

Chapter 1

INTRODUCTION

Eutrophication of South Africa's natural waters is greatly accelerated by human activities, resulting in the discharge of the nutrients nitrogen (N) and phosphorus (P). Nutrients are introduced into the water from point sources, e.g. wastewater treatment works, and diffuse sources, e.g. from fertilizers or the excreta of animals and birds (Lilley *et al.*, 1997). Phosphate is recognised as one of the major nutrients contributing to the eutrophication of aquatic environments (Momba and Cloete, 1996a,b). Gross eutrophication is marked by large visible blooms of algae which make water treatment difficult (Atlas and Bartha, 1993; Lilley *et al.*, 1997). Degradation in the water quality of lakes is noticed by an offensive odour, appearance, taste, as well as depletion of oxygen from the lower water, resulting in extensive fish kills.

Many algae have the ability to fix nitrogen in water and therefore phosphorus is the element that should be restricted in order to minimize eutrophication (Lilley *et al.*, 1997). Sources of phosphorus include fertilizers, synthetic detergent and excreta. In the United States of America the concentration of phosphate in raw municipal wastewaters has been significantly reduced by the implementation of detergent "phosphate bans" (Martin and Wilson, 1994).

Phosphate is a major component of nucleic acids and therefore no organism can reproduce without phosphate. Phosphate is however a limiting nutrient, and therefore the removal of phosphate from effluents should limit one of the basic causes of eutrophication or algal blooms (Momba, 1995).

Both chemical and biological nutrient removal may be used in limiting eutrophication of water (Lilley *et al.*, 1997). Phosphate removal by chemical precipitation always remains an option,

although its high cost relative to the biological route reduces its appeal (Lötter and Murphy, 1988).

Activated sludge processes are currently the most widely used biological wastewater treatment process in the developed world treating both domestic and industrial wastewaters (Gray, 1989). Activated sludge systems, modified for enhanced P removal during effluent treatment are now operative in at least nine countries spanning the globe: Australia, Brazil, Canada, France, New Zealand, South Africa, United States, Zimbabwe and Namibia (Toerien *et al.*, 1990).

The activated sludge process relies on a dense microbial population being mixed in suspension with the wastewater under aerobic conditions (Gray, 1989). Kuba *et al.* (1997) indicated that biological P removal is achieved by recirculation of activated sludge through anaerobic and aerobic or anoxic conditions. There is consensus that enhanced P removal is a result of microbial action (Toerien *et al.*, 1990) and the ability of certain organisms (for e.g. *Acinetobacter* spp.) to accumulate large quantities of polyphosphates has been well elucidated by Fuhs and Chen (1975). However it has been illustrated that no single species of bacteria is capable of removing all the phosphate from wastewater.

Biological phosphate removal plants in South Africa have not always given reliable and satisfactory performance (Osborn *et al.*, 1986). In 1983, an extensive review of 11 of the South African Bardenpho plants was conducted and it was found that only one was achieving consistently good phosphorus removal, while a further 3 plants achieved good removal only after improving operational and control procedures (Oldham *et al.*, 1994). The reasons are not well elucidated, but a significant feature is dilution of substrate in sewage influents by continuous wet weather and storm periods. Other microorganisms can compete with polyphosphate accumulating organisms (PAO) in anaerobic-aerobic activated sludge systems, leading to system failure.

Sludge, in which PAO dominate, consumes carbohydrates while taking up short-chain fatty acids in the anaerobic phase and accumulates poly- β -hydroxy-alkanoates (PHA). Significant

release of phosphate was not observed because polyphosphate was not utilized. Energy was rather obtained from the utilization of glycogen (Erasmus, 1997).

One of the factors contributing to poor performance of biological treatment plants (especially for P removal) is the so-called nitrate feedback whereby the anaerobic conditions required for phosphate removal are lost (Lilley *et al.*, 1997). In South African activated sludge systems it has been indicated that high concentrations of sulphide also has a detrimental effect on enhanced biological P removal. Apart from being toxic, excessive sulphide concentrations may reduce the concentrations of essential trace elements to sub-critical concentrations, thus interfering directly with biochemical reactions occurring during P release (Toerien *et al.*, 1990).

It has been indicated that no population differences have been observed between P removing and non-P removing activated sludge systems and neither does the community differ from one zone to another (Ehlers, 1997). Bosch (1992), Momba (1995) and Muyima (1995) indicated that biomass was related to phosphorus removal. Preliminary research has indicated that biomass quantity is more important than the microbial community of the biomass (Bosch, 1992). The higher the biomass the better the P-removal. This suggested that the main difference between P removing and non-P removing systems was biomass related and not due to the microbial community structure.

The secret to designing and running an activated sludge plant for successful P removal, therefore, lies in creating conditions in the plant which favour propagation and growth of biomass capable of removing P (Lilley *et al.*, 1997).

To this end simple, fast and effective measures of determining, not only total biomass, but also viable biomass should be developed, which could assist in the operation and management of wastewater treatment plants.

Although research effort has been directed towards improving the understanding of biological excess phosphorus removal (BEPR), designs of activated sludge systems to accomplish BEPR

are still based on experience and semi-empirical methods. Current research in wastewater treatment has been directed towards mathematical modelling of basic design and operational procedures. Quantification, as well as kinetic models of biomass in activated sludge are routinely used in design of wastewater treatment plants, in spite of the limitations which are currently encountered in biomass determination. One important parameter in such models has been the amount of viable biomass, therefore attempts have been made to find simple, fast and reliable methods of determining biomass in wastewater and activated sludge. The simplest and most often used method is to measure suspended solids (SS) or volatile suspended solids (VSS). These methods, however, do not distinguish between living cells and debris of either organic or inorganic origin. One key component of the mixed liquor suspended solids (MLSS) is the heterotrophic active biomass, mediating the biodegradation processes of chemical oxygen demand (COD) removal and denitrification. However, this parameter has only been hypothetical within the structure of models, and has not been measured directly, primarily due to the lack of simple, suitable measurement techniques (Ubisi *et al.*, 1997). In literature, principally microbiological techniques have been proposed for biomass estimation (Ubisi *et al.*, 1997), including pour plate or other culturing techniques, DNA analysis, the use of fluorescent probes for rRNA and sequencing of rDNA. These techniques, however, have not yet been adequately integrated with design and kinetic modelling, while culturing techniques have been widely criticized for their unreliability (Cloete and Steyn, 1988). With the use of traditional plate count techniques, an underestimation of biomass is done due to the selectivity of the media employed (Jørgensen *et al.*, 1992), as well as the concept of flocs acting as colony forming units (cfu), which may consist of thousands of cells. Although good progress has been made with the RNA and two DNA approaches, these methods are still in their infancy. All the methods, with exception of culturing techniques, also require sophisticated equipment and experimental techniques not widely available (Ubisi *et al.*, 1997).

Microbial activity has also been used as a parameter to determine the microbial potential. Methods include respirometry, ATP content and different enzyme assays. However, there have been very few attempts to relate activity to biomass. ATP is a fundamental component of living matter, involved in metabolic activities and disappearing immediately after cell death (Atlas, 1982). Specific proportions of ATP in relation to total cellular carbon are constant

with variations not exceeding 17 % (Atlas, 1982). ATP has been found to reflect viable biomass (Patterson *et al.*, 1970; Kucknerowicz and Verstraete, 1979; Jenkinson and Ladd, 1981; Jørgensen *et al.*, 1992).

Extracellular polymeric substances (EPS), also referred to as extracellular polysaccharides are macromolecules formed by polymerization of similar or identical building blocks. Building blocks include monosaccharides, uronic acids and amino sugars (containing various substituents), proteins (for example glycoproteins and lipoproteins), nucleic acids, phospholipids and humic substances (Wingender *et al.*, 1999). Among activated sludge EPS, proteins dominate, which, on the basis of their relatively high content of negatively charged amino acids, are supposed to be more involved than sugars in electrostatic bonds with multivalent cations, underlining their key role in floc structure (Wingender *et al.*, 1999). Functions attributed to EPS include sorption of exogenous organic compounds and inorganic ions. Adsorptive properties of exopolymers have been well documented especially in terms of biosorption of pollutants and toxics (Beech and Cheung, 1995; Loaec *et al.*, 1997).

Although the macro-environmental conditions of activated sludge have been well described, very little is known about the micro-environment in activated sludge flocs, including diffusion gradients and the role of EPS in EBPR. However, the role of EPS in biological phosphorus removal has not been well studied. In previous studies, Buchan (1980) used EDS to determine the location of phosphorus volutin granules in activated sludge, without attention to the phosphorus content of EPS. As far as the author could establish, no attempts have been made as yet to elucidate the phosphorus content of activated sludge EPS by means of *in situ* methods. Results could be included in modelling of activated sludge systems to streamline the process and to attain constant and reproducible effluents conforming to standards set by Government.

EDS entails the bombardment of a sample with electrons, producing, along with various secondary signals, X-rays. The release of X-rays is produced by ionization of atoms in the sample. This ionization creates a vacancy in one of the energy levels of the atoms, which is almost immediately filled by an electron from a higher energy level. As these electrons

transfer to lower energy levels, the excess energy is emitted as X-rays (Buchan, 1983). Each element in the periodic table contains electrons in orbits with particular discrete energy levels, and it is on the basis of the differences between these specific energies that each element can be identified (Buchan, 1983). The spectrometer plots energies of different X-rays against counts of each specific energy, giving both qualitative and quantitative information about the elemental composition of a sample. EDS was therefore chosen as a technique to study the role that EPS plays in phosphate removal from wastewater in the activated sludge process.

The objectives of the study were therefore as follows:

- * to investigate ATP, TPC, MLSS and MLVSS of activated sludge, as measures of viable biomass, and its relation to phosphorus removal;
- * to investigate the *in situ* phosphorus content of EPS by means of EDS of air-dried sludge and bulk EDS analysis of extracted EPS.

REFERENCES

- Atlas R.M. (1982). Enumeration and estimation of microbial biomass. In: *Experimental Microbial Ecology*. Blackwell Scientific, Oxford. Pp. 84-101.
- Atlas R.M. and Bartha R. (1993). *Microbial Ecology-Fundamentals and Applications*. Third Edition. The Benjamin/Cummings Publishing Company, Inc., Redwood City, California, U.S.A.
- Beech I.B. and Cheung C.W.S. (1995) Interactions of exopolymers produced by sulphate-reducing bacteria with metal ions. *International Biodeterioration & Biodegradation* **35**: 59.
- Bosch M. (1992). P uptake kinetics of *Acinetobacter* in activated mixed liquor. M.Sc. Thesis, University of Pretoria, Pretoria, South Africa.

Kuba T., Van Loosdrecht M.C.M., Murnleitner E. and Heijnen J.J. (1997). Kinetics and stoichiometry in the biological P removal process with short cycle times. *Water Research* **31**(4): 918-928.

Kucknerowicz F. and Verstraete W. (1979). Direct measurement of microbial ATP in activated sludge samples. *Journal of Chemical Technology and Biotechnology* **29** :707-712.

Lilley I.D., Pybus P.J. and Power S.P.B. (1997). Operating manual for biological nutrient removal wastewater treatment works. Water Research Commission of South Africa. WRC Report No. TT 83/97.

Loaëc M., Olier, R. and Guezennec, J. (1997) Uptake of lead, cadmium and zinc by a novel bacterial exopolysaccharide. *Water Research* **31**: 1171-1179.

Lötter L.H. and Murphy M. (1988). Microscopic evaluation of carbon and P accumulation in nutrient removal activated sludge plants. *Water Science and Technology* **20**: 37-49.

Martin R.F. and Wilson T.E. (1994). Design and operation of nutrient removal facilities in the U.S. In: *Nutrient Removal From Wastewaters*. N.G. Horan, P. Lowe and E. Stentiford (eds.). Technomic Publishing Company, Inc., Lancaster, Pennsylvania, U.S.A. Pp. 173-186.

Momba M.N.B. (1995). Phosphate removal in activated sludge and its relationship to biomass. M.Sc. Thesis, University of Pretoria, Pretoria, South Africa.

Momba M.N.B. and Cloete T.E. (1996). Biomass relationship to growth and phosphate uptake of *Pseudomonas fluorescens*, *Escherichia coli* and *Acinetobacter radioresistans* in mixed liquor medium. *Journal of Industrial Microbiology* **16**: 364-369.

Muyima N.Y.O. (1995). Phosphate uptake by immobilized activated sludge in activated sludge mixed liquor. Ph.D. Thesis, University of Pretoria, Pretoria, South Africa.

Osborn D.W., Lötter L.H., Pitman A.R. and Nicholls H.A. (1986). Enhancement of Biological Phosphate Removal by Altering Process Feed Composition. Report to the Water Research Commission of South Africa by the City Health and City Engineers Departments of Johannesburg City Council. WRC Report no. 137/1/86.

Oldham W., Abraham K., Dawson R.N. and McGeachie G. (1994). Primary sludge fermentation design and optimization for biological nutrient removal plants. In: *Nutrient Removal from Wastewaters*. N.G. Horan, P. Lowe and E. Stintford (eds.). Technomic Publishing Company, Inc., Lancaster, Pennsylvania, U.S.A. Pp. 187-198.

Patterson J.W., Brezonik P.L. and Putnam H.U. (1970). Measurement and significance of adenosine triphosphate in activated sludge. *Environmental Science & Technology* **4**: 569-575.

Toerien D.F., Gerber A., Lötter L.H. and Cloete T.E. (1990). Enhanced biological P removal in activated sludge systems. *Advances in Microbial Ecology* **1**: 173-230.

Ubisi M.F., Jood T.W., Wentzel M.C. and Ekama G.A. (1997). Activated sludge mixed liquor heterotrophic active biomass. *Water SA* **23**(3): 239-248.

Wingender J., Neu T.R and Flemming H-C. (1999). What are bacterial extracellular polymeric substances? In: *Microbial Extracellular Polymeric Substances – Characterization, Structure and Function*. Wingender J., Ney T.R and Flemming H-C. (eds.). Springer-Verlag, Berlin, Germany. Pp. 1-19.