mixed liquor were used.**

When using 20 g of the bioaugmentation product per 1000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 183.34 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 49.42 mg.l^{-1} after 9 h (Figure 3.3). The amount of phosphate removed per gram of the bioaugmentation product added was 13.41mg.l^{-1} (Table 3.2).

When using 40 g of the bioaugmentation product per 1000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 283.17 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 95.72 mg.l^{-1} after 9 h (Figure 3.3). The amount of phosphate removed per gram of the bioaugmentation product added was 9.46 mg.l^{-1} (Table 3.2).

When using 80 g of the bioaugmentation product per 1000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 186.65 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration increased to 338.00 mg.l^{-1} after 9 h (Figure 3.4).
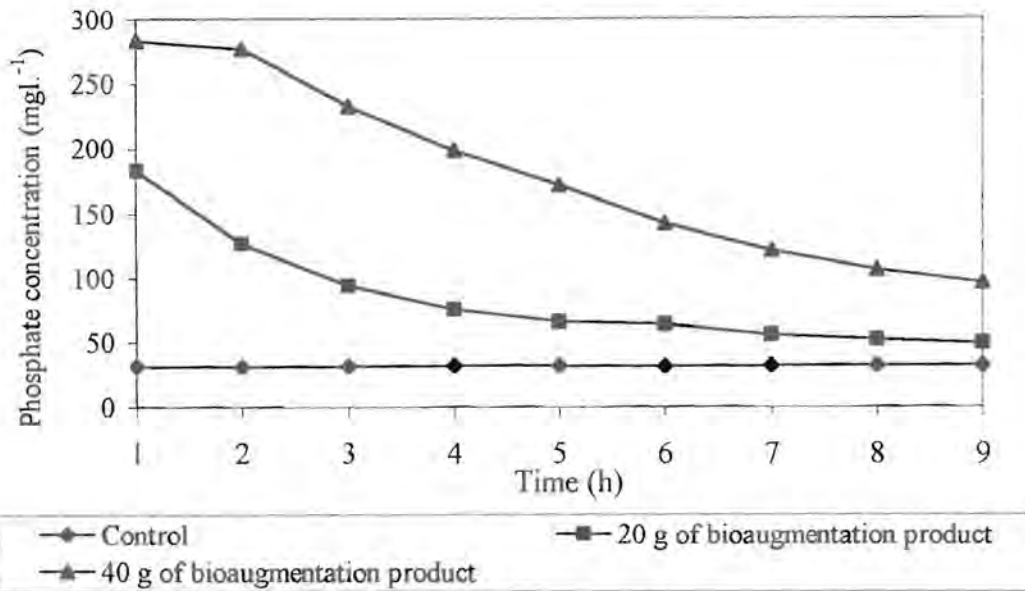
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Figure 3.3: **Phosphate removal when 20 g and 40 g of bioaugmentation product per 1000 ml of mixed liquor were used.**

When using 100 g of the bioaugmentation product per 1000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 306.92 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration increased to 751.5 mg.l^{-1} after 9 h (Figure 3.4).

When using 160 g of the bioaugmentation product per 1000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 949.90 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration increased to 1129.90 mg.l^{-1} after 9 h (Figure 3.4).

When using 200 g of the biosupplement per 1000 ml of mixed liquor, the phosphate concentration increased from 30.00 mg.l^{-1} to 946.60 mg.l^{-1} after the addition of the bioaugmentation product (time 0 h). The phosphate concentration decreased to 834.15 mg.l^{-1} after 9 h (Figure 3.4). The amount of phosphate removed per gram of the bioaugmentation product added was 1.124 mg.l^{-1} (Table 3.2).
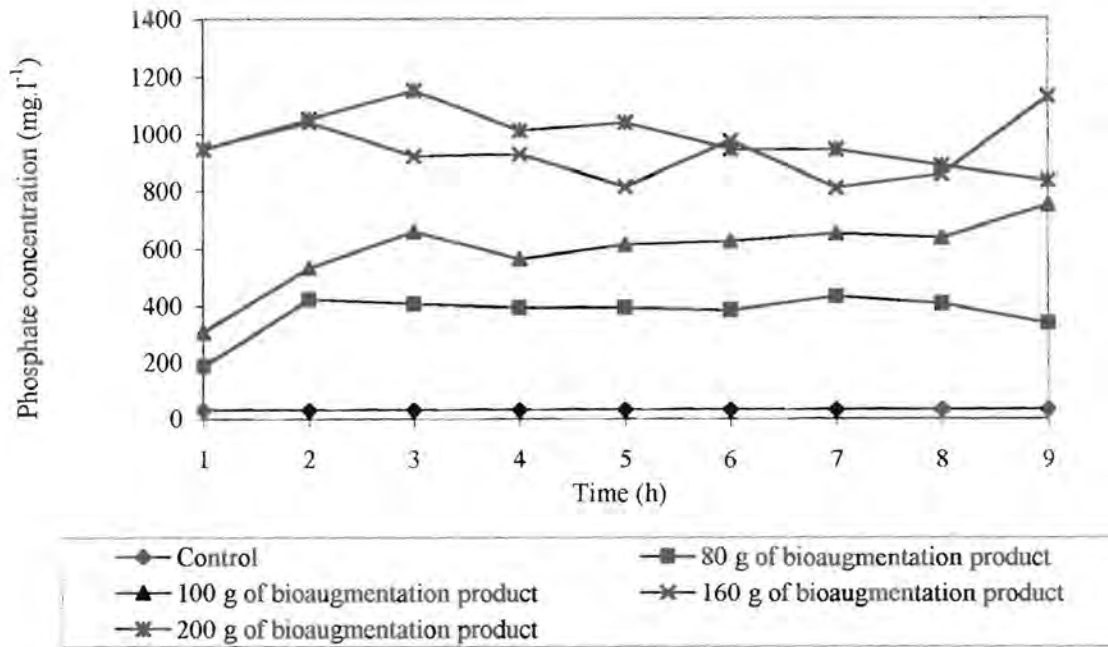
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Figure 3.4: **Phosphate removal when 80 g, 100 g, 160 g and 200 g of bioaugmentation product per 1000 ml of mixed liquor were used.**

When sterile bioaugmentation product was used as inoculum, for 2 g per 1000 ml, initially, the phosphate concentration was 32 mg.l^{-1} and after 9 h it remained 32 mg.l^{-1} (Figure 3.5). When using 6 g of the sterile biosupplement per 1000 ml of mixed liquor, phosphate concentration increased from 32 mg.l^{-1} to 35.2 mg.l^{-1} after the addition of the product (time 0 h). The phosphate concentration increased to 43.3 mg.l^{-1} after 9 h (Figure 3.5). The amount of phosphate released per gram of the bioaugmentation product added was 8.1 mg.l^{-1} (Table 3.3).

When using 8 g and 10 g of the sterile bioaugmentation product per 1000 ml of the mixed liquor, phosphate concentration increased from 32 mg.l^{-1} to 35.6 mg.l^{-1} and 37.6 mg.l^{-1} after the addition of the bioaugmentation product, respectively. The phosphate concentration increased to 40.3 mg.l^{-1} and 43.3 mg.l^{-1}, respectively, after 9 h (Figure 3.5). The amount of phosphate released per gram of the bioaugmentation product added was 4.7 mg.l^{-1} and 5.7 mg.l^{-1}, respectively (Table 3.3).
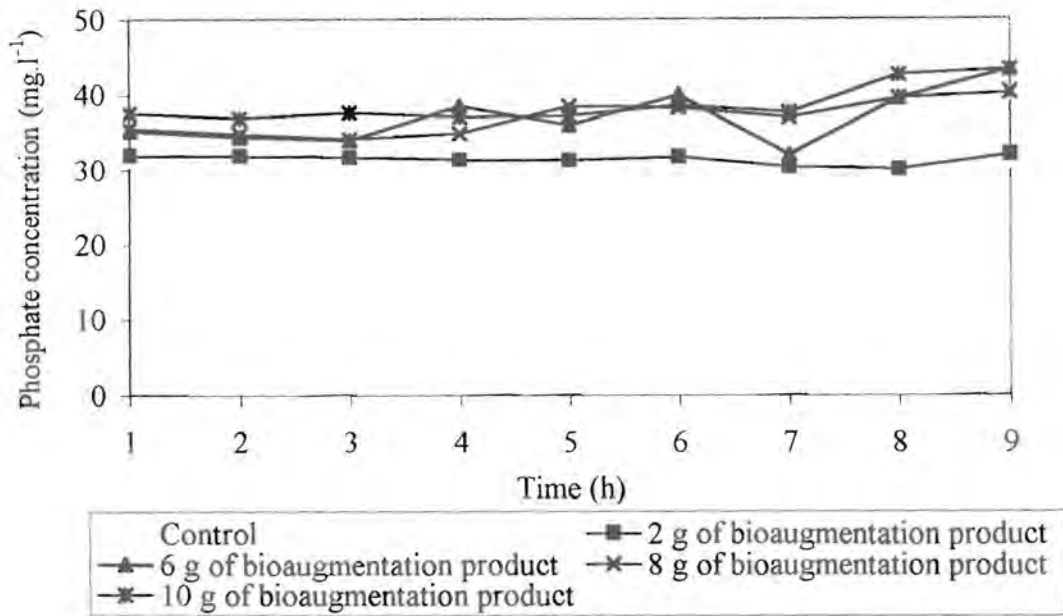
Figure 3.5: **Phosphate removal when 2 g, 6 g, 8 g and 10 g of sterile bioaugmentation product per 1000 ml of mixed liquor were used.**

Table 3.3: **Phosphate removed per gram of the sterile bioaugmentation product added.**

Bioaugmentation product added per 1000 ml of mixed liquor	Phosphate (P) concentration (mg.l^{-1}) T = 0 h	Phosphate (P) concentration (mg.l^{-1}) T = 9 h	Change in phosphate concentration, $\Delta P*$ (mg.l^{-1})	Phosphate removed per gram of bioaugmentation product added*** (mg.l^{-1})
2g.l^{-1}	32.00	32.00	0.00	0.00
6g.l^{-1}	35.20	43.30	8.10	2.70
8g.l^{-1}	35.60	40.30	4.70	1.175
10g.l^{-1}	37.60	43.30	5.70	1.14
Control	31.30	31.00	0.30	-

*ΔP = (P concentration)$_{T9}$ − (P concentration)$_{T0}$, ***ΔP/gram(s) of the bioaugmentation product added

The sterile bioaugmentation product did not remove phosphate.

3.4.3 Evaluation of bioaugmentation product B

When 2.5 ml of bioaugmentation product and anaerobic sludge were inoculated in 250 ml mixed liquor, to determine the growth curve of the product, there was no increase in the absorbance readings. The absorbance reading of the medium under aerobic conditions of the bioaugmentation product was initially 0.20 and decreased to 0.03 and that under anaerobic conditions was 0.20 and decreased to 0.02. The absorbance reading of the medium under aerobic conditions inoculated with anaerobic sludge was initially 0.04 and increased to 0.06. The medium under anaerobic conditions indicated the absorbance reading of 0.14 and decreased to 0.12 (Figure 3.6).
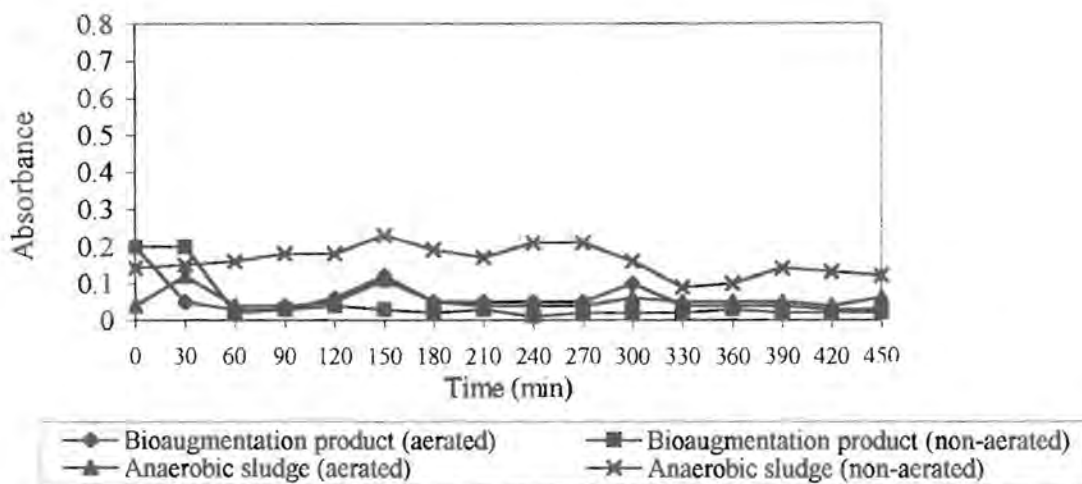

Figure 3.6: **The growth of bioaugmentation product (2.5 ml) and anaerobic sludge (2.5 ml) inoculated in 250 ml sterile mixed liquor, during aerobic and anaerobic conditions.**

When using 10 ml of the bioaugmentation product and anaerobic sludge per 90 ml of mixed liquor, there was no increase in the absorbance readings, that is, the readings remained the same. The absorbance reading of the medium under aerobic conditions, inoculated with the bioaugmentation product, was initially 0.12 and increased to 0.16 after 24 h and under anaerobic conditions the initial absorbance was 0.06 and decreased to 0.05 after 24 h. In the medium inoculated with anaerobic sludge, for aerobic conditions, the absorbance reading was initially 0.12 and decreased to 0.00 after 24 h and for anaerobic conditions, the absorbance reading was 0.17 and decreased to 0.02 after 24 h (Figure 3.7).
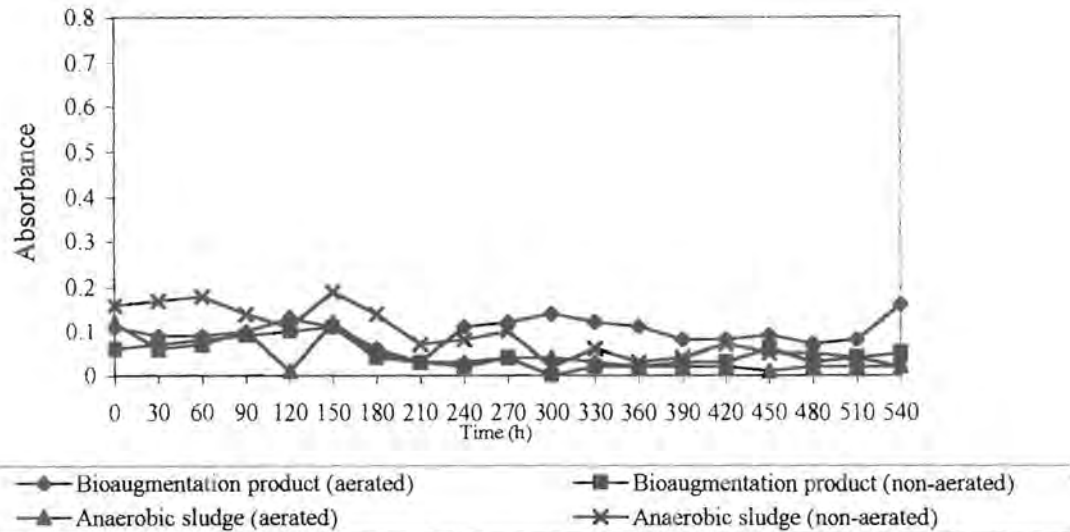
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Figure 3.7: **The growth of bioaugmentation product (10 ml) and anaerobic sludge (10 ml) inoculated in 90 ml of sterile mixed liquor, during aerobic and anaerobic conditions.**

The absorbance readings of the inoculation of the bioaugmentation product and the anaerobic sludge in 90 ml of Nutrient broth showed an increase. The absorbance readings of the medium incubated under aerobic conditions, inoculated with the bioaugmentation product, were initially 0.04 and increased to 2.00 after 24 h and for anaerobic conditions medium, the absorbance reading was initially 0.03 and increased to 0.76 after 24 h. The absorbance reading for the medium inoculated with the anaerobic sludge, incubated under aerobic conditions, was 0.56 and it increased to 2.00 after 24 h. During anaerobic conditions, the absorbance was 0.55 and it increased to 1.35 after 24 h (Figure 3.8).

There was no growth of the microorganisms in the medium when using 10 ml of the bioaugmentation product per 90 ml of the mixed liquor with added nutrients (see materials and methods).
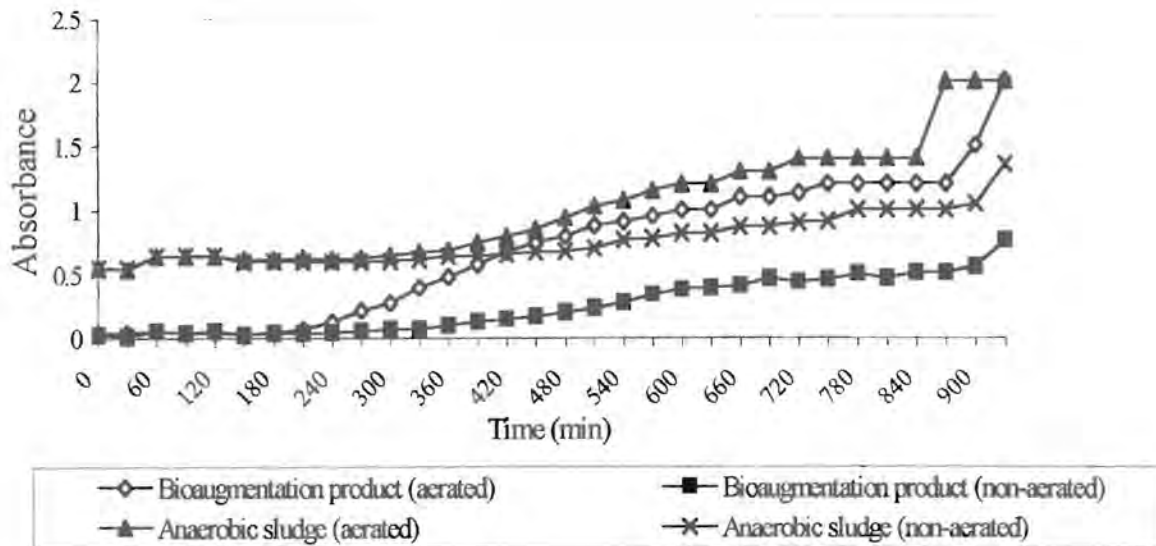
Figure 3.8: The growth of bioaugmentation product (10 ml) and anaerobic sludge (10 ml) inoculated in 90 ml Nutrient broth, during aerobic and anaerobic conditions.

3.5   Discussion and Conclusion

Bioaugmentation products A and B contained phosphate. This increased the phosphate concentration of the mixed liquor in all the experiments upon addition. Initially, the phosphate removal increased proportional to the quantity of bioaugmentation product added. Eight g and ten g of bioaugmentation product per 1000 ml of mixed liquor resulted in 17.90 mg.l^{-1} and 18.86 mg.l^{-1} phosphate removal per g of bioaugmentation product added, respectively. Hereafter, the rate of phosphate removal per g of bioaugmentation product decreased. This could be ascribed to a number of factors. The first factor could be the presence of organic matter which was introduced by the bioaugmentation product which contained 14 542 mg.l^{-1} COD. Previous studies have indicated that the presence of organic carbon matter would inhibit phosphate removal (Bosch and Cloete, 1993). The second limiting factor could be the presence of nitrate, also introduced during bioaugmentation. The bioaugmentation product A contained 39 mg.l^{-1}

nitrate. It is known that nitrate limits phosphate uptake in activated sludge (Barnard, 1976). Oxygen limitations could also have occurred limiting phosphate uptake in the experiments, due to the elevated organic carbon content as previously discussed. From our results, it became clear that there was a relationship between phosphate uptake and the quantity of biougmentation product added up to a point where other factors became limiting.

Since 8 g and 10 g per 1000 ml of mixed liquor removed phosphorus, full-scale applications will need between 8 kg and 10 kg of bioaugmentation product per 1000 liters to remove phosphorus from the activated sludge. Even at these concentrations, the limit of 1 $mg.l^{-1}$ phosphate was never reached. Full-scale applications of the bioaugmentation product would be impractical and too expensive. It was therefore concluded that the bioaugmentation product evaluated was unsuitable for use in activated sludge systems.

When determining the possibility of culturing a bioaugmentation product B and anaerobic sludge in sterile mixed liquor medium, as reserve biomass, no growth of the microorganisms was observed in the sterile mixed liquor and sterile mixed liquor with added nutrients. Growth only occurred in Nutrient broth. The hypothesis was to use an inexpensive substrate to grow this product in separate tanks to have a reserve of biomass, which could then serve as biomass inoculum to increase the already existing biomass in the system when necessary. The results indicated that there were not enough nutrients in the sterile mixed liquor to support the growth of the microorganisms of the bioaugmentation product, or it was not suitable for their growth. In order for this approach to be successful, alternative inexpensive substrates will have to be evaluated in order to increase biomass.

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CHAPTER 4

PHOSPHATE REMOVAL CAPACITY OF AEROBIC, ANAEROBIC AND RETURN SLUDGE MLSS

4.1   Abstract

It has been reported that biological phosphorus removal (BPR) often fails. Various reasons for the failure of activated sludge systems have been reported. Biomass depletion has been suggested as one of the reasons for system failure in BPR. The role of biomass in biological excess phosphate removal from activated sludge is widely accepted, though its role is not yet clearly understood. To better understand how activated sludge systems remove phosphate five sets of experiments were conducted. In the first experiment (40 g.l^{-1}, 80 g.l^{-1}, 120 g.l^{-1} and 160 g.l^{-1}) and (33 g.l^{-1}, 66 g.l^{-1}, 100 g.l^{-1} and 133 g.l^{-1}) aerobic sludge mass was used as inocula in sterile mixed liquor containing 32 mg.l^{-1} and 100 mg.l^{-1}PO$_4^{3-}$ respectively. Also 100 g.l^{-1} and 133 g.l^{-1} aerobic sludge mass was used in sterile mixed liquor containing 200 mg.l^{-1} PO$_4^{3-}$. In the second experiment, 33 g.l^{-1}, 66 g.l^{-1}, 100 g.l^{-1} and 133 g.l^{-1} of anaerobic sludge mass was used in sterile mixed liquor containing 200 mg.l^{-1} PO$_4^{3-}$. In the third experiment, 33 g.l^{-1}, 66 g.l^{-1}, 100 g.l^{-1} and 133 g.l^{-1} of return sludge mass was used in sterile mixed liquor containing 200 mg.l^{-1} PO$_4^{3-}$. The fourth and the fifth experiments were done simulating the actual MLSS of the aerobic, anaerobic and return sludge. When using aerobic sludge mass in mixed liquor at a concentration of 32 mg.l^{-1} PO$_4^{3-}$, all the phosphate was removed within 6 h for all the concentrations. This indicated that the sludge to PO$_4^{3-}$ ratio was too high to determine the relationship between the sludge PO$_4^{3-}$ removal. When using aerobic sludge mass from the same system at different concentrations of mixed liquor media containing 100 mg.l^{-1} PO$_4^{3-}$ and 200 mg.l^{-1} PO$_4^{3-}$, sludge concentration was linearly related to PO$_4^{3-}$ removal. However, there was no difference in the removal of phosphate by different quantities of anaerobic and return sludge mass from the same system. When simulating the actual MLSS concentration in a specific plant, in the first experiment, no difference could be detected between the phosphate removal capacity

of aerobic and anaerobic sludge although the use of return sludge resulted in a higher phosphate removal when compared to aerobic and anaerobic sludge. In the second simulation experiment, the anaerobic sludge indicated variable results. However, the return sludge performed consistently well at phosphate removal than the aerobic and anaerobic sludges. The return and the anaerobic sludges were more effective than aerobic sludge.

4.2 Introduction

Activated sludge is a widely used process for the biological removal of phosphorus. Biological nutrient removal often fails to meet the required legislated levels (Osborn *et al.*, 1986; Oldham *et al.*, 1994). Some of the known factors contributing to system failure include shortage of volatile fatty acids as carbon sources, nitrate feedback and accumulation of hydrogen sulfide. For these reasons, attempts have been made to redress system failure. Biomass depletion has been suggested as a parameter that can contribute towards system failure in biological phosphate removal (Momba, 1995; Muyima, 1995).

One approach in redressing system failure has focused on understanding the role of the microbial community structure in relation to phosphate (P) removal (Erasmus, 1997; Ehlers, 1998). Research has indicated that no microbial population differences could be observed between P removing and non-P removing activated sludge systems (Ehlers, 1998). The protein profiles also indicated that microbial communities from the same activated sludge system did not differ from one zone to the other (Erasmus, 1997; Ehlers, 1998). This suggested that P removal was not the function of a specific microbial community, but rather a lack of sufficient biomass of polyphosphate accumulating organisms.

The involvement of biomass in activated sludge is widely accepted, though its role is not yet clearly understood (Momba and Cloete, 1996). Bosch (1992) and Muyima (1995) have all indicated that biomass is related to P removal. This suggested that the main difference between P removing and non-P removing systems were biomass related and not due to the microbial community structure. It is thus possible that in certain instances the failure for biological P removal could be biomass related.

The objectives of the study were to evaluate the anaerobic sludge, return sludge and aerobic sludge as supplements and to determine the phosphate removal capacity of a system based on wet sludge mass and also MLSS concentrations similar to that found in full-scale plants.

4.3 Materials and Methods

The purpose of the experiments was to determine the relationship between biomass and phosphate removal using aerobic, anaerobic and return sludge mass as supplements.

4.3.1 Samples

Grab mixed liquor samples of aerobic (10 l), anaerobic (20 l) and return (10 l) sludge were collected from the Daspoort wastewater treatment plant in Pretoria, South Africa. The total plate count, mixed liquor suspended solid (MLSS), chemical oxygen demand (COD), mixed liquor volatile suspended solids (MLVSS), phosphate, nitrate and ammonium concentration and pH were performed immediately after collection, for experiment 4. The samples were stored at 4 ^{0}C overnight for further use.

4.3.2 Preparation of the mixed liquor medium

The same procedure for preparation of the growth medium was followed as described in section 3.3.4.1. However, the following changes were made:

The filtrate was adjusted to 32 mg.l $^{-1}$, 100 mg.l^{-1} and 200 mg.l^{-1} for experiment 1 (aerobic sludge) and 200 mg.l^{-1} for experiment 2 (anaerobic sludge) and for experiment 3 (return sludge) by the addition of KH_2PO_4, respectively.

4.3.3 Inoculum

Grab mixed liquor samples of aerobic sludge (for experiment 1), anaerobic sludge (for experiment 2), return sludge (for experiment 3) and the experiment simulating the actual MLSS of the plant (experiment 4 and 5) were centrifuged at 3000 rpm for 20 min using a Beckman Model J 6 Centrifuge, respectively. For the sludge mass experiments, specific quantities of concentrated sludge were weighed. For the experiment simulating the actual MLSS of the

system, the sludge was concentrated as follows: for half the sludge concentration, 500 ml of the aerobic sludge was used. For the standard sludge concentration, 1 l was concentrated and for double the sludge concentration, 2 l of the sludge was concentrated. The aerobic, anaerobic and return pellets were then put in 1 l glass beakers, respectively. To induce phosphate uptake, the pellets were stored at 4 ^{0}C overnight for anaerobic/microaerophilic conditions to develop.

4.3.4 Experimental setup and running

4.3.4.1 Experiments with sludge mass

The prepared sludge pellets, which were stored overnight were weighed off into specific concentrations into sterile 1 l Erlenmeyer flasks for the sludge mass experiments. For experiment 1, the sterile mixed liquor medium (500 ml) containing 32 mg.l^{-1} phosphate concentration was dispensed into 5 l litre flasks containing 20 g, 40 g, 60 g and 80 g of aerobic sludge pellets. A control, which was not inoculated, was also included. The same experimental procedure was repeated using aerobic sludge pellets (20 g, 40 g, 60 g and 80 g) and (60 g and 80 g) per 600 ml of sterile mixed liquor containing 100 mg.l^{-1} and 200 mg.l^{-1} phosphate concentration, respectively. The experiments were conducted in triplicate. For experiment 2 (anaerobic sludge), the sterile mixed liquor (600 ml) containing 200 mg.l^{-1} of phosphate was dispensed into 5 1 l Erlenmeyer flasks containing 20 g, 40 g, 60 g and 80 g of sludge mass. A control, which was not inoculated, was included. The experiment was done in triplicate. For experiment 3 (return sludge), the sterile mixed liquor medium (600 ml) containing 200 mg.l^{-1} of phosphate was dispensed into 5 1 l Erlenmeyer flasks containing 20 g, 40 g, 60 g and 80g of sludge mass. A control was also included. The flasks were constantly shaken on a Labotec shaker at room temperature, respectively. In experiment 1, where aerobic sludge was used per 500 ml of mixed liquor medium containing 32 mg.l^{-1} phosphate, the mixed liquor was aerated by means of aquarium pump (Elite 802). Samples (6 flasks) were constantly shaken by the use of a six-plate magnetic stirrer device (Instrulab). In the experiment where aerobic sludge per 600 ml of mixed liquor medium containing 100 mg.l^{-1} and 200 mg.l^{-1}, mixed liquor was aerated by means of a compressor-vacuum pump (Edwards E.B.3). In other experiments, the mixed liquor media were not aerated but shaken on a Labotec shaker (Figure 4.1).

4.3.4.2 Experiment with MLSS concentrations simulating full-scale MLSS concentrations.

In the experiment simulating the actual MLSS of the plant, the half, standard and double mass of the aerobic, anaerobic and return sludge was inoculated in 3 1 l Erlenmeyer flasks containing sterile mixed liquor (600 ml) containing 32 mg.l^{-1} phosphate concentration, respectively. A control was also included. The experiment was done in duplicate. The flasks were constantly shaken on a Labotec shaker at room temperature, respectively (Figure 4.2).

4.3.5 Total Bacterial Count

Immediately after inoculation, 0.1 ml of sample was extracted from each flask. The total bacterial count was determined on the original sample, at time 0 h and again at time 8 h as described in section 3.3.2. The bacterial count was also determined using the ATP bioprobe (Merck) for aerobic sludge.

4.3.6 Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS)

Mixed liquor and volatile suspended solids were determined using standard methods (Standard Methods, 1995). This was conducted on the original sample, at time 0 h and time 8 h.

4.3.7 Oxygen concentration

Oxygen concentration was determined at time 0 h and time 8 h of the experiment using an oxygen probe (Oxygen meter YSI Model 54).

Preparation of the sterile mixed liquor medium   **Preparation of the inoculum**

Sampling - anaerobic zone of Daspoort

Sampling - aerobic, anaerobic and
return zones - Daspoort

Settling of the sludge

Centrifugation (3000 rpm, 20 min)

Filtration of clear liquid with

Whatman no. 1 filter papers

Weigh off specific concentration of Sludge mass into 1 l Erlenmeyer
flasks

Sterilize the supernatant by autoclaving (121 ^{0}C for 1 h)

Store at 4 ^{0}C overnight

Adjust pH (conc. H_2SO_4) and PO_4^{3-} (KH_2PO_4)

For experiment 1 (32 mg.l^{-1}, 100 mg.l^{-1} and 200 mg.l^{-1})

For experiment 2 (200 mg.l^{-1})

For experiment 3 (200 mg.l^{-1})

Dispense into flasks containing the pellets

Microbiological and physico-chemical analyses (before the start of the
experiment)

Shaking and aeration

Phosphate analysis (every hour)

Microbiological and physico-chemical analyses (T = 8 h)

Figure 4.1:   **Schematic representation of the experimental protocol used in the constant
biomass experiments.**

Preparation of the mixed liquor medium

Sampling - anaerobic zone Daspoort

↓

Settling of the sludge

↓

Vacuum filtration of the clear liquid

↓

Sterilize the supernatant by autoclaving (121 ^{0}C for 1 h)

↓

Adjust the pH (conc. H_2SO_4) and PO_4^{3-} (KH_2PO_4)

↓

Dispense into flasks containing the pellets

↓

Microbiological and physico-chemical analyses (before the start of the experiment, T = 0)

Preparation of the inoculum

Sampling - aerobic, anaerobic and return sludges Daspoort

↓

Microbiological analyses: Total plate count

Physico-chemical analyses: COD, MLVSS, MLSS, pH, PO_4^{3-}, NH_4^+, NO_3^-

↓

Concentration of the pellets, aerobic, anaerobic and return sludge (3000 rpm, 20 min):

For 1/2 x concentration: 500 ml was used,

For standard concentration: 1 l was used

For 2 x concentration: 2 l was used

↓

Weigh off sludge mass into sterile 1 l Erlenmeyer flasks

↓

Store at 4 ^{0}C overnight

↓

Shaking and aeration

↓

Phosphate analysis (every hour)

Microbiological and physico-chemical analyses (T = 8)

Figure 4.2: **Schematic representation of the experimental protocol used in the biomass simulation experiments.**

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4.3.8 Phosphate removal studies

The same procedure was followed as described in section 3.3.4.3. The first samples were drawn at time 0 h (i.e. prior to aeration and shaking) and also at time 8 h (before termination of the experiment) as well as hourly during the experiment. The phosphate concentration was also determined on the original sample prior preparation of the experiment.

4.3.9 Chemical analysis

The same procedure was followed as described in section 3.3.3. The chemical analyses were determined on the original sample, at time 0 h and time 8 h.

4.3.10 pH

pH of the mixed liquor media was determined on the original sample, at time 0 h and time 8 h. The pH was determined by a Beckman Φ34 pH meter and a relevant probe.

4.4 Results and Discussion

4.4.1 Experiments with sludge mass

4.4.1.1 Experiment 1 (Aerobic sludge)

In experiment 1, where an average of 32 mg.l^{-1} PO_4^{3-} was used in the mixed liquor medium, the inoculum led to the removal of all the PO_4^{3-} within relatively short period of time ($\pm$ 3 h). In some cases (80 g.l^{-1}, 120 g.l^{-1} and 160 g.l^{-1} of sludge mass), PO_4^{3-} removal was in less than 1 hour (Figure 4.3). No difference could be detected between 40 g.l^{-1}, 80 g.l^{-1}, 120 g.l^{-1}, and 160 g.l^{-1} sludge mass after 3 h. Hence, no quantitative conclusion could be made with regards to sludge mass and its relationship to PO_4^{3-} removal excepting that the PO_4^{3-} concentration was too low in relation to the sludge mass. Hence the PO_4^{3-} concentration was increased in subsequent experiments.
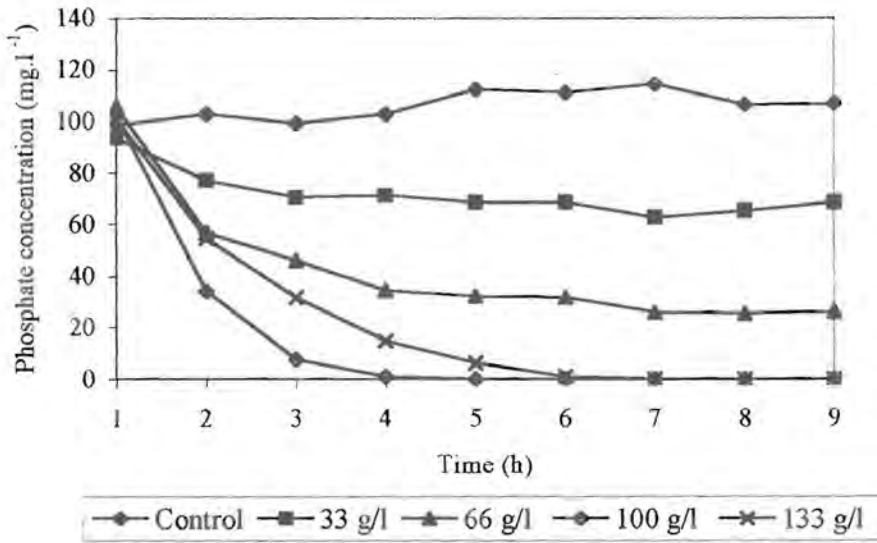
Figure 4.3: Phosphate removal by 40 g.l⁻¹, 80 g.l⁻¹, 120 g.l⁻¹ and 160 g.l⁻¹ of aerobic sludge mass in sterile mixed liquor containing 32 mg.l⁻¹.
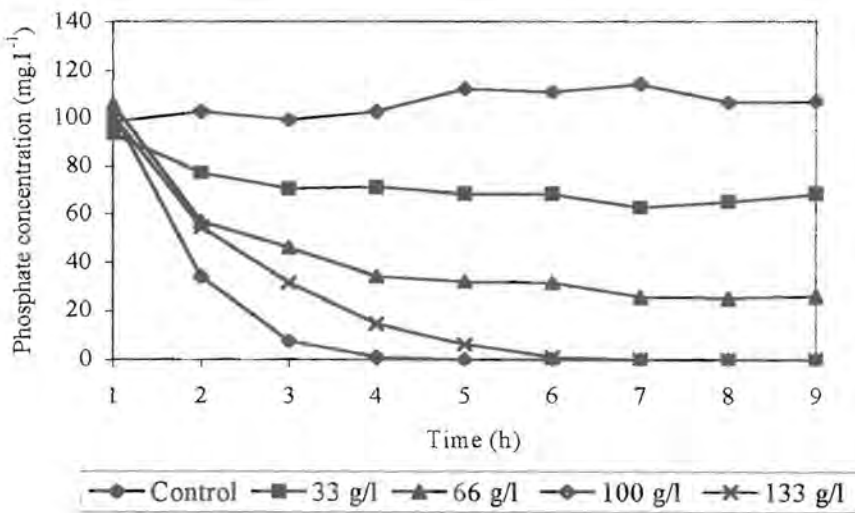

Figure 4.4: Phosphate removal by 33 g.l⁻¹, 66 g.l⁻¹, 100 g.l⁻¹ and 133 g.l⁻¹ of aerobic sludge mass in sterile mixed liquor containing 100 mg.l⁻¹ P.

When different quantities (33 g.l^{-1}, 66 g.l^{-1}, 100 g.l^{-1} and 133 g.l^{-1}) of aerobic sludge mass in sterile mixed liquor containing 100 mg.l^{-1} of phosphate was used, the results indicated that 100 g.l^{-1} and 133 g.l^{-1} of sludge mass removed all the phosphate within 4 h and 6 h respectively. There was also an increase in phosphate removal as the sludge mass increased (Figure 4.4). It was decided to repeat the experiment for the 100 g.l^{-1} and 133 g.l^{-1} of sludge mass, using a higher phosphate concentration (200 mg.l^{-1}).
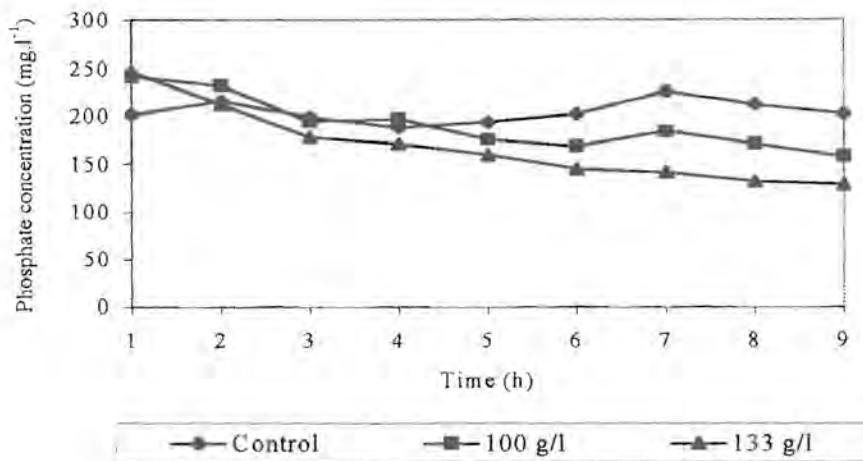

Figure 4.5: **Phosphate removal by 100 g.l^{-1} and 133 g.l^{-1} of aerobic sludge mass in sterile mixed liquor containing 200 mg.l^{-1} P.**

The 133 g.l^{-1} of aerobic sludge mass removed more phosphate than the 100 g.l^{-1} (Figure 4.5). These results also indicated that an increase in sludge mass resulted in increase in phosphate removal.

The increase in PO$_4$$^{3-}$ removal with an increase in aerobic sludge mass indicated a direct relationship between sludge mass and PO$_4$$^{3-}$ removal from mixed liquor (Figures 4.4 and 4.5). In terms of phosphate removed per gram of sludge mass, values were in the range of 1.25 mg PO$_4$$^{3-}$ .g^{-1} of sludge to 1.99 mg PO$_4$$^{3-}$.g^{-1} of sludge (Table 4.1). However, in terms of PO$_4$$^{3-}$ removed per gram of initial MLSS, a larger range of values was observed (10.69 mg PO$_4$$^{3-}$.g^{-1} sludge to 19.91 mg PO$_4$$^{3-}$.g^{-1}sludge). In the experiment with 100 mg.l^{-1} phosphate, the optimum sludge

concentration of 66 g.l^{-1} was indicated (Table 4.1). For experiment pertaining 200 mg.l^{-1} phosphate, an increase in phosphate removal was observed for an increase in sludge mass (Table 4.1). Although the optimum sludge concentrations could not be determined.

Table 4.1: Phosphate removal by 40 g.l^{-1}, 80 g.l^{-1}, 120 g.l^{-1} and 160 g.l^{-1} of aerobic sludge mass in sterile mixed liquor containing 100 and 200 mg.l^{-1} phosphate concentration.

$[PO_4^{3-}]$ (mg.l^{-1})	Wet Sludge mass (g.l^{-1})	PO_4^{3-} (mg.l^{-1}) T = 0 h	T = 8 h	PO_4^{3-} removed (mg.l^{-1})	$\ast PO_4^{3-}$ removed per g of sludge (mg.g^{-1})	PO_4^{3-} Removed per initial MLSS (mg.g^{-1})
100	Control	98.66	107.00	-8.34	-	-83.4
	33	93.33	68.33	25.00	1.25	11.26
	66	105.66	26.00	79.66	1.99	19.91
	100	102.33	0.00	102.33	1.70	18.05
	133	101.33	0.00	101.33	1.27	13.84
200	Control	200.00	202.00	-2.00	-	0.57
	100	242.00	157.67	84.33	1.40	10.96
	133	246.67	128.67	118.00	1.48	13.28

* P removed per gram of sludge = P removed/Amount of sludge mass used.

We assumed that an increase in sludge mass would also result in a proportional increase in the biomass since the sludge was taken from the same system. Therefore, we concluded that phosphate removal was proportional to the biomass. Our results are in agreement with that of Lemos *et al.* (1997) and others (Momba and Cloete, 1996; Muyima, 1995) who stated that a phosphorus free effluent could be obtained when there was enough biomass in a wastewater process. This suggested that the failure of EBPR under certain conditions could be due to insufficient biomass.

Table 4.2: ATP, Total Plate Count and MLSS of aerobic sludge mass in sterile mixed liquor containing 100 mg.l⁻¹ and 200 mg.l⁻¹ phosphate concentration.

$[PO_4^{-3}]$ (mg.l⁻¹)	Wet Sludge mass (g.l⁻¹)	ATP		Total Plate Count (cfu.ml⁻¹)		MLSS (mg.l⁻¹)	
		T = 0 h	T = 8 h	T = 0 h	T = 8 h	T = 0 h	T = 8 h
100	Control	1.23×10^4	4.51×10^6	0	*ND	100	200
	40	3.34×10^6	4.93×10^7	1.69×10^6	1.44×10^6	2220	3260
	80	5.41×10^6	5.36×10^7	5.34×10^6	6.17×10^7	4001	6080
	120	5.81×10^6	2.93×10^7	8.71×10^6	1.49×10^7	5670	7570
	160	8.92×10^6	1.60×10^7	6.50×10^6	1.93×10^7	7320	10240
200	Control	7.18×10^2	1.60×10^4	0.0	1.17×10^5	3486	3508
	120	2.13×10^7	3.75×10^7	6.43×10^6	1.01×10^7	7691	12101
	160	3.11×10^7	4.78×10^7	5.26×10^6	1.13×10^7	8886	21811

*ND - Not determined

The ATP results indicated the biomass fraction in the MLSS of wet aerobic sludge mass. The ATP values increased during the experimental period (Table 4.2). At time 0 h, when using 100 mg.l⁻¹ of phosphate in sterile mixed liquor, the 160 g.l⁻¹ of wet sludge mass concentration had the highest ATP value. After 8 h, the 80 g.l⁻¹ of wet sludge concentration had the highest value of ATP followed by 40 g.l⁻¹, 120 g.l⁻¹ and 160 g.l⁻¹, when 100 mg.l⁻¹ phosphate was used. The increase in ATP concentrations in all the experiments indicated that bacterial growth occurred during the experimental period. The increase in MLSS values was attributed to the increase in ATP values, reflecting bacterial growth (Table 4.2). The total plate count of aerobic sludge in all the experiments also increased during the experimental period (Table 4.2). The 120 g.l⁻¹ of sludge mass had the highest initial TPC values for both the 100 mg.l⁻¹ and the 200 mg.l⁻¹ experiments at time 0 h. After 8 h, the 80 g.l⁻¹ sludge concentration had the highest TPC value for 100 mg.l⁻¹ phosphate followed by 160 g.l⁻¹, 120 g.l⁻¹ and 40 g.l⁻¹ of wet sludge mass. For mixed liquor containing 200 mg.l⁻¹ phosphate, the 160 g.l⁻¹ sludge concentration had the highest value of TPC followed by the 120 g.l⁻¹ sludge mass. These results are in agreement with the ATP values indicating bacterial growth and thus increasing biomass.

Table 4.3: Phosphate removal by 40 g.l^{-1}, 80 g.l^{-1}, 120 g.l^{-1} and 160 g.l^{-1} of anaerobic sludge mass in sterile mixed liquor containing 200 mg.l^{-1} phosphate concentration.

[PO$_4^{3-}$] (mg.l^{-1})	Wet Sludge mass (g.l^{-1})	PO$_4^{3-}$ (mg.l^{-1})		PO$_4^{3-}$ removed (mg.l^{-1})	PO$_4^{3-}$ removed per g of sludge (mg.g^{-1})	PO$_4^{3-}$ removed per initial MLSS (mg.g^{-1})
		T = 0 h	T = 8 h			
200	Control	197.28	217.8	-20.52	-	1.17
	33	229.00	196.67	32.33	1.62	12.13
	66	250.76	167.33	83.43	2.08	17.50
	100	244.00	190.10	53.90	0.89	8.69
	133	265.27	179.00	86.27	1.07	14.10

The ATP and the TPC values of the anaerobic sludge mass indicated an increase in cell numbers during the experimental period (Table 4.4). This indicated an increase in the biomass fraction of the anaerobic sludge mass during the experimental period. The 80 g.l^{-1} of anaerobic sludge mass had the highest value of ATP, followed by the 40 g.l^{-1}, 120 g.l^{-1} and 160 g.l^{-1} concentrations at time 0 h. After 8 h, the 80 g.l^{-1} concentration still had the highest value followed by the 120 g.l^{-1}, 160 g.l^{-1} and 40 g.l^{-1} concentrations. The increase in MLSS was attributed to an increase in ATP and TPC of the anaerobic sludge.

Table 4.4: ATP, Total Plate Count and MLSS of anaerobic sludge mass in sterile mixed liquor containing 200 mg.l^{-1} phosphate concentration.

PO$_4^{3-}$ concentration (mg.l^{-1})	Sludge mass (g.l^{-1})	ATP		Total Plate Count (cfu.ml^{-1})		MLSS (mg.l^{-1})	
		T = 0 h	T = 8 h	T = 0 h	T = 8 h	T = 0 h	T = 8 h
200	Control	9.40×10^2	1.00×10^5	0.00	1.74×10^4	1750	2000
	40	2.06×10^7	5.30×10^7	4.90×10^6	3.77×10^6	2666	2866
	80	2.26×10^7	7.33×10^7	1.06×10^6	5.80×10^6	4766	5166
	120	1.60×10^7	6.50×10^7	4.10×10^6	7.73×10^6	6200	7933
	160	1.39×10^7	6.20×10^7	1.99×10^6	2.21×10^7	6116	10133

Higher ATP values were observed then those for TPC. This was ascribed to the fact that TPC relies on colony formation. The activated sludge bacterial suspension occurs in flocs, which may contain thousands of individual bacteria. The floc size and distribution in an activated sludge sample will vary and one would expect a variance in the results. On the other hand, ATP relies on chemical extraction and is hence not influenced by floc size or distribution. These results indicated that ATP is a better method than TPC for determining the bacterial numbers in activated sludge.

Jorgensen *et al.*, (1992) determined the biomass of activated sludge growth cultures in terms of dry weight and compared to with ATP content, the oxygen utilization rate and fluorescein hydrolysis data. ATP content showed the best correlation with biomass. ATP was also estimated to suspended solids ratio at maximum viability in activated sludge, it was found that viability varied with mean cell residence time. This showed that ATP is the best and reliable method to estimate bacterial numbers in activated sludge.

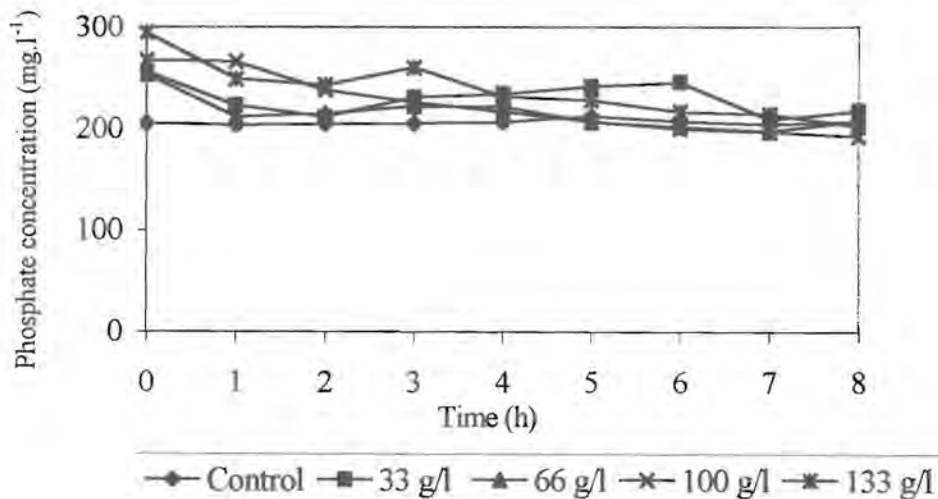### 4.4.1.3 Experiment 3 (Return sludge)

Figure 4.7: Phosphate removal by 33 g.l^{-1}, 66 g.l^{-1}, 100 g.l^{-1} and 133 g.l^{-1} of return sludge mass in sterile mixed liquor containing 200 mg.l^{-1} P.

When different quantities (33 g.l^{-1}, 66 g.l^{-1}, 100 g.l^{-1} and 133 g.l^{-1}) of return sludge mass was used, very little phosphate removal was detected (Figure 4.7). No quantitative conclusion could be made by phosphate removal of this sludge mass, hence the actual MLSS of the plant was used in subsequent experiments.

4.4.2 Experiment with MLSS concentrations simulating full-scale MLSS concentrations (experiment 4).

The original samples were chemically and microbiologically analyzed immediately after sampling (Table 4.5). The results indicated low concentrations of phosphate and ammonium. The nitrate concentration was below the lower limit (5 mg.l^{-1}) of the spectrophotometric method. The results also indicated a total plate count of 1.25 x 10^6 cfu.ml^{-1} (return sludge), 1.89 x 10^6 cfu.ml^{-1} (anaerobic sludge) and 1.45 x 10^6 cfu.ml^{-1} (aerobic sludge). The return sludge had higher MLSS (6447 mg.l^{-1}) and COD (6012 mg.l^{-1}) than the aerobic and anaerobic sludge. The MLVSS of the return sludge was also higher (8117 mg.l^{-1}) than that of the aerobic and anaerobic sludge.

Table 4.5: **Chemical and microbiological analyses of the original sludge from Daspoort used in experiment 4.**

Sample	[PO$_4^{3-}$] (mg.l^{-1})	pH	COD (mg.l^{-1})	NH$_4^+$ (mg.l^{-1})	Total plate count (cfu/ml)	MLSS (mg.l^{-1})	MLVSS (mg.l^{-1})
Influent	6.46	*ND	ND	ND	ND	ND	ND
Effluent	1.33	ND	ND	ND	ND	ND	ND
Return sludge	3.00	7.16	6012	0.60	1.25 x 10^6	6447	8117
Anaerobic sludge	6.90	7.39	2298	9.61	1.89 x 10^6	3053	5307
Aerobic sludge	ND	7.27	2848	0.34	1.45 x 10^6	3073	2263

*ND = Not Determined

Experiment with Aerobic sludge

Figure 4.8: Phosphate removal by ½ x aerobic (1045 mg.l^{-1}MLSS), standard aerobic (2190 mg.l^{-1}MLSS) and 2 x aerobic (4430 mg.l^{-1}MLSS) sludge in sterile mixed liquor containing 32 mg.l^{-1} P.

The 2 x aerobic sludge (4430 mg.l^{-1}) removed phosphate (0.22 mg PO$_4^{3-}$.g^{-1} MLSS) better than the ½ x aerobic (0.12 mg PO$_4^{-3}$ g^{-1}MLSS) and the standard aerobic sludge (0.02 mg PO$_4^{3-}$.g^{-1}MLSS) (Figure 4.8 and Table 4.6).
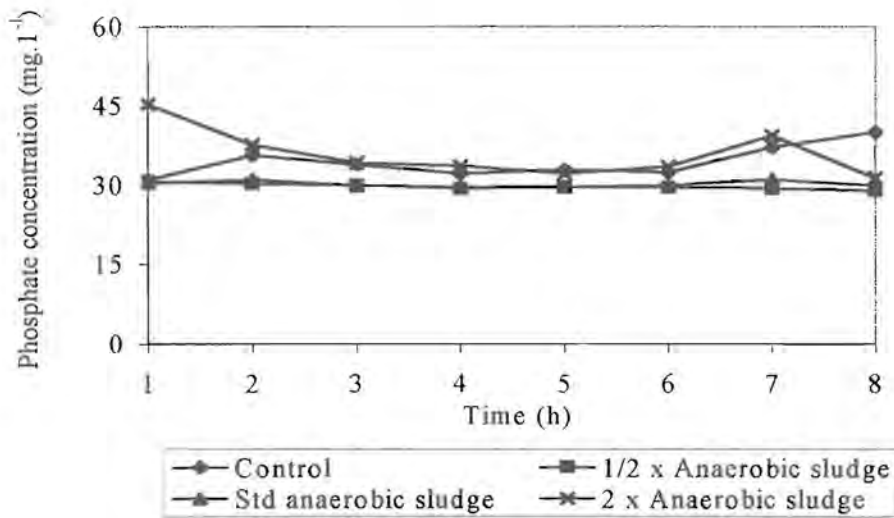
Experiment with anaerobic sludge

Figure 4.9: Phosphate removal by ½ x anaerobic (1095 mg.l^{-1} MLSS), standard anaerobic (2095 mg.l^{-1} MLSS) and 2 x anaerobic (4240 mg.l^{-1} MLSS) sludge in sterile mixed liquor containing 32 mg.l^{-1} P.

When the actual MLSS of the plant for anaerobic sludge was used, the 2 x anaerobic sludge (4240 mg.l^{-1}) removed higher concentration of phosphate (0.33 mg PO_4^{3-}.g^{-1}MLSS) compared to ½ x anaerobic (0.14 mg PO_4^{3-}.g^{-1}MLSS) and standard anaerobic sludge (0.02 mg PO_4^{3-}.g^{-1}MLSS) (Figure 4.9 and Table 4.6).
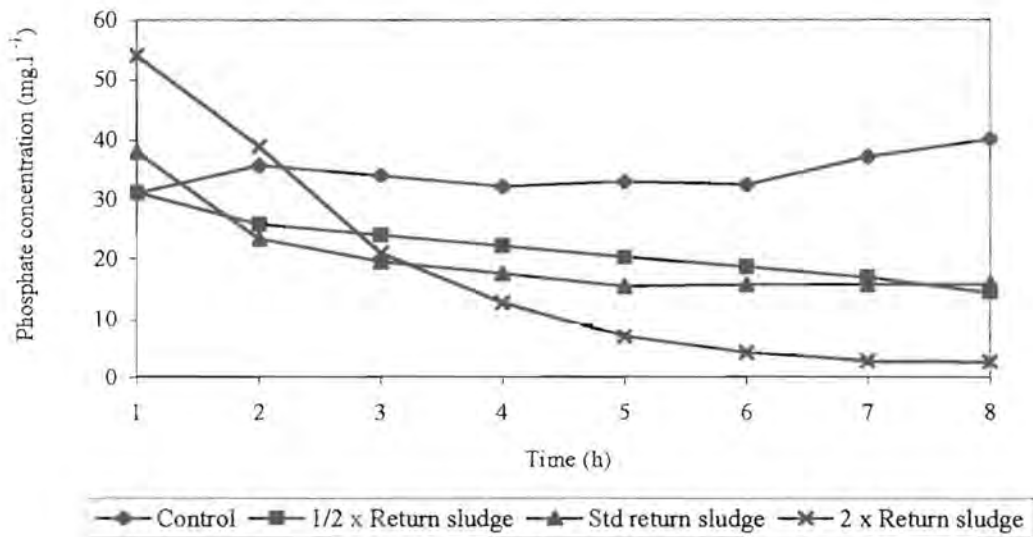
Experiment with return sludge

Figure 4.10: **Phosphate removal by ½ x return (2590 mg.l^{-1} MLSS), standard return (4790 mg.l^{-1} MLSS) and 2 x return (7840 mg.l^{-1} MLSS) sludge in sterile mixed liquor containing 32 mg.l^{-1} P.**

The phosphate removal by the actual MLSS of the plant for the return sludge indicated that the ½ x return (2590 mg.l^{-1}) and the 2 x return (7840 mg.l^{-1}) sludge removed phosphate better than the standard return sludge (Figure 4.10 and Table 4.6). The ½ x return, the standard return and the 2 x return sludge removed 0.69 mg PO$_4^{3-}$.g^{-1}MLSS, 0.51 mg PO$_4^{3-}$.g MLSS and 0.67 mg PO$_4^{3-}$.g^{-1} MLSS, respectively.

Table 4.6: Phosphate removed by ½ the actual, the actual and double the actual MLSS of the Daspoort plant inoculated into sterile mixed liquor containing 32 mg.l^{-1} phosphate concentration.

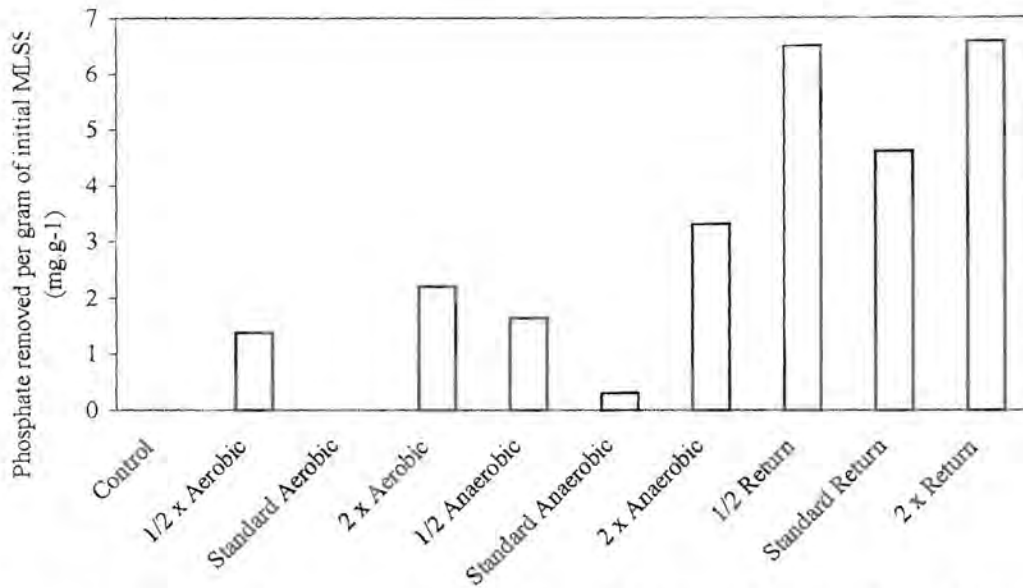| Sample	Mass (g.l^{-1})	$[PO_4^{3-}]$ (mg.l^{-1}) T =0 h	$[PO_4^{3-}]$ (mg.l^{-1}) T =7 h	PO_4^{3-} removed (mg.l^{-1})	PO_4^{3-} removed per gram sludge (mg.l^{-1})	PO_4^{3-} removed per initial MLVSS (mg.g^{-1})	PO_4^{3-} removed per initial MLSS (mg.l^{-1})
Control	-	31.10	40.05	-8.95	-	-447.5	0.00
½ x Aerobic sludge	20.00	30.05	28.60	1.45	0.12	1.946	1.387
Standard Aerobic sludge	39.58	29.90	30.45	-0.55	0.02	-0.346	-0.251
2 x Aerobic sludge	75.16	41.75	32.00	9.75	0.22	2.607	2.200
½ Anaerobic sludge	21.50	30.80	29.00	1.8	0.14	1.895	1.644
Standard Anaerobic sludge	37.91	30.50	29.85	0.65	0.02	0.445	0.310
2 x Anaerobic sludge	70.33	45.40	31.40	14.00	0.33	4.409	3.302
½ Return sludge	40.33	31.20	14.40	16.8	0.69	8.865	6.486
Standard Return sludge	72.00	38.0	15.95	22.05	0.51	6.602	4.603
2 x Return sludge	128.50	54.15	2.70	51.45	0.67	8.809	6.562

Figure 4.11: **Phosphate removed per gram of initial MLSS of aerobic, anaerobic and return sludge in sterile mixed liquor containing 32 mg.l^{-1} P (experiment 4). A negative value of 0.251 mg.g^{-1} was obtained for standard aerobic sludge concentrations (not indicated).**

The mixed liquor suspended solids increased with increasing inoculum size and time (Figure 4.11 and Tables 4.2, 4.4 and 4.7) in all the experiments. The return sludge removed the highest concentration of phosphate (6.48 mg PO_4^{3-}.g^{-1} for ½ x return, 4.60 mg PO_4^{3-}.g^{-1} for standard return and 6.56 mg PO_4^{3-}.g^{-1} of MLSS for 2 x return sludge) (Table 4.6). The results suggested that the return sludge had a higher biomass than aerobic and anaerobic sludge. These results also indicated that the return sludge removed phosphate better than aerobic and anaerobic sludge.
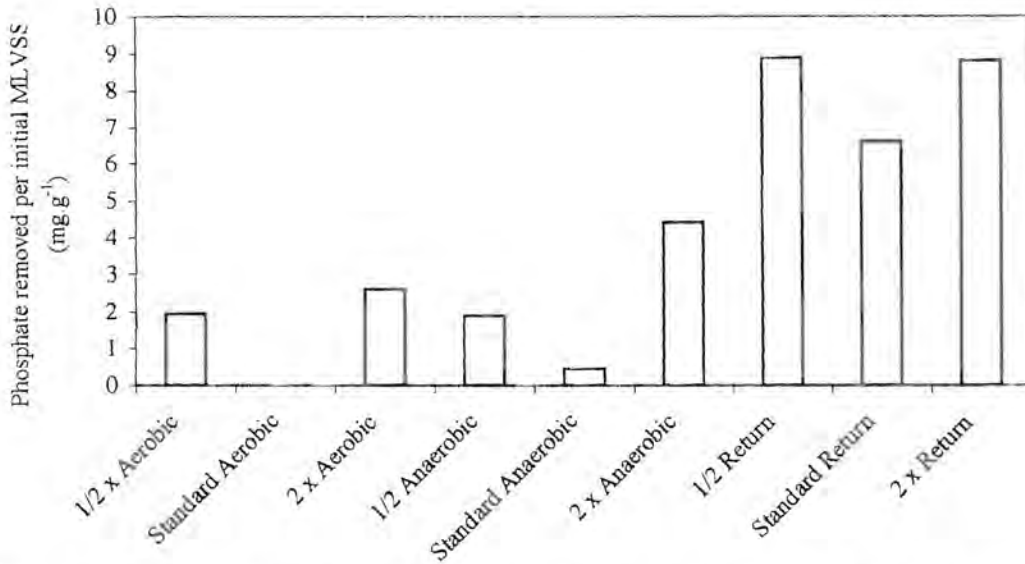
Figure 4.12: **Phosphate removed per gram of initial MLVSS of aerobic, anaerobic and return sludge in sterile mixed liquor containing 32 mg.l^{-1} P (experiment 4). A negative value of 0.346 mg.g^{-1} was obtained for standard aerobic sludge concentration (not indicated).**

The MLVSS followed the same phosphate removal trend as the MLSS for all the sludges (Figure 4.12). Average phosphate removal calculated in terms of MLVSS indicated that the return sludge removed the highest concentration of phosphate. The ½ x return sludge removed 8.865 mg PO$_4$$^{3-}$.g^{-1} of MLSS, standard return removed 6.602 mg PO$_4$$^{3-}$.g^{-1} MLSS and the 2 x return removed 8.809 mg PO$_4$$^{3-}$.g^{-1} of MLSS (Table 4.6). The return sludge performed better than the aerobic and anaerobic sludges. This was attributed to the larger initial bacterial numbers resulting in higher MLVSS values.
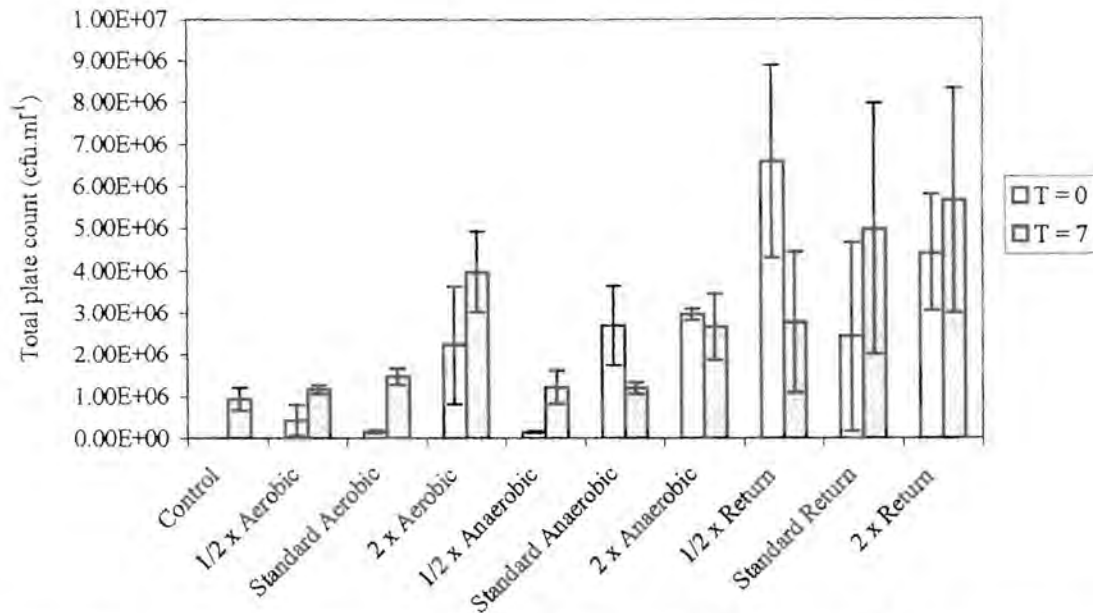
Figure 4.13: The total plate count of ½ x aerobic (1045 mg.l^{-1} MLSS), standard aerobic (2190 mg.l^{-1} MLSS), 2 x aerobic (4430 mg.l^{-1} MLSS), ½ x anaerobic (1095 mg.l^{-1} MLSS), standard anaerobic (2095 mg.l^{-1} MLSS), 2 x anaerobic (4240 mg.l^{-1} MLSS), ½ x return (2590 mg.l^{-1} MLSS), standard return (4790 mg.l^{-1} MLSS) and 2 x return (7840 mg.l^{-1} MLSS) sludge.

The TPC of the actual MLSS of the plant increased during the experimental period (Figure 4.13). On average, the return sludge had the highest bacterial numbers followed by the anaerobic and then the aerobic sludge (Table 4.7). The ½ x return sludge had high TPC value (6.60 x 10^6 cfu.ml^{-1}) followed by 2 x return sludge (4.42 x 10^6 cfu.ml^{-1}), 2 x anaerobic sludge (2.9 x 10^6 cfu.ml^{-1}) and the standard anaerobic sludge (2.69 x 10^6 cfu.ml^{-1}) for time 0 h. After 7 h, the ½ x return sludge had a high TPC value followed by 2 x return (5.67 x 10^6 cfu.ml^{-1}), 2 x aerobic (3.97 x 10^6 cfu.ml^{-1}), standard return (5.00 x 10^6 cfu.ml^{-1}) and the 2 x anaerobic (2.65 x 10^6 cfu.ml^{-1}) sludge.

Table 4.7: Total Plate Count, MLSS and MLVSS of aerobic, anaerobic and return sludge inoculated in sterile mixed liquor containing 32 mg.l^{-1} phosphate concentration (experiment 4).

| Sample	Biomass (g.l^{-1})	Total Plate Count (cfu.ml^{-1})		MLSS (mg.l^{-1})		MLVSS (mg.l^{-1})	
		T = 0 h	T = 7 h	T = 0 h	T = 7 h	T = 0 h	T=8 h
Control	-	< 10	0.93×10^6	0.00	-10.00	20	-1590
½ x Aerobic sludge	20.00	4.25×10^5	1.16×10^6	1045	1060	745	755
Standard Aerobic sludge	39.58	1.58×10^5	1.47×10^6	2190	2095	1590	1620
2 x Aerobic sludge	75.16	2.22×10^6	3.97×10^6	4430	4440	3740	3220
½ Anaerobic sludge	21.50	1.43×10^5	1.22×10^6	1095	1070	950	800
Standard Anaerobic sludge	37.91	2.69×10^6	1.20×10^6	2095	2080	1460	1550
2 x Anaerobic sludge	70.33	2.95×10^6	2.65×10^6	4240	4415	3175	3085
½ Return sludge	40.33	6.60×10^6	2.77×10^6	2590	2745	1895	1860
Standard Return sludge	72.00	2.42×10^6	5.00×10^6	4790	5030	3340	3430
2 x Return sludge	128.50	442×10^6	5.67×10^6	7840	8540	5840	5575
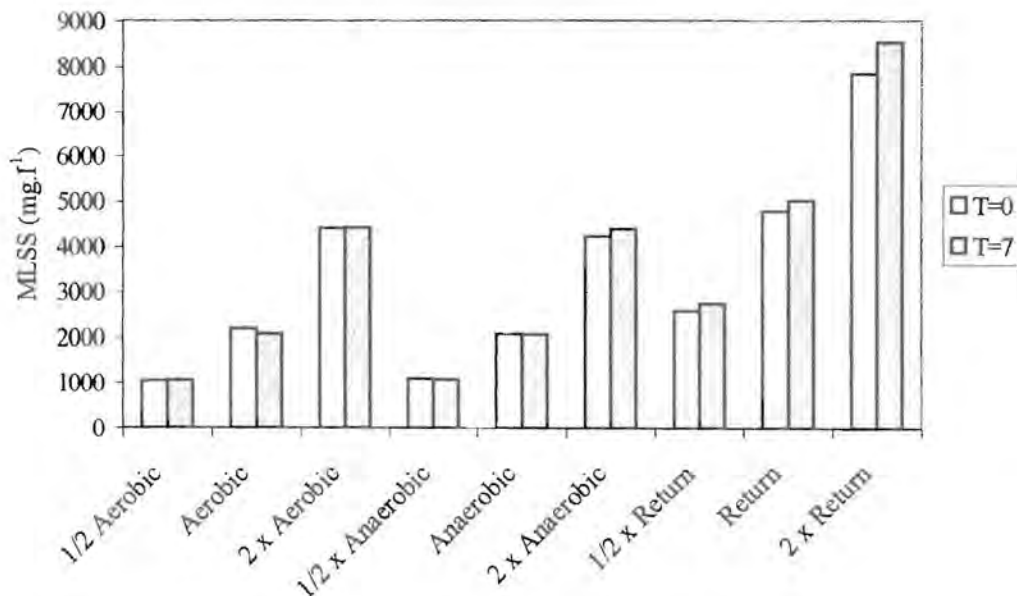
Figure 4.14: Average MLSS values at time 0 h and time 7 h.

The MLSS increased in all the samples except in the standard aerobic sludge and ½ x aerobic sludge (Figure 4.14 and Table 4.7). The 2 x return sludge mass indicated the highest MLSS values (7840 mg.l⁻¹ and 8540 mg.l⁻¹) for both time 0 h and time 7 h and similar MLSS values for standard return sludge, 2 x aerobic and the 2 x anaerobic sludge were observed for both time 0 h and 7 h. The increase in MLSS was attributed to an increase in bacterial cell numbers indicated by TPC. The results also indicated that microbial growth occurred during the experimental period.
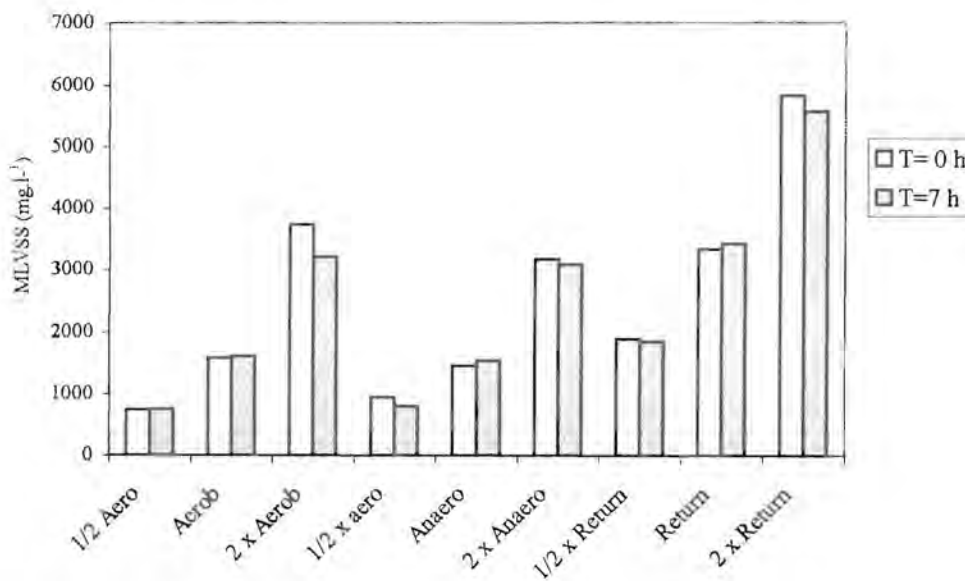

Figure 4.15: Average MLVSS values at time 0 h and time 7 h.

The 2 x return sludge concentration had highest MLVSS values at time 0 h and time 7 h followed by 2 x aerobic and standard return sludge concentrations (Figure 4.15). The results of the MLVSS were lower than those for MLSS (Figure 4.14 and Table 4.7) since it represents the volatile fraction of the MLSS. The increase in MLVSS of the standard aerobic, standard anaerobic and standard return sludge concentrations was attributed to an increase in bacterial cell numbers indicated by the TPC analyses. The MLVSS is a standard parameter of biomass in activated sludge, although it is recognized as an indirect and incomplete measure of the viable sludge floc (Patterson et al., 1970).

Experiment with MLSS concentrations simulating full-scale MLSS concentrations (Experiment 5)

To check the constancy of the MLSS and MLVSS simulation results, a second experiment using the same experimental layout was done three months after the first experiment.
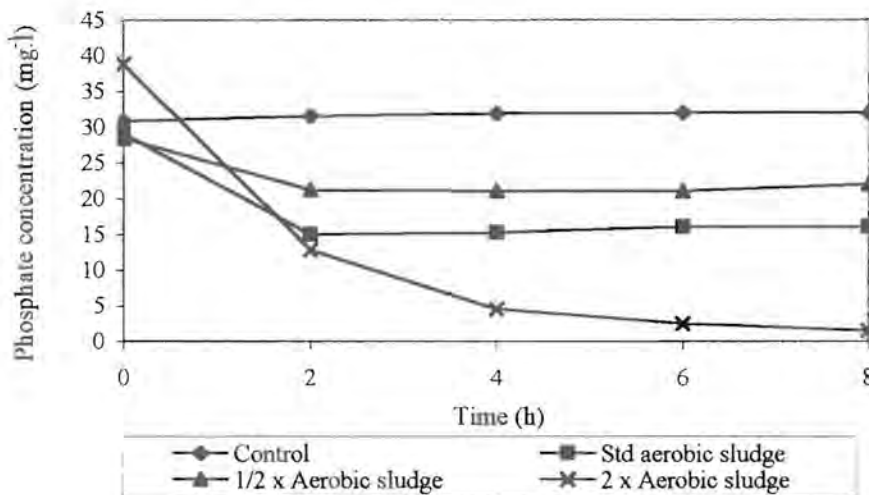
Experiment with aerobic sludge

Figure 4.16: **Phosphate removal by ½ x aerobic (1140 mg.l^{-1} MLSS), standard aerobic (3230 mg.l^{-1} MLSS) and 2 x aerobic (4030 mg.l^{-1} MLSS) sludge in sterile mixed liquor containing 32 mg.l^{-1} P.**

The phosphate removal by the actual MLSS of the plant for the aerobic sludge indicated that 2 x aerobic sludge removed phosphate better (0.40 mg PO_4^{3-}.g^{-1} MLSS) than the standard aerobic (0.21 mg PO_4^{3-}.g^{-1} MLSS) and the ½ x aerobic (0.28 mg PO_4^{3-}.g^{-1} MLSS) sludge (Figure 4.16 and Table 4.8). The 2 x aerobic sludge was most effective after 8 h whereby it removed almost the phosphate in the mixed liquor.
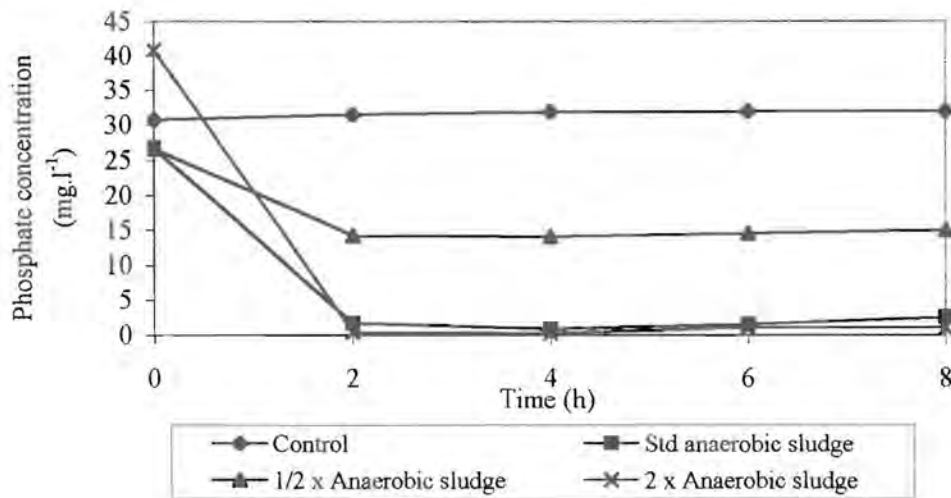
Experiment with anaerobic sludge

Figure 4.17: **Phosphate removal by ½ x anaerobic (1650 mg.l⁻¹ MLSS), standard anaerobic (3320 mg.l⁻¹ MLSS) and 2 x anaerobic (5600 mg.l⁻¹ MLSS) sludge in sterile mixed liquor containing 32 mg.l⁻¹ P.**

When the actual MLSS of the plant for anaerobic sludge was used, the standard anaerobic and the 2 x anaerobic sludge removed almost all the phosphate in the mixed liquor after 2 h of the experiment, at the same rate (Figure 4.17 and Table 4.8). Both removed 0.41 mg $PO_4^{3-}.g^{-1}$ MLSS compared to the ½ x anaerobic sludge which removed 0.39 mg $PO_4^{3-}.g^{-1}$ MLSS.
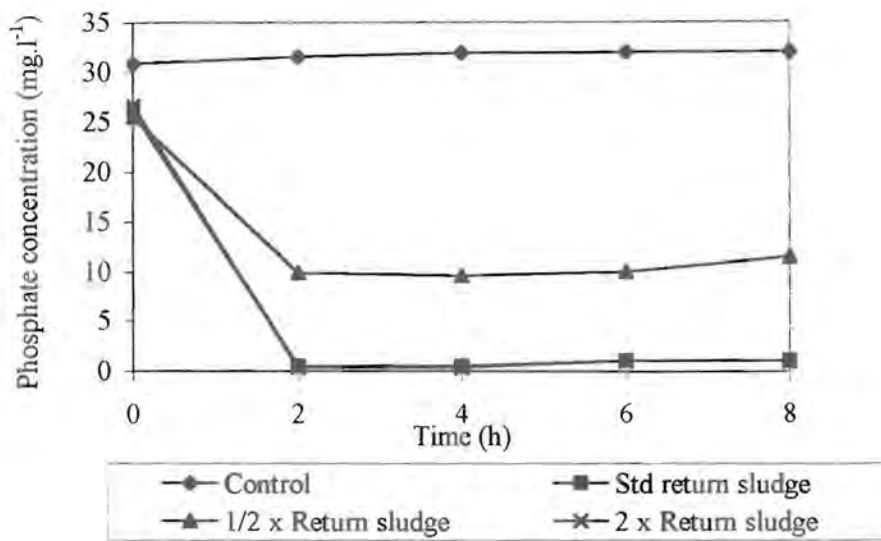
Experiment with return sludge

Figure 4.18: Phosphate removal by ½ x return (2430 mg.l⁻¹ MLSS), standard return (4010 mg.l⁻¹ MLSS) and 2 x return (4800 mg.l⁻¹ MLSS) sludge in sterile mixed liquor containing 32 mg.l⁻¹ P.

The phosphate removal by the actual MLSS of the plant for the return sludge indicated the same pattern as the anaerobic sludge (Figure 4.18 and Table 4.8). The standard return and the 2 x return sludge removed all the phosphate (0.30 mg $PO_4^{3-}.g^{-1}$ MLSS and 0.20 mg $PO_4^{3-}.g^{-1}$ MLSS) in the mixed liquor after 2 h of the experiment, respectively. The ½ x return sludge removed the least phosphate.

Table 4.8: Phosphate removed by ½ the actual, the actual and double the actual MLSS of the Daspoort plant inoculated into sterile mixed liquor containing 32 mg.l^{-1} P.

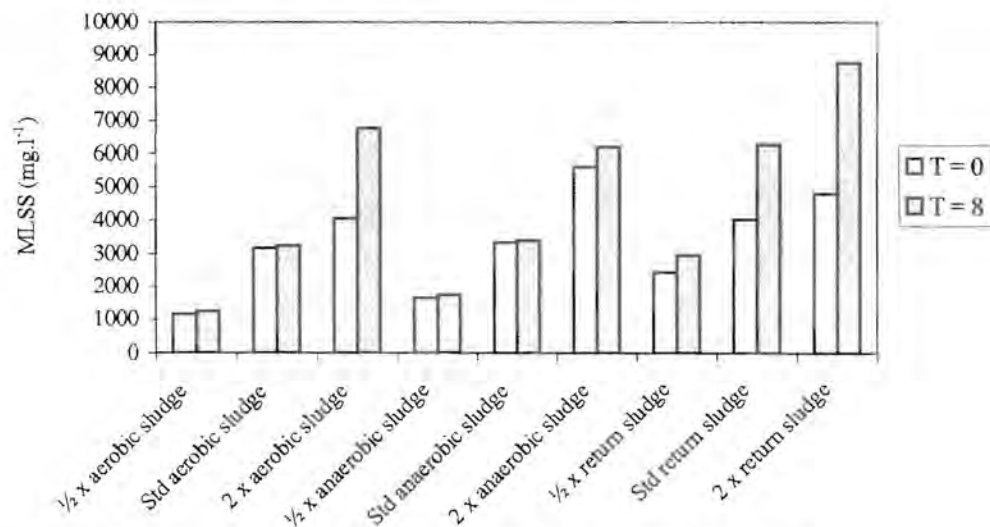| Sample	Sludge Mass (g.l^{-1})	[PO$_4^{3-}$] (mg.l^{-1}) T=0h T=8h		PO$_4^{3-}$ Removed (mg.l^{-1})	PO$_4^{3-}$ removed per gram of sludge (mg.l^{-1})	PO$_4^{3-}$ removed per initial MLVSS (mg.g^{-1})	PO$_4^{3-}$ removed per initial MLSS (mg.g^{-1})
Control	-	30.80	32.00	-1.2		-12.12	0.00
½ x aerobic sludge	23.50	28.55	22.00	6.55	0.28	5.776	5.771
Std aerobic sludge	60.00	29.10	16.00	13.1	0.21	4.057	4.056
2 x aerobic sludge	92.70	38.85	1.50	37.35	0.40	11.202	11.199
½ x anaerobic sludge	29.50	26.70	15.00	11.7	0.39	7.073	7.069
Std anaerobic sludge	58.70	26.80	2.50	24.3	0.41	7.321	7.319
2 x anaerobic sludge	96.00	40.80	1.00	39.8	0.41	7.112	7.100
½ x return sludge	45.90	25.50	11.50	14.00	0.30	5.756	5.773
Std return sludge	83.70	26.15	1.00	25.15	0.30	6.275	6.271
2 x return sludge	131.50	26.60	1.00	25.6	0.20	5.340	5.344

Figure 4.19: Average MLSS values at time 0 h and time 8 h.

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The MLSS increased with the increasing inoculum and size in all the sludges (Figure 4.19 and Table 4.9). The 2 x anaerobic sludge indicated the highest MLSS value (5600 mg.l^{-1}) at time 0 h followed by 2 x return (4800 mg.l^{-1}) and standard return (4010 mg.l^{-1}) sludge. After time 8 h, the highest MLSS value was indicated by the 2 x return sludge (8730 mg.l^{-1}) followed by the 2 x aerobic (6760 mg.l^{-1}), standard return (6270 mg.l^{-1}) and 2 x anaerobic (6190 mg.l^{-1}) sludge. The results indicated that microbial growth occurred during the experiment.
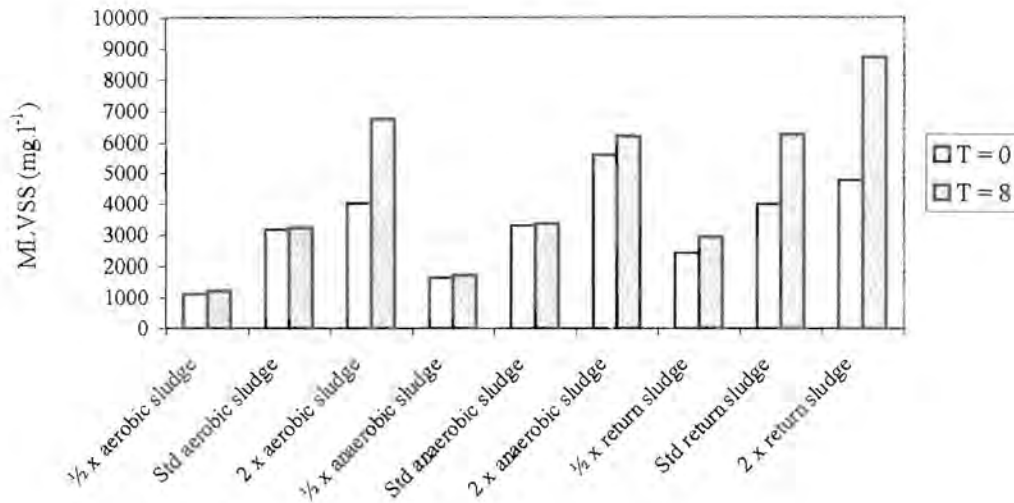

Figure 4.20:   Average MLVSS values at time 0 h and time 8 h.

The MLVSS followed the same trend as the MLSS for all the sludges (Figure 4.20). The 2 x anaerobic sludge indicated the highest MLVSS value (5596 mg.l^{-1}) at time 0 h followed by 2 x return (4794 mg.l^{-1}) and standard return (4008 mg.l^{-1}) sludge. After time 8 h, the highest MLVSS value was indicated by the 2 x return sludge (8722 mg.l^{-1}) followed by the 2 x aerobic (6753 mg.l^{-1}), standard return (6263 mg.l^{-1}) and 2 x anaerobic (6188 mg.l^{-1}) sludge. The MLVSS results were lower than those for MLSS (Table 4.9) since it represents the volatile fraction of the MLSS. But in some cases, the MLVSS were greater than the MLSS numbers. This could be due to the analytical error during the experimental procedure.

Table 4.9: MLSS and MLVSS of aerobic, anaerobic and return sludge inoculated in sterile mixed liquor medium containing 32 mg.l^{-1} phosphate concentration.

Sample	Sludge mass (g.l^{-1})	MLSS (mg.l^{-1})		MLVSS (mg.l^{-1})	
		T = 0 h	T = 8 h	T = 0 h	T = 8 h
Control	-	0.000	20.00	-99	-199
½ x aerobic sludge	23.50	1140	1230	1134	1229
Std aerobic sludge	60.00	3170	3230	3169	3229
2 x aerobic sludge	92.70	4030	6760	4034	6753
½ x anaerobic sludge	29.50	1650	1740	1654	1739
Std anaerobic sludge	58.70	3320	3390	3319	3384
2 x anaerobic sludge	96.00	5600	6190	5596	6188
½ x return sludge	45.90	2430	2950	2432	2949
Std return sludge	83.70	4010	6270	4008	6263
2 x return sludge	131.50	4800	8730	4794	8722

Current research in wastewater treatment has been directed towards mathematical modeling of basic design and operational procedures (Jorgensen *et al.*, 1992). One important parameter in such models is the amount of viable biomass. For this reason, attempts have been made to find methods to determine the biomass in wastewater and activated sludge. The choice of a method is a crucial decision to make. One parameter, which is used to measure biomass in activated sludge is the MLSS. The MLSS is made of heterotrophic active biomass, endegenous residue and inert material. Historically, the MLSS has been measured as a lump parameter via the VSS test (Standard methods, 1985).

The viable counting methods such as the total plate count, can also be used to differentiate between living and dead cells, by assessing their ability to grow either in liquid or solid media (Herbert 1990). The viable counting methods substantially underestimate the true microbial populations presence, since there is no universal growth medium on which microorganisms will grow.

Momba and Cloete (1996) indicated that an increase in biomass of pure cultures resulted in increase in phosphate removal. They proved that the high initial cell concentration of the phosphate accumulating organisms removed more phosphate than low cell concentrations and phosphate uptake was directly related to biomass and high nutrient availability.

4.5 Conclusions

- ATP was a more reliable method for biomass determination in activated sludge than TPC, due to higher bacterial counts.
- An increase in sludge mass resulted in a proportional increase in phosphate removal.
- In the first simulation experiment, the return sludge MLSS removed phosphate better than the aerobic MLSS and anaerobic sludge MLSS.
- In the second simulation experiment, the anaerobic and the return sludges indicated the same trend in phosphate removal and were more effective than the aerobic sludge.
- The MLSS and the MLVSS indicated the same trend in phosphate removal, although the MLVSS was lower since it represents the volatile fraction of the MLSS.

4.6 References

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CHAPTER 5

CONCLUSIONS

The results of the study indicated that bioaugmentation, with natural biomass not commercial bioaugmentation products, could be a possible solution for removing phosphate in activated sludge. The bioaugmentation products tested in the study contained a high phosphate concentration, making them unsuitable for bioaugmentation.

Experiments with different concentrations of a commercially available bioaugmentation product A indicated a relationship between phosphate removal and the quantity of bioaugmentation product added. The samples containing 8 g and 10 g of the product per 1000 ml of mixed liquor removed 17.90 mg.l^{-1} and 18.86 mg.l^{-1} phosphate per g of product added, respectively. At concentrations exceeding 80 g per 1000 ml of mixed liquor, no phosphorus was removed, instead the phosphate concentration increased as a result of the phosphate content of the product. Bioaugmentation product A had a high phosphate content, making it unsuitable for bioaugmentation.

When the growth of the bioaugmentation product B and anaerobic sludge were determined for addition to activated sludge, no growth was observed in the mixed liquor media. The media did not contain enough nutrients for microorganisms to grow. Nevertheless growth occurred in the Nutrient broth but it will not be used to grow microorganisms since it is expensive. Alternative inexpensive substrates have to be evaluated in order to increase biomass. From an economical point of view, the bioaugmentation products tested would be impractical and too expensive for bioaugmentation in activated sludge.

When determining the phosphate removal capacity of aerobic, anaerobic and return sludge from the same system, an increase in biomass resulted in an increase in phosphate removal. When calculating the quantity of phosphate removal per g of sludge mass, no significant difference was observed. The same trend was observed for phosphate removal in terms of MLSS, indicating

that there was also a direct relationship between phosphate removal and MLSS for a specific system.

In one experiment simulating the actual MLSS from the same plant, the anaerobic and aerobic sludges removed phosphate at the same rate when calculated in terms of initial MLSS and MLVSS. In another experiment, the anaerobic sludge performed better at phosphorus removal than aerobic sludge. The return sludge mass performed consistently better at phosphorus removal than the aerobic and anaerobic sludges.

The study also indicated that ATP was the most reliable method for biomass determination in activated sludge due to its higher yield. The MLVSS showed the same trend in phosphate removal as the MLSS.