

Chapter 2

LITERATURE REVIEW

2.1 Introduction

General removal of phosphorus (P) from wastewater was introduced in Scandinavia in the late 1960's (Atkinson, 1999). At the time it was believed that P alone was limiting to algal growth and that only P removal would solve the problem of eutrophication. It is now known that both nitrogen (N) and P contribute to this phenomenon, although P is still regarded as the limiting growth factor of algae (Lilley *et al.*, 1997). Much research regarding both the chemical and biological removal of these nutrients from wastewaters have been conducted (Atkinson, 1999). Enhanced biological P removal (EBPR), which can be described as biological accumulation of soluble P as polyphosphate (poly-P) from the bulk liquid in excess of normal metabolic requirements, tends to be sensitive to many external parameters and, thus, is subject to fluctuations (Atkinson, 1999). This creates difficulties for wastewater treatment plants (WTP) to achieve and maintain full compliance with discharge regulations (Atkinson, 1999). A more comprehensive understanding of the microbial communities within mixed liquors of wastewater treatment systems is therefore required, which will ultimately assist in the improvement of system design and performance (Atkinson, 1999).

Chemical and civil engineers only consider the biological or chemical processes taking place in reactors when designing biological wastewater treatment systems, with little or no regard for individual microbial species or the entire microbial community involved (Atkinson, 1999). Process design seems to be tackled empirically, with biological reactions or processes occurring within a system (such as wastewater treatment) all lumped together and attributed to a single organism, of which the response accounts for the total system response (Atkinson, 1999). If one were to consider every microbial species present in a highly organized

community such as activated sludge, process models, designed to make quantitative and qualitative predictions as to the effluent quality from a particular design, would become increasingly complex and superfluous (Atkinson, 1999). It is evident from accomplishments to date that engineers have succeeded, to a certain degree, in modelling wastewater treatment systems, especially if one considers the tremendous successes achieved with biological P (bio-P) removal and nitrification denitrification processes at full-scale (Atkinson, 1999). However, there are limitations to this empirical approach and EBPR processes occasionally deteriorate in P removal efficiency (Atkinson, 1999), or even routinely fails (Bond *et al.*, 1995). In order to further optimize biological processes (for example oxidation of organics, bio-P removal, nitrification and denitrification), biological community analyses will have to play a more significant role in design (Atkinson, 1999). The better the microbial community structure and function of a system is known, the better the control and management of the system will be (Atkinson, 1999). Also the precise role and optimum concentration of biomass in systems should be determined to attain effluent standards and in broader terms make systems more efficient. The precise role of extracellular polysaccharides (EPS), beyond aggregation properties for cells in activated sludge has also not yet been determined and/or included in modelling. Elucidation of these factors will lead to a better-understood and optimized EBPR process.

2.2 Water

Water is the essence of life on earth. However, despite being the precious commodity it is, this resource is grossly ignored, only being considered under severe droughts and/or other crises. Water resources are simply too precious to be exploited. Umgeni Water (1996) states that 97.5 % of the earth's water is present as salt water, followed by frozen water and fresh water with 1.7 % and 0.8 %, respectively. Of the 0.8 % fresh water, two-thirds are lost due to evapo-transpiration, leaving approximately 0.3 % remaining fresh water. Some 20 % of this amount of potentially potable water is located in areas too remote for human access, and three-quarters of the remaining 80 % is not effectively captured for use as it arrives in the form

of monsoons and floods. Therefore, we only get to use less than 0.05 % of the total amount of water on the planet (Serageldin, 1998).

Available fresh water for human consumption is rapidly becoming scarcer due to massive amounts of pollutants being discharged directly into waterways. Subsequently, these pollutants contaminate ground water supplies and aquifers, diminishing reserves even further (Atkinson, 1999). The number of countries in the world today confronted by a water deficit total 26, equivalent to a total population of 300 million. However, this figure will increase drastically to approximately two-thirds of the world's population by the year 2050 if pro-active steps to curb the trend are not implemented (Abu-Zeid, 1998). It has been estimated that South Africa alone already utilizes approximately 60 % of its useable water supplies, a demand which will increase as the country strives for first-world status (Asmal, 1998). It is evident that water resource demands have escalated dramatically with population explosions, urbanization and the subsequent growth of mega-cities. Thus, the concept of sustainability of our natural resources has now become a matter of urgency and should no longer be viewed as an ideological philosophy (Atkinson, 1999).

N and P are the two key nutrients determining the productivity (trophy) of lakes and other water impoundments. Greater human activities in catchment areas (whether by urban development or agriculture) will lead to greater annual loads of N and P entering an impoundment. P is usually the limiting nutrient, since the planet's reservoir of P is generally much lower than that of N. P compounds also tend to be less soluble than N compounds (Atkinson, 1999).

2.3 Eutrophication

Eutrophication is a natural ageing process which usually occurs in lakes and other quiescent bodies of water through introduction of plant nutrients (P and N) to the impoundment (Atkinson, 1999). The process occurs naturally over hundreds of years, but the process has

been greatly accelerated by human activities in sensitive areas (Atkinson, 1999), including the use of detergents and fertilizers. Eutrophication of natural and man-made water impoundments is a problem encountered in many countries, including South Africa (Atkinson, 1999). However, problems experienced in South Africa which promulgate eutrophication are long storage times of dams and reservoirs, high summer temperatures and long daylight hours (Bolitho, 1976).

A water body can be classified according to its trophic status, describing the rate at which organic material is supplied to or by the relevant impoundment per unit of time (Wetzel, 1983). Thus, the term refers to the “productivity” of a water body, and therefore the amount of new organic biomass formed per unit time within the aquatic ecosystem (Atkinson, 1999). The trophic status of water is directly influenced by the concentration of P and N in solution (Atkinson, 1999).

In contrast to other nutritional components in the hydrosphere, P is the least abundant and usually limits biological productivity (Atkinson, 1999). The most significant form of P in lake systems is inorganic P (orthophosphate or PO_4^{3-}), of which the uptake satisfies metabolic requirements for living organisms (Muyima *et al*, 1997). Organic P is then synthesized intracellularly. The sum of inorganic and organic forms of P in a body of water (in particulate or solubilized form) is known as total P (TP). Release of phosphate from aquatic sediments is primarily accomplished by bacterial metabolism of organic matter at the bottom of the lake (Atkinson, 1999). This metabolism usually creates conditions conducive to the resolubilization of P by a decrease in ambient pH through the production of acids (Wetzel, 1983). Low trophic states in an impoundment can exist despite high P loadings, provided that water residence times are low (Thornton, 1980).

A classic example of eutrophication in South Africa is the deterioration of the Hartbeespoort Dam. Constructed 66 years ago, the dam became so overgrown with water hyacinth (60% of surface area) during the mid 1970's, that the Department of Water Affairs was compelled to spend R200 000 on various rehabilitation programmes (Rudd, 1979). Constant clearing of water weeds from the canal system amounted to R40 000 per annum. Hypereutrophic

conditions intensified during the 1980's, resulting in algal hyperscums (crusts of algal biomass) which were concentrated up to a metre thick at the dam wall (Haarhoff *et al.*, 1992). The dam now enjoys oligotrophic status, mainly by greater understanding of the prevailing conditions and implementation of intensive remedial programmes (Atkinson, 1999). Speculators, however, agree that the improvement might only be temporary (Atkinson, 1999).

Enrichment of water in an impoundment results in undesirable effects, the primary being the establishment of profuse algal blooms and excessive growth of nuisance causing aquatic plants (Atkinson, 1999). These include several species of blue-green (*Cyanobacteria*) and green (*Chlorophyta*) algae, the diatoms and flagellates, water grasses, rooted broad leaf plants as well as floating water plants (hyacinth)(Rudd, 1979). Joska and Bolton (1994) found *Cladophora glomerata* to be a major problem algal weed in South Africa. The algal population which blooms as a result of the lake or pond becoming rich in nutrients, eventually crashes as a result of exhaustion of micro-nutrients or accumulation of toxic by-products. The dead algal biomass is decomposed by heterotrophic microbes, which exhaust the dissolved oxygen in water, resulting in extensive fish kills and septic conditions (Atlas and Bartha, 1993). Secondary effects (a direct result of weed and algal growth) include (Rudd, 1979):

- * rapid oxygen uptake from the water causing lower layers of water to become anaerobic;
- * stratification with respect to oxygen and temperature of the water occurs due to the exclusion of sunlight and heat from the lower waters;
- * production of methane and sulphides from anaerobic digestion of dead plants which sink to the bottom of the water body;
- * these anaerobic acid conditions which occur cause the release of bound phosphates, iron and manganese from sediments;
- * production of sulphides results in fish kills (especially in winter) and a negative aesthetic appeal of the dam due to overturning of the water and the appearance that the water is black;
- * sulphides also cause a strong rotten egg smell to the water; the water can no longer be used for potable consumption purposes for livestock or humans as certain species of the algae are toxic.

Costs of purification of such “over-fertilized” waters escalate dramatically and the chemical quality of these waters become altered, resulting in the need for alternative treatment methods and higher chlorine demands (Atkinson, 1999). Many limnological studies have been conducted concerning eutrophication, concerned primarily with its causes and effects (Walmsley and Thornton, 1984; Chutter, 1990; Dillon and Molot, 1996). And results have conclusively indicated that eutrophication is promoted if P and N is released into a reservoir or a catchment area. These studies have also shown that eutrophication can be effectively controlled and curtailed if the P load to receiving waters is controlled. Gross eutrophication becomes marked when the inorganic soluble N and P concentrations in waters are in excess of 0.3 mg.l^{-1} and 0.015 mg.l^{-1} , respectively (Lilley *et al.*, 1997). It is virtually impossible to control eutrophication by limiting N, due to the ability of algae to fix and assimilate atmospheric N, which is made available to other aquatic life forms when these cells die and the assimilated N is released (Atkinson, 1999). The increased awareness that P is the limiting nutrient has led to the introduction of more stringent legislation governing the discharge of P to receiving water bodies (Atkinson, 1999).

It is not necessary to enforce nutrient limitation when effluents are to be used directly for irrigation purposes, industrial use or to be discharged directly to sea (Atkinson, 1999). Only if the effluent is to be discharged to a lake or impoundment where eutrophication and its effects are undesirable, for example sensitive catchment areas, is nutrient removal strictly enforced (Atkinson, 1999). In 1980, the Department of Water Affairs introduced legislation limiting the dissolved orthophosphate content of point source discharges to seven sensitive catchment areas to 1 mg.l^{-1} (Government Gazette, 1984) and this became known as the Special Phosphate Standard. A five-year grace period was, however, permitted before legislation enforcement in order to encourage P removal technology development and implementation. Excessive chemical precipitation was not encouraged due to its mineralising effect on water. The mechanism of biological P removal was studied intensely due to its attractive features and, especially low operating costs. During this period many existing WTP in South Africa were modified or new plants constructed to incorporate EBPR (Atkinson, 1999). Although initial capital outlay is high, the cost of EBPR operations in terms of operation and maintenance is significantly lower than chemical dosing plants, purely because chemical precipitants cannot be

recovered and are expensive (Atkinson, 1999). However, although the biological P removal mechanism is understood to a certain extent, it is still difficult to achieve full compliance with the Special Phosphate Standard and the biological process often has to be enhanced by simultaneous chemical dosing (Atkinson, 1999).

Problems caused by eutrophication are extensive in South Africa, being a country with limited water resources, and cannot be ignored due to the eminent threat on her limited water resources. To this end legislation have been made (Water Act, 1956). Continual failure of some activated sludge systems to remove limiting nutrients in their effluents continues to threaten our limited water resources. It is therefore empirical that effluents from wastewater treatment plants comply with the stipulated regulations of the Water Act of 1956. On the one hand it is also necessary that extensive research be carried out as to comprehend the phosphate removal mechanisms in order to assist in process design of wastewater plants which will optimally remove phosphate and other eutrophication-causing nutrients. The prevention of eutrophication can be achieved by removing phosphate from wastewater by chemical as well as by biological or by combination both methods (Momba and Cloete, 1996a,b). The biological methods due to their low maintenance expenses and by virtue of their environmental friendliness have become most popular of recent.

2.4 Algae

Algae are an essential component of the aquatic environment and play a significant role in eutrophication. Algae are defined as an assemblage of eukaryotic organisms that contain chlorophyll and carry out oxygenic photosynthesis. Most algae are of microscopic size and hence are clearly microbes. There are, however, a number of forms, which are macroscopic, for example species of seaweed, which can grow over 100 m in length.

Algae are important autochthonous members of freshwater ecosystem. In large, deep lakes, phytoplankton contributes most of the organic carbon, which supports the growth of the heterotrophic organisms in freshwater ecosystems (Atlas and Bartha, 1993).

Algae are largely, if not exclusively photoautotrophic, their growth inevitably depending upon intercepting sufficient light energy to sustain photosynthetic carbon fixation in excess of immediate respiratory needs. Radiant energy of suitable wavelengths (photosynthetically-active radiation) is neither universally nor uniformly available in water but it is attenuated hyperbolically with depth, through absorbency by the water and scattering by particulate matter there is less available photosynthetically-active radiation with increasing depth. For any given autotroph in a given water mass, there is likely to be a critical depth (the light compensation point) below which nett accumulation of photosynthetate is impossible. It is therefore implicit that the long-term survival of the algae depends upon its ability to enter or remain in the upper illuminated part of the water mass, for at least parts of its life.

2.5 Wastewater

Wastewater consists of materials derived from domestic sewage or industrial processes, which for reasons of public health and for recreational, economic and aesthetic considerations cannot be disposed of merely by discarding them untreated into lakes or streams. Rather, the undesirable and toxic materials in water must either be removed or rendered harmless. Gray (1989) defines wastewater as consisting of domestic (sanitary) or industrial (trade) effluents. Domestic wastewater comes exclusively from residences, commercial buildings and institutions such as schools and hospitals, while industrial wastewater comes from manufacturing plants. Large towns and cities have a mixture of both domestic and industrial wastewater which is commonly referred to as municipal wastewater and normally include effluents from the service industries such as dairies, laundries and bakeries, as well as a variety of small factories (Gray, 1989). Sewage is a complex mixture of natural inorganic and organic materials with a small proportion of man-made substances.

The main source of pollution in sewage is human excreta with smaller contributions from food preparation, personal washing laundry and surface drainage. The chemical and physical nature of wastewater can be further complicated by the inclusion of industrial wastes which are composed of strong spent liquors from main industrial processes and comparatively weak wastewater from rinsing, washing and condensation (Gray, 1989).

Sewage composition is normally measured in terms of BOD₅ (the five-day biochemical oxygen demand test), COD (chemical oxygen demand), suspended solids (SS), ammonia (NH₄⁺) and the P content (Gray, 1989). Enrichment of waters with nutrients, notably nitrogen and P in conjunction with carbon dioxide results in the prolific growth of algae (algal blooms), a process previously defined as eutrophication (Lilley *et al.*, 1997).

2.5.1 Inorganic properties of sewage

Inorganic components of sewage are sodium, calcium, potassium, magnesium, chlorine, sulphur (as sulphates and other forms), phosphate, bicarbonates and ammonia. Traces of heavy metals are also found. Domestic wastewater contains a very wide range of inorganic salts and trace elements, including all those necessary for biological growth and activity. Among the major ions in wastewater are chloride, nitrogen and P (Gray, 1989).

N and P are both essential nutrients for plant growth. N is generally necessary for the synthesis of protein and biological growth. In fresh wastewater, N is primarily present as proteinaceous matter and urea. This organic N is rapidly decomposed by bacterial action in the case of proteins, or by hydrolysis in the case of urea to ammonia, the concentration of which, in wastewater, is indicative to some extent of its age (Gray, 1989).

Ammonia exists in aqueous solution as either the ammonium (NH₄⁺) or as ammonia (NH₃) depending on the pH of the wastewater. At pH values of >7 the equilibrium of the reaction $\text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-$ is displaced to the left so that ammonia predominates and at pH

values < 7 equilibrium moves to the right and ammonium predominates. The oxidized forms of ammonia, nitrite and nitrate are normally absent from fresh sewage as they are products of the biological oxidation process within the treatment plant.

P is present in sewage in three distinct forms, i.e. orthophosphate, polyphosphate and organic phosphate. Organic P is a minor constituent of sewage and like the polyphosphates requires further decomposition to the more assimilable orthophosphate form, which is normally fairly slow. Sources of phosphate in sewerage are the alkyl-benzene-sulphonate detergents and surfactants (Gray, 1989).

2.5.2 Organic properties of sewage

Organic matter comprises of carbon, hydrogen, and oxygen with N frequently present. Carbohydrates comprise the largest group in sewage followed by non-volatile and volatile acids. Urea is a major component of urine but is hydrolyzed so rapidly to ammonia that it is only found in very fresh sewage. Fats are the major organic constituents in the suspended solids fraction, together with carbohydrates and proteins account for 60-80% of the organic carbon present (Gray, 1989).

2.6 Wastewater treatment

Effective removal of pollutants from wastewater prior to discharge is of prime importance and the production of high quality effluents can have an economic advantages by supplementing existing water resources (Slim, 1987).

Wastewater treatment is necessary before disposal of effluent into rivers, lakes or ponds so as to avoid excessive eutrophication. If the wastewater was recycled, additional costs would be incurred by water purification works using this water due to problems associated with tastes and odours, filter and screen clogging, slime accumulation in pipes and toxicity caused by

certain algae. In addition to these treatment problems, the appeal of the water for recreational purposes is also reduced (Lilley *et al.*, 1997).

Wastewater treatment is a process achieved by physical, chemical and biological nutrient removal referred to as Biological Nutrient Removal (BNR). This process is divided into three levels (Alas and Bartha, 1993):

- * primary treatment
- * secondary treatment
- * tertiary treatment.

In wastewater treatment, the objective of BNR is to remove the primary nutrients which cause eutrophication namely carbon, N and P (Lilley *et al.*, 1997).

Some of the floc material is then returned to the aerator to serve as inoculum, while the rest is sent to the sludge digester. The residence time in an activated sludge system is generally 5-10 h. The main process occurring during this time is adsorption of soluble organic matter to the floc and incorporation of some of the soluble material into microbial cell material. The BOD of the liquid is considerably reduced by this process (75-90%) but the overall BOD liquid plus solids is only slightly reduced, because most of the absorbed organic matter still resides in the floc. The main process of BOD reduction thus occurs in the sludge digester, to which the floc is transferred (Atlas and Bartha, 1993).

2.6.1 Conventional activated sludge systems

Conventional activated sludge systems have been used to purify domestic wastewater for many decades (Toerien *et al.*, 1990). Conventional activated sludge systems consist primarily of an aeration basin and a settling basin. Microbes are retained in the system as an activated sludge. It should be noted that the conventional activated sludge process is not designed or operated to

achieve biological excess P removal (BEPR). The suspended bacteria of this system use P in quantities which satisfy only their basic metabolic requirements (Pitman, 1984).

Activated sludge systems have become an integral part of municipal wastewater treatment. The process relies upon the dense growth of microbes in a reactor where air is continuously supplied to allow for carbonaceous oxidation (Atkinson, 1999). The term “activated sludge” refers to an aerobic slurry of microbes which can be removed from the process by sedimentation and returned in quantifiable amounts to the wastewater stream (Grady and Lim, 1980). All activated sludge systems operate with the following characteristics in common (Grady and Lim, 1980):

- * utilization of a flocculent slurry of microbes to remove organic matter from the surrounding wastewater;
- * prior to effluent discharge from the plant, microbes are removed by sedimentation, thereby reducing outgoing solids loads;
- * settled microbes are recycled to the biological reactor via a clarifier underflow;
- * dependency of plant performance on the mean cell residence time (MCRT or sludge age) in the system.

In the presence of both oxygen and nutrients, high rates of microbial growth are achieved. Microbial metabolism of the organic matter present results in the production of oxidised end-products such as carbon dioxide, nitrates, sulphates and phosphates, as well as the biosynthesis of new microbial biomass (Gray, 1989; Horan, 1990; Bitton, 1994; Muyima *et al.*, 1997, Atkinson, 1999). In conventional activated sludge systems, the aspects of the process that can be varied independently to achieve different responses, are the process layout, the loading rate and the method of aeration (Atkinson, 1999).

The basic ecological unit of activated sludge is the floc. Microbial floc formation is essential to the success of activated sludge processes, as it allows for rapid and efficient separation of sludge from treated wastewater in the sedimentation tank. The exact mechanism of formation of flocs is not well understood, but seems to be almost entirely bacterially mediated (Muyima

et al., 1997). The model proposed by Forster and Dallas-Newton (1980), explaining the structure of the floc, has gained acceptance and forms the basis of our perception of floc arrangement. According to this model, filamentous microbes form the matrix or backbone of the structure to which zooglear (floc-forming) microbes attach (Bitton, 1994). Attachment is thought to be brought about by exopolysaccharides present in the form of a capsule or discrete slime layer (Horan, 1990). These polymers are composed of sugars, amino sugars, uronic acids and amino acids, are produced during the endogenous growth phase and form the three-dimensional matrix (Bitton, 1994). As extracellular polysaccharide production gradually continues, other microbes and colloidal material become entrapped in the matrix and the floc diameter increases. Surface charges on the microbial cells, as well as bridge formation by polyvalent cations, also contribute to flocculation (Gray, 1989). The very rigid structure of the floc has impeded the quantitative analysis of the activated sludge community structure, as complete dispersion of the floc is extremely difficult. Clumping of cells in the floc leads to an underestimation of the number of active cells present in the mixed liquor when using viable plate count techniques (Atkinson, 1999). Problems attributable to poor floc formation can be one of two causes: non-filamentous bulking and filamentous bulking. These characteristics are undesirable and will have negative economic consequences to the plant in question (Atkinson, 1999).

The activated sludge process has been developed primarily for carbon, N and P removal (Momba, 1995). The activated sludge process is capable of producing an effluent of high quality at reasonable cost (Saayman, 1995). Activated sludge reactors are relatively resistant to shock loads and can achieve acceptable effluent in spite of dynamic inputs (Grady and Lim, 1980). The activated sludge system is efficient and flexible and is able to withstand considerable variations in sewage flow rate and concentration (Atlas and Bartha, 1993). The potential of activated sludge in controlling eutrophication is well known so is its capability to prevent salinity as a result of discharging in the receiving water body. Increasing industrial and population demands on water supplies have ensured that the activated sludge process has had to undergo various modifications in order to cope with the increase in carbonaceous and nutrient loadings (Atkinson, 1999).

Single aerobic systems are no longer sufficient to treat domestic and industrial wastes and changes incorporated into the process relate to size, number and configuration of the reactors, flow direction and mixed liquor recycle and flow regime within the reactors (Wentzel *et al.*, 1992). P and N removal from wastewater is essential to ensure the sustainability of potable water supplies. However, in conventional systems, phosphate is only taken up in quantities, which satisfy basic bacterial metabolic requirements. Thus, nutrient loads to receiving waters would subsequently remain excessive (Atkinson, 1999). Therefore, in order to encourage resident microflora in the system to accumulate P in excess of basic metabolic needs, modifications and extensions to conventional reactor configurations have been implemented.

Nitrification denitrification systems were developed in 1974 by Barnard, who recognized the importance of an anaerobic zone at the head of the biological reactor to encourage excess biological P uptake (Wentzel *et al.*, 1992). He realized that biological P removal was induced if the organism mass was stressed by subjecting it to conditions of anaerobiosis, thereby releasing P to the bulk liquid (Ekama *et al.*, 1984). This particular system was termed the Bardenpho system, and, since its inception, a number of further modifications have taken place to enhance biologically mediated nutrient removal (Atkinson, 1999).

2.6.2 Biological P removal

Biological treatment of wastewater can be accomplished in a number of ways, the determining factors depending upon efficiency and economics of wastewater contact with the microbes concerned (Atkinson, 1999). Some technologies depend upon immobilization techniques (such as biofilm systems), suspended growth systems (such as activated sludge) and lagoon systems (such as anaerobic and aerobic lagoons, maturation ponds and high-rate algal ponds)(Muyima *et al.*, 1997). However, in this section only the activated sludge process will be discussed.

Although P can be removed from wastewater by chemical precipitation (precipitants including ferrous and ferric ions, aluminium salts, lime and polyelectrolytes), biological P removal has

several advantages over chemical precipitation. These include low sludge production, the fertilizer value of the sludge, less expensive operation costs and the avoidance of anion enrichment of the treated water (Wentzel, 1992; Lilley *et al.*, 1997; Romanski *et al.*, 1997). Although biological P removal (BPR) can effectively compete with chemical removal in terms of cost, the characteristics of the influent wastewater will dictate the performance of the removal mechanism (Atkinson, 1999).

However, a distinction must be made between P removal by additional chemical dosing by the plant operator in addition to biological action and P removal by means of chemical changes of the wastewater as a result of biological action like changes in alkalinity, acidity and pH (Atkinson, 1999). It can be assumed that when treating municipal wastewater by an appropriately designed activated sludge process, excess P removal is principally mediated by the biological mechanisms of adsorption and absorption (Ekama *et al.*, 1984).

There exists within the activated sludge mixed liquor a microbial population capable of accumulating soluble P as poly-P granules (volutins). These microbes are termed P accumulating organisms (PAO) (Wentzel *et al.*, 1988) and are able to take up P in excess of normal metabolic requirements. The process is, therefore, termed biological excess P removal (BEPR). These organisms will, however, only proliferate in the system, and the mechanism of poly-P accumulation will only be induced if certain structural alterations are made to the aeration basin (for example an anaerobic zone at the head of the basin). Research has shown that the anaerobic zone leads to the enrichment of fermentative organisms such as *Enterobacter*, *Klebsiella*, *Citrobacter*, *Pasteurella*, *Proteus* and *Aeromonas* (Lötter and Murphy, 1985). The PAO are then able to accumulate the fermentation products (including acetic, lactic, succinic, propionic, butyric acids) and store them intracellularly as carbon and energy reserves, for example poly- β -hydroxyalkanoates (PHA's), of which poly- β -hydroxybutyrate (PHB) is an example (Satoh *et al.*, 1992; Lilley *et al.*, 1997). As a result of internal carbohydrate accumulation, the PAO release P back into solution and the orthophosphate concentration in the anaerobic zone increases. In the subsequent aerobic reactor, the PAO utilize the internally stored carbon for growth, which increases their biomass in the sludge. PHB is also used as an energy source in the aerobic reactor to take up P from

the bulk solution and to re-synthesize the poly-P degraded in the anaerobic reactor. P uptake, together with an increase in the quantity of PAO in the system, leads to a net removal of P from the wastewater (Wentzel, 1992). Soluble P, accumulated as biological poly-P in the solid phase, is then removed from the system with the waste sludge.

According to current models, uptake of P in the aerobic zone is directly related to the quantity of P released in the anaerobic zone, in other words, the more P released in the anaerobic zone, the greater the ability of the biomass to take up P under aerobic conditions (Helmer and Kunst, 1998). The amount of P which can be removed by biological P activity is also directly coupled to the amount of volatile fatty acid (VFA) that the PAO accumulate in the anaerobic reactor (Henze, 1996).

Although the process of EBPR is well understood from a technical aspect, it remains difficult to achieve consistent and reproducible removal rates at the full-scale due to a lack of understanding of the process from a biochemical and microbiological point of view (Satoh *et al.*, 1996; Wang and Park, 1998). Without understanding the correlation between the PAO community structure within activated sludge mixed liquor and the wastewater plant performance, reliable and efficient biological P removal operations will remain difficult to design (Atkinson, 1999).

2.6.3 Modified activated sludge systems

Various modifications have been made on the activated sludge process in order to meet most wastewater treatment needs (Toerien *et al.*, 1990). The need for nutrient removal from wastewater has been stated and thus modifications of the activated sludge processes have been developed to achieve maximum potential in terms of nutrient removal. Barnard (1975) reviewed the available technology for biological nitrification and denitrification and proposed the Bardenpho process (Figure 2.1). The Bardenpho process consists of four stages i.e.: primary anoxic, primary aerated, secondary anoxic and secondary aerated followed by a

clarifier. Carbon removal and nitrification takes place in the main aeration basin. Nitrified mixed liquor is recycled from this basin to the primary anoxic basin where, in the absence of free dissolved oxygen, denitrification occurs, using organic compounds as the carbon source. Mixed liquor not recycled to the primary anoxic basin passes on to the secondary anoxic basin, where additional denitrification takes place at a slow rate under conditions of endogenous respiration. Before entering the secondary clarifier, the mixed liquor from the secondary anoxic basin passes through a small re-aeration basin, the function of which is to ensure that:

- * $\text{NH}_3\text{-N}$ formed during endogenous respiration in the secondary anoxic basin is converted to $\text{NO}_3\text{-N}$;
- * aerobic conditions exists in the secondary clarifier, as any denitrification that occurs under anoxic conditions would produce nitrogen gas that could cause rising sludge;
- * aerobic conditions exists in the secondary clarifier to prevent P release from the sludge into the effluent (Toerien *et al.*, 1990).

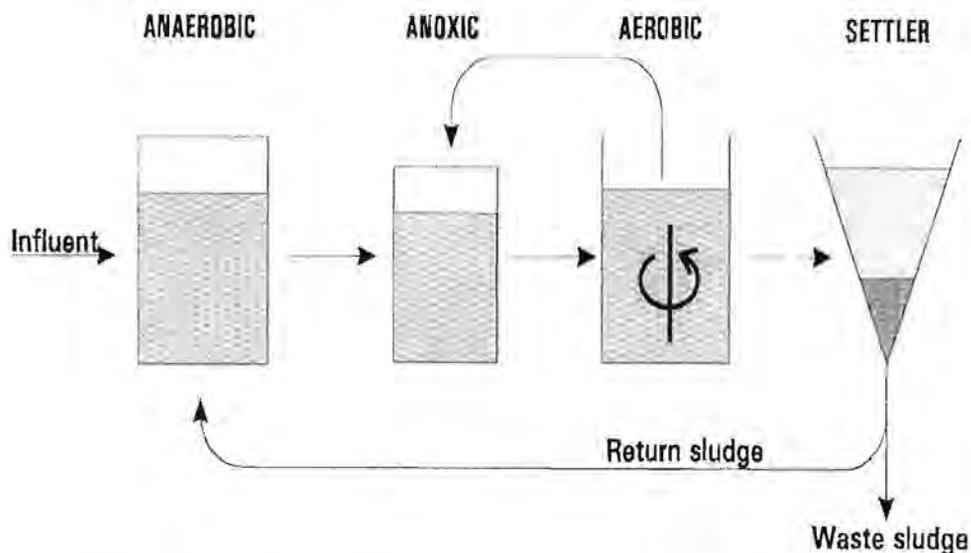


Figure 2.1: The three-stage Phoredox system (modified Bardenpho)(Erasmus, 1997).

2.6.3.1 Anaerobic zone

The term “anaerobic zone” (in the activated sludge process) means that the contents of the zone are kept, as far as possible, deficient of nitrate and dissolved oxygen and the input of nitrate and oxygen to this zone is severely restricted (Buchan, 1984; Lilley *et al.*, 1997). The anaerobic zone is characterized by an oxidation-reduction potential that excludes any oxidative processes. The most important reactions are anaerobic fermentation (especially acido- and aceto-genesis), poly-P depolymerization and desulphation. Readily degradable organic substrate from wastewater is converted to low-molecular organic compounds, which are then stored by PAO in the form of PHB (Wanner and Grau, 1988). The anaerobic zone is essential for phosphate removal, as the bacteria in the activated sludge passing through this zone are preconditioned to take up excess phosphate under aerobic conditions (Momba, 1995).

The presence of nitrate in an anaerobic zone has been reported as a handicap to the phosphate removing potential of the activated sludge systems and there is need to exclude nitrate in the anaerobic zone. In the anaerobic zone, sludge from the clarifier jointly flows with the influent wastewater. Influent nitrate levels has to be kept low in order to ensure that nitrates returned with the underflow of the final clarifier does not negatively affect the performance of the initial oxygen limiting zone (Momba, 1995).

2.6.3.2 The anoxic zone

The anoxic zone refers to the presence of nitrates and the absence of dissolved oxygen (Buchan, 1984; Pitman, 1984; Streichan *et al.*, 1990). Substrate is oxidised anoxically in the anaerobic zone (i.e. electrons from organic compounds are transferred to nitrate- and/or nitrite-nitrogen which is reduced to elementary N) (Wanner and Grau, 1988).

The anoxic zone is the main denitrification reactor in the activated sludge process. It is fed by the effluent from the anaerobic zone and the mixed liquor recycled from the aerobic zone.

This zone is fundamental to the biological removal of N and due to the absence of oxygen (O₂). Non-poly-P organisms utilize nitrate as electron acceptors, reducing it to N gas, thus carrying out denitrification of the mixed liquor and allowing the elemental N formed to escape as a gas (Lilley *et al.*, 1997).

2.6.3.3 Primary aerobic zone

The primary aerobic zone within the activated sludge plant, is the zone which is aerated by introducing either air or oxygen (Lilley *et al.*, 1997). Various mechanical devices are used for aerating this zone (Nogaj, 1980). The main function of this zone is to oxidize organic material in the sewage, oxidize ammonia to nitrite and to nitrate, and provide an environment in which the biomass can take up the phosphate released in the anaerobic zone, plus the phosphate which enters the process in the feed sewage. (Momba, 1995; Wanner and Grau, 1988). The aeration rate seems to be the principal operational determinant of the efficiency of phosphate removal (Pitman 1984).

Ammonia is oxidised in the aerobic zone. Chemoautotrophs are responsible for this oxidation. Ammonia is oxidized to nitrate by *Nitrosomonas*, *Nitrospira* and *Nitrosolobus* spp., whereas nitrite is oxidized to nitrate by *Nitrobacter*, *Nitrospira* and *Nitrococcus* spp. (Momba, 1995). The nitrifying organisms are sensitive to the pH and alkalinity of the wastewater. The growth rate of these organisms is severely inhibited outside the pH range of 7 to 8,5. During the conversion of ammonia to nitrate, hydrogen ions are released resulting in a decrease in the alkalinity of the wastewater. If the alkalinity of wastewater decreases to below 40 mg.l⁻¹ (as CaCO₃), the pH becomes unstable resulting in a sharp decrease in nitrification efficiency due to the retarded growth rate of the autotrophs (Lilley *et al.*, 1997).

2.6.3.4 The clarifier

The clarifier is used for producing 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). The operation of a clarifier can greatly affect its performance. However, sludge settling properties have an overriding effect with the clarifier operations having to be adapted to the sludge settling characteristics. The latter are largely influenced by the operating conditions in the biological reactor (Pitman, 1984).

2.6.4 Preconditioning of the poly-P organisms for EBPR

Under aerobic conditions the PAO are not able to compete with non-PAO for substrate (food sources) such as glucose or other saccharides. Under anaerobic conditions (no nitrate or oxygen present) and in the presence of short chain fatty acids (SCFA or VFA) (Osborn *et al.*, 1986) the poly-P-organisms break down and hydrolyze stored poly-P. This process releases orthophosphate to the surrounding liquid and leads to the phenomenon known as P release in the anaerobic zone. The bound energy released in the hydrolyzing poly-P is utilised by the poly-P organisms to absorb, process and store the SCFA within the organisms, thereby reserving substrate for their exclusive use when they enter an environment which contains external electron acceptors such as nitrate or oxygen. In this way they do not have to compete with the non-poly-P-organisms which are unable to utilize SCFA under the anaerobic conditions because of lack of a suitable electron acceptor (Alexander *et al.*, 1994, Lilley *et al.*, 1997; Osborn *et al.*, 1986).

Upon re-entering the aerobic environment the poly-P organisms utilise the reserved SCFA both for growth and to replenish their poly-P pool by abstracting orthophosphate from the surrounding medium. This gives rise to the phenomenon known as excess P uptake which occurs in aerobic environments (Alexander *et al.*, 1994; Lilley *et al.*, 1997).

Alexander *et al.* (1997) postulated that in order to promote the growth of poly-P organisms the following conditions are required:

- * an anaerobic environment which receives or generates an adequate supply of SCFA, the mass of released P being proportional to the mass of SCFA utilised by the poly-P system; followed by
- * an aerobic environment for P uptake by the poly-P organisms.

2.7 Biogeochemical cycles related to wastewater treatment

Pathways by which nutrients are circulated within ecosystems are referred to as biogeochemical cycles. For every element the cycling process involves (Mader, 1998):

- * a reservoir where the source normally remains unavailable to the surrounding biota (for example rocks and sediments); assimilable forms of the element are only released by various geological components;
- * an exchange pool, represented by an elemental source from which organisms are able to accumulate nutritional components (for example hydrosphere and atmosphere);
- * a biotic community through which elements move along either simple or elaborate food chains.

N is involved in a gaseous cycle where the element is assimilated from and returned to the atmosphere. P, on the other hand, is sedimentary, where the element is absorbed from the soil by plant roots, exchanged to heterotrophs and returned to the soil by decomposers (Mader, 1998). Both P and N are interchangeable with reference to ecosystems they inhabit, as both elements are capable of moving between terrestrial and aquatic systems (Atkinson, 1999).

Microbes like all biological systems respond to their environment. Microbes have diverse metabolic capabilities and high enzymatic activity rates, and thus they play an important role in

biogeochemical cycling. Microbes are vital for the functioning and maintenance of the earth's ecosystems (Ehlers, 1995). Biogeochemical cycling describes the movement and conversion of materials by biochemical activities within the ecosphere. Biogeochemical cycles include physical transformation (e.g. dissolution, precipitation, volatilization and fixation), chemical transformation (e.g. biosynthesis, biodegradation, and oxido-reductive biotransformations) and various other combinations of physical and chemical changes (Atlas and Bartha, 1993). Microbes play a major role in biogeochemical cycling because of their ubiquity, diverse metabolic capabilities and high enzymatic activity rates (Jørgensen 1989; Pomeroy, 1984). Wastewater treatment by biological nutrient removal or activated sludge processes fulfills the biogeochemical cycles of the nutrients involved in the process. In wastewater treatment the objective of BNR is to remove the primary nutrients which cause eutrophication namely carbon, N and P from wastewaters (Lilley *et al.*, 1997).

2.7.1 The carbon cycle

Carbon is one of the important nutrients contributing to the growth of algae and other macrophytes in aquatic environments and thus is directly associated with eutrophication. Wastewater treatment processes have to minimise the discharge of carbon to water bodies in order to minimize eutrophication. Carbon in wastewater streams occurs as organic and inorganic compounds. Organic compounds can be utilised by heterotrophs, while inorganic compounds are utilised by autotrophs.

Boths forms of carbon are removed from wastewater through a series of redox reactions, oxidising the carbon source to carbon dioxide and water (Lilley *et al.*, 1997). It is empirical that an understanding of the carbon cycle be established in order to maximize knowledge on carbon removal in wastewater treatment.

In the carbon cycle, carbon moves from reservoirs in the atmosphere and oceans through living organisms back to these reservoirs. Carbon is introduced into the atmosphere by aerobic

respiration, fossil fuel burning and volcanic eruptions which release carbon from rocks deep in the earth's crust (Starr and Taggart, 1981). In the atmosphere carbon exists as bicarbonates or carbonate ions (Purves, *et al.*, 1995).

Carbon undergoes oxidation and reduction during the carbon cycle. Carbon is assimilated by autotrophs in gaseous form (CO_2). The involvement of microbes in the carbon cycling can be best discussed within the context of the food web. Autotrophic organisms carry out the net fixation of carbon dioxide to form organic compounds, this includes both the photosynthetic and chemolithotrophic microbes. The most important groups of microbes, in terms of their ability to convert carbon dioxide to organic matter are the algae, the cyanobacteria, and the green and purple photosynthetic bacteria. Chemolithotrophic microbes contribute to a lesser extent (Atlas and Bartha, 1993). Photosynthetic microbes fix CO_2 via the pentose-phosphate or Calvin cycle.

Microbes are capable of incorporating CO_2 through the phospho-enol pyruvate carboxylase system. In the case of heterotrophic microbes, exchange, but no net CO_2 fixation occurs, but some chemolithotrophic microbes use this system instead of, or in addition to the pentose cycle for net CO_2 fixation (Atlas and Bartha, 1993).

Methanogenic archaeobacteria play an important role in the anaerobic reduction of carbon dioxide. The resulting methane can be utilised only by a limited number of microbes (Gottschalk, 1979); these methylotrophs are important in carbon cycling, however, especially as it relates to atmospheric carbon transfers.

2.7.2 The nitrogen cycle

Matter required for maintaining and reproducing life is obtained from five major nutrient sources, carbon, hydrogen, oxygen, N and P are more often the limiting nutrients, although N can be readily obtained from the air. The availability of N and P (and sometimes carbon)

therefore often limits the growth of algae and plant life. This in turn limits the growth of the heterotrophs (Lilley *et al.*, 1997).

N is essential to life and is a component of proteins and nucleic acids in microbial, animal, and plant cells. In fresh wastewater, N is primarily present as proteinaceous matter and urea. This organic N is rapidly decomposed by bacterial action in the case of proteins, or hydrolysis in the case of urea to ammonia, the concentration of which in wastewater is indicative to some extent of its age (Gray, 1989). N is the most abundant gas in the atmosphere. Most organisms are, however, incapable of utilizing N gas unless it is first converted to ammonia. This is because N gas is a very stable molecule that can undergo changes only under extreme conditions (for example electrical discharge, high temperatures and pressures).

2.7.2.1 Microbiology of the nitrogen cycle

Atlas and Bartha, 1987 and Grady and Lim, 1980 agree that microbiologically the N cycle consists of several steps which include:

- * N fixation
- * N assimilation
- * mineralization
- * nitrification
- * denitrification

2.7.2.1.1 Nitrogen fixation

N fixation is a process only a few species of bacteria and cyanobacteria are capable of carrying out. N fixation results with the production of ammonia. Organisms capable of N fixation are classified into two categories:

- * nonsymbiotic N-fixing microbes. Included in this category are members of *Azotobacter* (for example *A. Agilis*, *A. chroocum*, *A. vinelandii*), gram-negative bacteria that form cysts and fix N in soils and other environments. Other N-fixing microbes are *Klebsiella*, *Clostridium* (anaerobic, spore-forming bacteria active in sediments) and cyanobacteria (for example *Anabaena* and *Nostoc*). The latter fix N in natural waters and soils and their fixation rate is said to be ten times higher than free N-fixing microbes in soils (Bitton, 1994).

- * Symbiotic N-fixing microbes. These organisms enter into symbiotic relationships with higher plants. A popular example is that of legumes and Rhizobia. *Rhizobium* infects the roots of legumes and fix N (Atlas and Bartha, 1993). N fixation in this symbiotic relationship is of great importance both in global N cycling and in agriculture.

2.7.2.1.2 Nitrogen assimilation

The term assimilation refers to the incorporation of nutrients into the biomass of an organism (Atlas and Bartha, 1993). Both heterotrophic and autotrophic microbes take up and assimilate NO_2^- and NO_3^- after reduction to NH_4^+ . Bitton (1994) states that assimilation is the process responsible for some N removal in wastewater treatment plants. Cells convert NO_3^- or NH_4^+ to proteins and grow until N becomes limiting. The proportion of carbon to N (C:N) assimilation is 100:10.

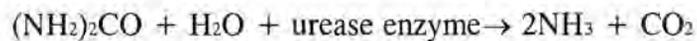
2.7.2.1.3 Nitrogen mineralization (ammonification)

The mineralization of N is also known as ammonification. Bitton (1994) defines ammonification as the transformation of organic nitrogenous compounds to inorganic forms. Ammonification is a process driven by a variety of microbes (e.g. acinomyces and fungi).

Proteins are mineralized to ammonium ions during ammonification in accordance with the following sequence:

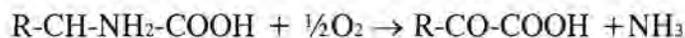
proteins → amino acids → deamination to NH_4^+

For example in the transformation of urea to ammonia:

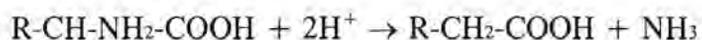


Ammonification usually involves deamination as one of the intermediate steps, prior to the formation of NH_3 or NH_4^+ . Deamination of ammonia takes place either oxidatively or by reduction depending on the availability of oxygen and other important extracellular proteolytic enzymes.

Oxidative deamination:



Reductive deamination:

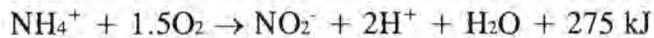


2.7.2.1.4 Nitrification

Nitrification is defined as the process in which ammonia is oxidized to nitrite and nitrite to nitrate (Atlas and Bartha, 1993; Bitton 1994). Nitrification is the first step in N removal in wastewater treatment processes (Lilley *et al.*, 1997). It is a process primarily carried out by aerobic, chemolithotrophic bacteria of the family *Nitrobacteraceae* (Atlas and Bartha, 1993).

Organisms responsible for nitrification may be divided into two groups:

- * organisms responsible for the conversion of NH_4^+ to NO_2^- which include *Nitrosomonas* species (for example *N. europaea* and *N. oligocarbogenes*). The conversion of ammonia to nitrite takes place via hydroxylamine. Included also amongst ammonium oxidizers are *Nitrosospira*, *Nitrosococcus* and *Nitrosolobus* (Bitton 1994).



- * organisms responsible for conversion of nitrite to nitrate (NO_2^- to NO_3^-) are the *Nitrobacter* species (for example *N. agilis* and *N. winogradsky*) as well as *Nitrospora* and *Nitrococcus*.



The oxidation of NH_4^+ to NO_2^- is an exogenic exothermic process. The generated energy is utilised by microbes to assimilate CO_2 . Carbon dioxide, bicarbonate and carbonate satisfy the carbon requirements of nitrifiers. Nitrification is favored by the presence of oxygen and sufficient alkalinity to neutralize the hydrogen ions produced during the oxidation process. Although autotrophic nitrifiers are predominant in nature, nitrification may also be carried out by heterotrophic bacteria (for example *Arthrobacter*) and fungi (for example *Aspergillus*). Nitrification occurs in the aeration tank (4-6 hr retention time), and sludge containing high numbers of nitrifiers is recycled to maintain nitrifier activity. Factors such as ammonia/nitrite concentration, oxygen concentration, pH, temperature, BOD_5 :TKN ratio and the presence of toxic chemicals all control nitrification in wastewater treatment plants (Bitton, 1994).

2.7.2.1.5 Denitrification

Atlas and Bartha (1993) defined denitrification as the formation of gaseous N or gaseous N oxides from nitrate or nitrite by microbes. Denitrification is the second stage in N removal culminating in the production of N gas, which eventually escape from the anaerobic zone

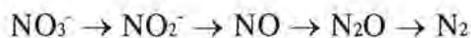
reactor in the activated sludge process. The two most important mechanisms for denitrification are accomplished by assimilatory and dissimilatory nitrate reduction (Bitton, 1994).

Assimilatory nitrate reduction:

During assimilatory nitrate reduction, nitrate is taken up and converted to nitrite and then to ammonium by microbes. This process involves several enzymes that convert NO_3^- to NH_3 , which is subsequently incorporated into proteins and nucleic acids.

Dissimilatory nitrate reduction:

Dissimilatory nitrate reduction is an anaerobic respiration by which NO_3^- serves as an electron acceptor. NO_3^- is reduced to nitrous oxide (N_2O) and N gas (N_2). N gas liberation is the predominant output of denitrification. N_2 , however, has low water solubility and thus tends to escape as rising bubbles. The microbes involved in denitrification are aerobic autotrophic or heterotrophic microbes that can switch to anaerobic growth when nitrate is used as the electron acceptor. The denitrification process is carried out according to the following sequence mediated by the nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase enzymes, respectively:



Denitrifiers belong to several physiological (organotrophs, lithotrophs, and phototrophs) and taxonomic groups and they can use various energy sources (organic or inorganic chemicals or light). Microbes that are capable of denitrification belong to the following genera: *Pseudomonas*, *Bacillus*, *Spirillum*, *Hyphomicrobium*, *Agrobacterium*, *Acinetobacter*, *Propionibacterium*, *Rhizobium*, *Cytophaga*, *Thiobacillus* and *Alcaligenes*. The most widely spread genus is probably *Pseudomonas* (*Ps. fluorescens*, *Ps. aeruginosa*, *Ps. denitrificans*).

The benefits of denitrification in wastewater treatment are the following:

- * it reduces the concentration of oxidized N in the water, not only as nitrate but also nitrite which may be present in high concentrations in certain industrial wastewater if *Nitrobacter* is inhibited;
- * it utilizes some of the organic matter during denitrification;
- * it releases oxygen into solution which can be used by heterotrophs thereby offsetting the extra aeration cost for nitrification (Gray, 1989).

2.7.3 The phosphorus cycle

P is a macronutrient that is necessary to all living cells. It is an important component of adenosine triphosphate (ATP), nucleic acids (DNA and RNA), and phospholipids in cell membranes. P can be stored in intracellular volutin granules as poly-P in both prokaryotes and eukaryotes. It is a limiting nutrient with regard to algal growth in lakes and thus contributes to eutrophication. The average concentration of TP (inorganic and organic forms) in wastewater is in the range of 10-20 mg.l⁻¹ (Bitton, 1994) and requirements for its removal calls for maximal concentration lower than 1 mg.l⁻¹ (Water Act 1954).

The weathering and mining of P-containing rocks makes phosphate ions available to plants, which accumulate the inorganic ion from the soil. Orthophosphate is the only directly utilisable form of soluble inorganic P. Some of the solubilised P is transferred from the terrestrial environment to the aquatic environment as a result of leaching. Aquatic algae take up the orthophosphate ion before sedimentation can occur, converting it to the organic form. Organically bound P can then be utilised by heterotrophic organisms, which feed off plants and algae, returning P to the extracellular environment once they die and are decomposed (Mader, 1998). Phosphate is referred to as a limiting nutrient because many of the sources consist of insoluble complexes such as calcium, iron and aluminium salts (Muyima *et al.*, 1997). At any given time, freshwater supplies usually only contain trace amounts of the phosphate ion.

However, human intervention increases the quantity of phosphate in the environment and includes the mining of phosphate ores for fertilizer production, animal feed supplementation and detergent production (Atkinson, 1999).

In this section, only the sources of P contamination will be discussed. The primary sources of P pollution entering the environment can be categorised as either diffuse or point. Basically, diffuse sources originate from rural and urban areas and agricultural fertilizers contribute the greatest amount to these sources (Atkinson, 1999). As the name implies, this pollution problem cannot be targeted to a specific area and treatment to remove the P content from diffuse sources is not usually economically feasible. However, quantification of the P load to an impoundment can be achieved (Atkinson, 1999). Atmospheric precipitation and dry fall-out are also classified as diffuse pollution (Rudd, 1979).

On the other hand, point source pollution is due to industrial and domestic effluents and includes effluents emanating from wastewater treatment plants (Atkinson, 1999). Point source pollution contributes the highest P load to the environment, although methods to treat the water do exist (Wentzel, 1992).

The difficulty in consistently achieving the mandatory effluent P standard in wastewater treatment has led to alternatives being considered, one option being to eliminate P from detergents. It has been calculated that P in detergents only constitutes approximately 40 % of the total P load in domestic wastes (Wentzel, 1992). In-depth feasibility studies rendered little economic incentive to remove P from detergents. The decision was therefore taken (in the South African context) not to introduce a P ban, but rather to investigate methods of improving existing P removal technologies or to develop new technologies (Atkinson, 1999).

The main sources of P in sewage are human waste (30-35 %), and phosphate builders in detergents (50-70 %). In the industrial wastewater field, the P content presents a problem in food processing and canning effluents, effluents containing poly-P used to prevent corrosion and scale formations, effluents from some fertilizer plants, etc. Human excretion contains between 226 to 1004 g P/cap/yr, and phosphate load in detergent builders is estimated at 1004

g P/cap/yr. (Ganczarzyk, 1983). P is present in sewage in three distinct forms: orthophosphate, poly-P, and organic phosphate. Organic phosphate is a minor constituent of sewage and like the poly-P requires further decomposition to the more assimilable orthophosphate form, which is normally fairly slow. About 25 % of the total P in settled sewage is present as orthophosphate in forms such as PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- and H_3PO_4 , which are available for immediate biological metabolism. Therefore, in terms of utilization, both in the treatment plant and subsequently in receiving waters, it is the inorganic phosphate concentration that is important rather than the total P concentration. After secondary treatment, about 80 % of the total P in a final effluent is in the orthophosphate form (Gray, 1989).

The major steps in the P cycle are:

- * mineralization
- * assimilation
- * precipitation

Microbes play an active role in the P cycle.

2.7.3.1 Mineralization

Mineralization of organic P compounds is carried out by a variety of microbes, which include bacteria (e.g. *Bacillus subtilis*, *Arthrobacter*), actinomycetes (e.g., *Streptomyces*) and fungi (e.g. *Aspergillus*, *Penicillium*). Organic P compounds such as phytin, inositol phosphates, nucleic acids and phospholipids are mineralized to orthophosphate. The enzymes responsible for the degradation of P compounds are the phosphatases (Bitton, 1994).

2.7.3.2 Assimilation

Microbes assimilate or absorb P, which enters into the composition of several macromolecules within the cell. P may be stored by some microbes as poly-P granules. The poly-P serves as both an energy and P source in microbes (Bitton, 1994). The natural ability of microbes to assimilate P has been exploited in terms of explaining the removal of P from activated sludge systems. To this end various theories have been formulated to explain the mechanism of P uptake by activated sludge microbes. P enters bacterial cells either by diffusion or biochemically by an active transport mechanism such as ATP. In the activated sludge process, where the concentration of P is normally low in comparison to the cell, active transport is more important. Gray (1989) showed that there was a constant movement of P into cells by active transport, but also out of cells by diffusion, with an overall gain in the P concentration in the cell due to the active transport mechanism.

In the activated sludge process, the mixed liquor sludge normally contains 1.0 - 2.5 % of P. However, under certain environmental conditions this can increase to 5.0 % or even more due to enhanced P accumulation by the microbial biomass. Some bacteria, when deprived temporarily of adequate concentrations of P for metabolism, rapidly accumulate the element once they are exposed to it again and store it as poly-P within their cells a process is known as luxury uptake.

Luxury uptake occurs when a bacterial cell takes up P more than for its normal metabolic requirements (Toerien *et al.*, 1990). Excess P is taken up and stored in the cell as long as sufficient energy is available (Carberry and Tenney, 1973). Enhanced P uptake from wastewater can be observed when obligate aerobic bacteria are exposed to the stress of an anaerobic environment. These cells take up P rapidly by luxury uptake reaction immediately on entry into the aerobic zone where the stress is released and there is an abundant supply of both energy and P.

A number of studies have indicated that P removing microbes proliferate in the aerobic zone as a result of the accumulation of fermentation breakdown products during anaerobiosis. The fermentation breakdown products consist of acetate and butyrate which are short chain carbohydrates (fatty acids), suitable for P removing bacteria. A model organism of this kind is *Acinetobacter* spp. (Buchan 1983; Lötter, 1985), which have been shown to accumulate phosphates under aerobic conditions of up to 25 % of their cell mass. Enhanced biological P removal plants have since been either designed or redesigned to employ luxury uptake of P (Atkinson, 1999). Vaker *et al.* (1967) reported EBPR in a full-scale plant and this was attributed to the phenomenon of luxury uptake of P by activated sludge bacteria.

2.7.3.3 Precipitation

The solubility of orthophosphate is controlled by the pH of the aquatic environment and the presence of Ca^{2+} , Mg^{2+} , Fe^{3+} and Al^{3+} . Precipitation results with the formation of insoluble hydroxy-apatite compounds such as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ or $\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$ (Lilley *et al.*, 1997)

Insoluble forms of P are solubilised by microbes through their metabolic activities. Mechanisms of solubilization used by microbes include: the use of enzymes, production of organic and inorganic acids (for example succinic acid, oxalic acid, nitric and sulphuric acid), production of CO_2 (which lowers pH), production of H_2S (which may react with iron phosphate and liberate orthophosphate), and the production of chelators that can complex calcium, iron or aluminium (Bitton, 1994). Chemical precipitation as a method for phosphate removal is gaining less popularity due to the high costs that are incurred for buying lime (Lilley *et al.*, 1997) and added to this, are the lime dumps that become characteristic of plants using chemical precipitation.

2.7.4 The sulphur cycle

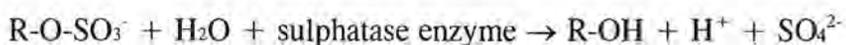
The South African experience in terms of EBPR (in the activated sludge process) indicates that high concentrations of sulphide are detrimental to the process. Apart from being toxic, excessive sulphide may reduce the concentration of essential trace elements to subcritical levels, thus interfering directly with biochemical reactions occurring during P release. Once sulphate reducing bacteria (SRB), such as *Desulfovibrio desulphuricans* develop, the net availability of SCFA declines because these bacteria utilize such compounds as carbon sources for growth. Furthermore, sulphide contributes to the phenomenon of bulking sludge due to the growth of filamentous organisms such as *Thiothrix* and is thus best avoided. It has, however been claimed that such organisms contribute to the process of enhanced P removal (Toerien *et al.*, 1990).

Sources of sulphur in wastewaters are organic sulphur found in excreta and sulphate, which is the most prevalent anion in natural waters.

2.7.4.1 Microbiology of the sulphur cycle

Organic sulphur compounds are mineralized by several types of microbes through aerobic and anaerobic pathways (Bitton, 1994)

Sulphatase enzymes are involved in the degradation of sulphate esters to SO_4^{2-} under aerobic conditions:



Sulphur containing amino acids (e.g. cysteine, cystine and methionine) are degraded to inorganic sulphur compounds or mercaptans, which are odorous sulphur compounds under anaerobic conditions (Bitton, 1994; Gray, 1989). Most anaerobic bacteria are able to produce

sulphide from protein, e.g. *Proteus* and *Bacteroides* spp., and some *Clostridium* spp. Although all can grow anaerobically, only *Bacteroides* spp., which can be present in feces at concentrations up to 10^{10} per gram, are obligate species (Gray 1989).

2.7.4.1.1 Assimilation

Microbes assimilate oxidized as well as reduced forms of sulphur. Anaerobic microbes assimilate reduced forms such as H_2S , whereas aerobes utilize more oxidized forms.

2.7.4.1.1.1 Oxidation reactions

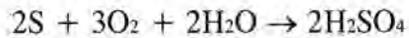
Several groups of microbes are involved in sulphur oxidation (Bitton, 1994). H_2S is oxidized to elemental sulphur under aerobic and anaerobic conditions. Under anaerobic conditions, *Thiobacillus thioparus* oxidizes S^{2-} to S^0 :



Under anaerobic conditions, oxidation is carried out by photoautotrophs and chemoautotrophs, such as *Thiobacillus denitrificans*. Photosynthetic bacteria use H_2S as an electron donor and oxidize H_2S to S^0 , which it stores within the cells of *Chromatiaceae* (purple sulphur bacteria) or outside the cells of *Chlorobiaceae* (green sulphur bacteria). Filamentous sulphur bacteria (e.g. *Beggiatoa*, *Thiotrix*) also carry out H_2S oxidation to elemental S, which is deposited in sulphur granules (Bitton, 1994).

Oxidation of elemental sulphur:

These reactions are carried out mainly by the aerobic, Gram negative, non-spore-forming thiobacilli (for example *Thiobacillus thiooxidans*) which grow at very low pH values.



Sulphur oxidation by heterotrophs:

Heterotrophs (for example *Arthrobacter*, *Micrococcus*, *Bacillus*, *Pseudomonas*) can also be responsible for sulphur oxidation in neutral and alkaline soils.

2.7.4.1.2 Sulphate reduction

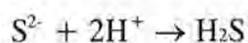
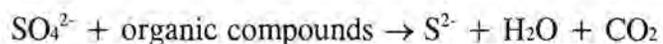
Sulphides are produced by assimilatory and dissimilatory sulphate reduction.

Assimilatory sulphate reduction:

H₂S may result from the anaerobic decomposition by proteolytic bacteria (for example *Clostridia*, *Vellionella*) of organic matter containing sulphur amino acids such as methionine, cysteine and cystine.

Dissimilatory sulphate reduction:

Sulphate reduction is the most important source of H₂S in wastewater. Sulphur reducing bacteria which are strict anaerobes, reduce sulphate:



H₂S is toxic to animals and plants including fish, as well as wastewater treatment operators (Bitton, 1994).

Sulphate reducing bacteria belonging to the following genera have been isolated from environmental samples (anaerobic sludge digestors, aquatic sediments and gastro-intestinal tracts): *Desulfovibrio*, *Desulfotomaculum*, *Desulfobulbus*, *Desulfobacter*, *Desulfococcus*, *Desulfonema*, *Desulfosarcina*, *Desulfobacterium* and *Thermodesulfobacterium*.

2.8 Pasteur effect and enhanced phosphate uptake

The Pasteur effect is a phenomenon associated mostly with the change in the metabolic rate of an organism when shifted from anaerobic to aerobic metabolism. For example, when yeast cells are transferred from anaerobic to aerobic conditions, they increase the rate of glucose breakdown by a factor of 3 to 4. This change is accompanied by a reduction of the fermentation rate and a stoppage of alcohol formation. The Pasteur effect is apparently the result of differences in the cell's energy charge under aerobic and anaerobic conditions. In the presence of oxygen, the respiratory chain and the sites of substrate-level phosphorylation in the glycolytic pathway compete for ADP. The activity of phosphofructokinase is known to be regulated by the level of ATP and citrate. Under anaerobic conditions the activity of phosphofructokinase increases because it is activated by ADP and AMP. Also, more ADP is available for enzymes catalyzing substrate-level phosphorylation reactions. The Pasteur effect could possibly be used to explain the logic behind the ability of activated sludge bacteria phosphate uptake phenomenon subsequent to stressful conditions of the anaerobic zone (Gottschalk, 1979).

2.9 Mechanisms of biological P removal

EBPR by bacteria present in the mixed liquor of activated sludge was first observed by Vaker *et al.* (1967). Since then this phenomenon has gained world-wide support and it utilized both in new and existing wastewater treatment plants, which are either constructed or upgraded to accommodate biological nutrient removal (Atkinson, 1999). The tendency to opt for biological

P removal is due to the low sludge production of the system, the fertilizer value of the waste sludge and the use of wastewater components like influent COD and its various fractions as process chemicals to control the mechanism (Henze, 1996).

Biological P removal is a direct result of the ability of certain microbes (PAO) to accumulate large quantities of poly-P intracellularly. The terms “enhanced” and “excess” are often incorporated to emphasize the ability of these microbes to accumulate poly-P in excess of their normal metabolic requirements. In order to encourage the growth and proliferation of these microbes, as well as to induce the biological P mechanism, two conditions are essential, namely sequential anaerobic and aerobic reactors, as well as the presence of VFA in the anaerobic reactor (Wentzel *et al.*, 1990).

2.9.1 Anaerobic zone

In wastewater technology, the term anaerobiosis describes an environment free both of dissolved oxygen and oxidized forms of nitrogen like nitrates and nitrites (Jenkins and Tandoi, 1991; Muyima *et al.*, 1997). The function of the anaerobic zone is two-fold:

- * the reduced redox potential induces the conversion of influent readily degradable COD (RBCOD) to SCFA via acidogenesis by non-PAO heterotrophs;
- * it provides an ideal environment where PAO are able to take up the SCFA and accumulate the intracellularly as PHA (the most common form being PHB)(Ekama and Wentzel, 1997).

The function of the anaerobic zone was originally thought to be one of stimulation of P release. However, it has now been realized that the zone is solely responsible for the production of suitable substrates (VFA) through fermentation, which allows for the proliferation of PAO in the system due to a lack of substrate competition from non-PAO (Fuhs and Chen, 1975; Muyima *et al.*, 1997). Due to the absence of terminal electron acceptors, PAO are able to

utilize the VFA exclusively through the energy generated from poly-P hydrolysis (Atkinson, 1999). This zone is essential for the net removal of P from the wastewater, as it preconditions the PAO to take up excess P under aerobic conditions, resulting in diminished intracellular poly-P storage granules (volutin), which must be replenished in the following aerobic zone. It is essential that neither nitrates nor dissolved oxygen are recycled to, or enter the anaerobic zone, as the effects on P removal are adverse. This may be due to (Henze *et al.*, 1995):

- * competition for VFA between PAO and other normal heterotrophs;
- * reduction in PAO activity due to reduced fermentation of RBCOD leading to a reduction in VFA;
- * some species of PAO are able to denitrify (in the case of nitrate infiltration) and all are aerobic (in the case of dissolved oxygen), so the organisms will preferably utilize these terminal electron acceptors to obtain energy, rather than switching their metabolism to poly-P hydrolysis (which directly affects P accumulation in the subsequent aerobic zone).

2.9.1.1 Volatile fatty acid synthesis and sequestration

In the anaerobic zone, facultative organisms (non-PAO) are able to derive a small amount of energy (sufficient only for survival) through the generation of intracellular electron acceptors. Through the Embden-Meyerhof pathway, they are able to degrade readily biodegradable organics such as glucose to fatty acids, including acetate, lactate, butyrate, succinate and fumarate. However, under anaerobic conditions, these fatty acids cannot enter the Krebs cycle and are subsequently released into the bulk liquid (Ekama *et al.*, 1984). PAO have a distinct advantage over other normal heterotrophs as they are able to utilize accumulated poly-P stores to supply the energy required to accumulate these fatty acids (Sato *et al.*, 1992). Wentzel *et al.* (1990) found that the rate of acetate sequestration by PAO is zero order with respect to soluble acetate and occurs rapidly. Although VFA are known to be a primary carbon source

accumulated by PAO in the anaerobic zone, other short chain organic compounds are also known to be taken up and accumulated as PHA (Satoh *et al.*, 1997).

VFA concentrations in the influent to wastewater treatment plants can be increased through the installation of primary sludge fermenters at the head of the activated sludge process. Acid-phase anaerobic digestion of primary sludge is used to boost the RBCOD and VFA fractions in the feed wastewater and can either be included as an in-line or side-stream facility (Banister and Pretorius, 1998). Primary sludge fermentation is therefore a practical solution for plants experiencing erratic biological P removal as a result of weak influent COD and the resultant low RBCOD and VFA concentrations. Seeding the fermenters with partially digested sludge (1-3 d digestion) has been shown to improve VFA production efficiency with maximum VFA yields of 10 % of the total influent COD being recorded after 6 d (Banister and Pretorius, 1998).

2.9.1.2 Poly- β -hydroxybutyrate synthesis

A consequence of the high organic carbon concentration in the anaerobic zone is that, provided both an electron and energy source is available, for example NADH/NAD and ATP/ADP, carbon can be taken up intracellularly and accumulated as PHB. Due to the inhibition of the tricarboxylic acid (TCA) cycle in the anaerobic zone due to the high NADH/NAD ratio, the synthesis of PHB acts as an electron sink, decreasing this ratio (Wentzel *et al.*, 1986). This promotes the TCA cycle, which generates more electrons. The interaction between the TCA cycle and PHB synthesis, mediated by the NADH/NAD ratio, ensures that all VFA taken up is stored as PHB. Reducing equivalents such as NAD(P)H₂ are also essential for PHB synthesis. Stoichiometrically, one NAD(P)H₂ molecule is required for the conversion of 2 mol acetate to PHB (Appeldoorn *et al.*, 1992). Before acetate can be stored as PHB, the molecule needs to be activated by acetyl-CoA. This is done at the expense of 1 mol ATP per mol acetate if acetate kinase is involved, or 2 mol ATP per mol acetate if the acetyl-CoA synthetase enzyme catalyzes the reaction (Appeldoorn *et al.*, 1992).

2.9.1.3 Orthophosphate release

A high external concentration of small organic molecules such as acetate allows for passive diffusion into the cell, without expenditure of energy. Activation of sequestered acetate to acetyl-CoA by coupled ATP hydrolysis has the effect of decreasing the ATP/ADP ratio to such an degree that ATP synthesis is stimulated via poly-P degradation (Wentzel *et al.*, 1986). On a molar basis, 1 mol intracellular P is released per mol VFA sequestered (Wentzel *et al.*, 1990). Therefore, if acetate is used as substrate, approximately 0.5 mg P is released per mg acetate (as COD) sequestered (Atkinson, 1999). The mass of P released will always be proportional to the VFA feedstock in solution, in other words, the greater the VFA concentration, the greater the mass of P released. A minimum prerequisite for P release in the anaerobic reactor is a biodegradable COD concentration of 60 mg COD.l⁻¹ (Punrattanasin and Randall, 1998) or a RBCOD fraction of 25 mg COD.l⁻¹ surrounding the organism (Ekama *et al.*, 1984). Compounds such as acetate, formate and propionate, are capable of inducing P release from phosphate-laden sludge under anaerobic, anoxic and aerobic conditions. This has compelled the belief that the release of P is primarily dependent on the nature of the substrate interacting with the biomass and not the prevailing anaerobic conditions (Gerber *et al.*, 1987).

2.9.2 Anoxic zone

Anoxia refers to an environment in which nitrates and nitrites are present, although dissolved oxygen is absent (Atkinson, 1999). This zone is responsible for denitrification in the activated sludge system and reduces the level of nitrates recycled to the anaerobic zone. The anoxic zone is fed by the effluent from the anaerobic zone and by the mixed liquor recycle from the aerobic zone. The variable nature of this recycle ensures that no dissolved oxygen enters the anoxic zone, resulting in the reduction and removal of NO_x from the system (Atkinson, 1999). It has been found that the PAO community can be divided into two fractions, those only capable of aerobic respiration with oxygen as an electron acceptor, and those capable of utilizing both oxygen and nitrate as electron acceptor (Kerrn-Jespersen and Henze, 1993). P

will therefore be taken up in the anoxic zone if no VFA or other low molecular organic compound is available for assimilation. Thus, if acetate leaks from the anaerobic zone into the anoxic zone, P release and PHB accumulation will continue (Artan *et al.*, 1997). Although considerable, anoxic P uptake is quantitatively lower than aerobic P uptake and is a first order reaction with respect to the size of anaerobically accumulated PHB stores (as is aerobic P uptake) (Kern-Jespersen and Henze, 1993; Artan *et al.*, 1997). Kern-Jespersen and Henze (1993) found a linear relationship between accumulated PHB concentration, denitrification rates and P uptake rates under anoxic conditions. Anoxic P uptake has the potential to reduce costs of wastewater treatment considerably due to simultaneous P removal and denitrification occurring in one zone (Atkinson, 1999).

2.9.3 Aerobic zone

The primary function of the aerobic zone is to oxidize organic material in the sewage. When a suitable sludge age is selected and the autotrophic organisms are able to establish themselves in the system, oxidation of ammonia N to nitrite and nitrate (nitrification) occurs simultaneously. Aerobiosis also provides an environment where the PAO are able to take up the P released in the anaerobic zone, as well as the P entering the system through the feed sewage (Atkinson, 1999).

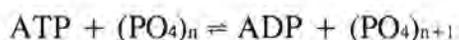
2.9.3.1 Poly- β -hydroxybutyrate degradation

The degradation of PHB proceeds via its hydrolysis to free β -hydroxybutyrate, the oxidation of the acid to acetoacetate and, finally, activation of acetoacetate to acetoacetyl-CoA. Two molecules of acetyl-CoA are then formed by the activated cleavage of acetoacetyl-CoA and these end products enter the TCA cycle (Wentzel *et al.*, 1986). This degradation pathway is regulated by high concentrations of pyruvate and/or a high NADH/NAD ratio and it can, therefore, be concluded that the pathway will only be functional in an environment where

concentrations of extracellular organic substrate is low and a terminal electron acceptor is present, for example in the aerobic zone (Wentzel *et al.*, 1986). Degradation of PHB to acetate provides a carbon and energy source for cell metabolism and function (Atkinson, 1999).

2.9.3.2 Phosphorus uptake and poly-P synthesis

Translocation of extracellular soluble phosphate ions occur via hydroxyl mediated antiport. The cations required for neutralizing the charge imbalance and stabilizing the phosphoryl bonds, for example Mg^{2+} , Ca^{2+} and K^{+} are taken up by the cell via proton mediated antiport (Wentzel *et al.*, 1986). A consequence of the presence of an external electron acceptor in the aerobic zone is a reduction in the NADH/NAD ration and an increase in the ATP/ADP ratio. A high and non-limiting ATP/ADP ration stimulates poly-P synthesis (Atkinson, 1999). The principal mechanism of poly-P synthesis is via the phosphorylation of accumulated phosphate by ATP:



It is evident that this pathway controls both poly-P synthesis and degradation, the direction of which is regulated entirely by intracellular ATP/ADP ratios. Poly-P synthesis will be promoted by high concentrations of ATP, a condition likely to be encountered in the aerobic zone where oxidative phosphorylation is able to proceed (Wentzel *et al.*, 1986).

2.10 Mixed liquor suspended solids (MLSS)

The concentration of suspended solids in the system, which to a large extent constitutes the resident biomass, is referred to as the MLSS. This value offers the system operator a crude measure of the biomass contained within the process (Atkinson, 1999). With the advent of

steady state and kinetic design modelling, the accurate determination of biomass in activated sludge systems has become a significant criterion (Wentzel *et al.*, 1990). Historically, sludge biomass and specific growth rates were calculated on the basis of MLSS, volatile suspended solids (VSS) or COD of the activated sludge (Liebeskind and Dohmann, 1994). In nitrifying denitrifying EBPR systems the mixed liquor organic suspended solids (MLOSS or mixed liquor VSS) is made up of four components: heterotrophic active biomass, endogenous residue, inert material and autotrophic active biomass (Ubisi *et al.*, 1997; Wentzel *et al.*, 1998). The heterotrophic active biomass component arises from the synthesis of heterotrophic organisms on influent biodegradable COD and is lost via endogenous respiration or death processes. The autotrophic active biomass arises from the synthesis of autotrophic organisms in the nitrification of ammonia and is also lost via endogenous respiration processes. Endogenous residue is generated as a result of the unbiodegradable fraction from heterotrophic and autotrophic biomass death processes, while inert material represents the unbiodegradable particulate COD fraction entering the system via the influent (Ubisi *et al.*, 1997). All four components are returned to the anaerobic reactor (in biological P removal systems) via the clarifier recycle and are removed via the waste flow. Ubisi *et al.* (1997) and Wentzel *et al.* (1998) described a simple batch test procedure for heterotrophic biomass determination in activated sludge mixed liquors. Liebeskind and Dohmann (1994) formulated an improved method of DNA extraction from activated sludge for quantitative biomass determination. Although the proposed method showed close correlation to the kinetic models when applied to pure cultures and high-rate aeration sludges, the full DNA complement could not be extracted from sludges containing precipitating agents such as ferric iron. Unfortunately, the procedures are unable to distinguish between heterotrophic PAO and non-PAO in mixed liquor samples originating from EBPR operations. The requirement for a simple method to determine the active PAO fraction therefore still exists and should be actively investigated.

2.11 Nutrients in sewage for microbes

In activated sludge, the relative number of each species is determined by its growth rate, availability of a suitable food source and predation. Furthermore, physical conditions prevailing in the plant have a different effect on the rate of proliferation of various species (Buchan, 1984).

Domestic sewage, and to a lesser extent industrial wastewater, contains a rich variety of organic and inorganic compounds, including important trace elements and organic growth factors. All the nutritional requirements for bacterial and fungal growth are present in the wastewater. Thus, provided the environmental factors are favourable, a wide range of heterotrophic microbes will develop within the biological reactor of a wastewater treatment plant. The microbes present are not only those species which can metabolize the raw constituents of the wastewater, but can also utilize the breakdown products of other microbes and species which prey upon other microbes (Gray, 1989).

Microbes require certain basic nutrient elements. Carbon is required as a source of energy and for the synthesis of various macromolecules. Important nutrients required by microbes include N, P, sulphur, potassium, calcium and magnesium. Trace elements such as iron, copper, zinc and cobalt are required by many species and more fastidious organisms require growth factors such as vitamins.

Carbon, N and P is required by microbes in balanced amounts. The ratio is COD:N:P of 100:6:1.5. Buchan (1984) states that only a fraction of the incoming constituents are involved in bacterial synthesis.

Large quantities of inert or non-biodegradable material can be present, which are removed by processes other than metabolism (such as adsorption onto floc particles). N is only considered fully available when present as ammonia and P as soluble phosphate, although other forms of these elements can be converted to ammonia and phosphate and thus become available.

Domestic sewage usually provides a nutritionally balanced food with the necessary elements and vitamins for bacterial activity. However, for the processes required for denitrification and enhanced P removal, the organic carbon source is often not available (Buchan, 1984). The most important nutrients for poly-P organisms involved in enhanced biological P uptake are the SCFA.

2.12 Microbial ecology of activated sludge systems

The activated sludge system, like all biological treatment processes, relies on a mixed culture of bacteria (in order to carry out basic oxidation of the substrate present) with higher grazing microbes also present, forming a complete ecosystem which has various trophic levels. The activated sludge aeration tank is a truly aquatic environment, although the high level of nutrients and high level of bacterial activity makes it unlike any natural aquatic habitat. Gray (1989), however, views the activated sludge aeration tank as an extreme environment or habitat for most aquatic species, due to the constant aeration and recirculation.

Aquatic species which find the activated sludge aeration tank being inhospitable or inhabitable include organisms which are larger than the smaller mesofauna, such as rotifers and nematodes, or those with long life cycles (Gray, 1989). Ganczarczyk (1983) views activated sludge as a representation of a complicated mixture of viruses, bacteria, protozoa and other organisms, found either singly or clumped together, often enmeshed in a fabric of organic debris, dead cells and other waste products.

Microbes which play an important role in the activated sludge system include groups of bacteria, fungi, protozoa, rotifers and nematodes (Gray, 1989). Activated sludge is a complex ecological system made up of species forming several trophic levels, which compete for food resources, with predator-prey and/or parasite-host relationships clearly discernible. Other species interactions, which effect species diversity and species dominance, such as antibiotic production and phage activity are also important (Gray, 1989). The response of this ecological

system to the environment is often difficult to predict as individual organisms have varying sensitivities to nutrient conditions, waste composition, and other stresses imposed upon the system (for example inorganic salt concentrations, turbulence, pH, temperature and the presence of competing microbes). These influences are poorly understood from a theoretical perspective and their control in any treatment unit may not be as meaningfully desired. This factor may be due to the fact that many of the characteristics indigenous to biological wastewater treatment systems, especially those related to floc formation, remain largely outside any direct control (Ganczarzyk, 1983). The origin of wastewater may limit the type of bacterial species present in the activated sludge system, for example wastewater from industry (Ganczarzyk, 1983).

The physical characteristics of the treatment system can also affect activated sludge population diversity by a washout of slow-growers, elimination of species more sensitive to turbulence, etc. (Ganczarzyk, 1983). Activated sludge as a habitat has its set of selective parameters which are characteristic of each zone in the process, hence the difference in terms of those organisms which constitute the bulk of the biomass and/or those that make the largest contribution to the total metabolic activity (Buchan, 1984). Some of the known parameters include pH, temperature, nutrients and growth stage of the microbes (Momba, 1995; Toerien *et al.*, 1990).

2.12.1 Bacteria

Bacteria constitute the major component of activated sludge flocs and are the most active organisms (Bitton, 1994; Ganczarzyk, 1983). Bacteria in the activated sludge system is the predominant group (Ganczarzyk, 1983) and more than 300 strains of bacteria are known to thrive in activated sludge (Bitton, 1994). Both heterotrophic and chemolithotrophic organisms are found in activated sludge systems (Ganczarzyk, 1993). Heterotrophic bacteria utilise organic material as a source of both carbon and energy, while chemolithotrophic bacteria generally depend on the oxidation of mineral compounds for energy requirements and utilize carbon dioxide as a carbon source (for example nitrifying bacteria).

The major genera found in the flocks are *Zooglea*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Bacillus*, *Achromobacter*, *Corynebacterium*, *Commomonas*, *Brevibacterium* and *Acinetobacter* as well as filamentous microbes. Some examples of filamentous microbes are the sheathed bacteria (for example *Sphaerotilus*) and gliding bacteria (for example *Beggiatoa*, *Vitreoscilla*) which are responsible for sludge bulking. *Caulobacter*, as well as *Zooglea*, has been isolated from wastewater treatment plants in general and activated sludge in particular. Activated sludge flocs also harbor autotrophic bacteria such as nitrifiers (*Nitrosomonas*, *Nitrobacter*) which convert ammonium to nitrate and phototrophic bacteria such as the purple non-sulphur bacteria (*Rhodospirillaceae*), which are detected at concentrations of approximately 10 cells per ml. The purple and green sulphur bacteria are found at much lower levels (Bitton, 1994).

In the anaerobic zone microbes of the species *Aeromonas*, *Citrobacter*, *Klebsiella*, *Pasteurella*, *Proteus* and *Serratia* are capable of fermentation and thus accumulate and produce organic compounds such as lactic acid, succinic acid, propionic acid, butyric acid, acetic acid and ethanol during fermentation. These organic compounds serve as electron donors and acceptors. However, these are not utilised under anaerobic conditions instead they are only consumed in the anoxic and aerobic zone. It appears thus that the anaerobic zone provides substances for the proliferation of aerobic phosphate-accumulating bacteria (Buchan, 1984; Fuhs and Chen, 1975).

Activated sludge represents a stable community where both the number of species and cell numbers of the representative species are high (Atkinson, 1999). These types of communities are best suited to overcome environmental stress like toxic shock. Wentzel *et al.* (1988) identified organisms from an enhanced PAO culture originating from laboratory-scale UCT and 3-stage Bardenpho/Phoredox systems by means of culture dependent methods of isolation and the Analytical Profile Index (API) system of identification, and discovered that >90 % of the bacterial community consisted of the genus *Acinetobacter*. On the other hand, Bond *et al.* (1997), by means of *in situ* molecular techniques, while operating a sequencing batch reactor employing BPR, detected very low numbers of *Acinetobacter* and concluded that bacteria other than *Acinetobacter* are responsible for EBPR.

The biomass of activated sludge is the active agent of biological wastewater treatment, responsible for carbonaceous material oxidation and nutrient removal. To date, process engineering has received particular attention and has subsequently practically become optimized. Yet, systems based on nitrifying denitrifying EBPR principles still regularly fail to achieve the desired end result (Atkinson, 1999). This is due to limitations in our current understanding of the complexities of microbiological interactions occurring within the sludge, as well as our inadequate knowledge of microbial community structure-function correlations (Wagner et al., 1993). To describe and control these microbial processes and mechanisms, Wanner (1997) suggests that activated sludge should be characterized from the following viewpoints:

- * characterization and quantification of microbial constituents according to metabolic activities;
- * identification and classification of microbes;
- * activated sludge quality, for example settleability and dewaterability.

Ecological studies of activated sludge are an integral part of creating a complete and more definitive understanding of the process, diversity and various functions performed by the constituent microflora. The microbial community of activated sludge consists of bacteria, algae, protozoa, fungi and filamentous organisms, although species from different sludges will vary considerably, depending on process design and influent wastewater characteristics (Bux *et al.*, 1994, Muyima *et al.*, 1997). Since organic carbon is the most important energy source entering these systems, it can be expected that heterotrophic bacteria will dominate the community structure (Kämpfer *et al.*, 1996). Probing of activated sludge with fluorescently labeled oligonucleotide probes specific for the alpha, beta and gamma subclasses of the *Proteobacteria* has revealed that the microbial consortia are dominated by these bacteria (approximately 80 %), a phylum containing the majority of the traditional Gram negative bacteria, the majority of which are heterotrophic (Wagner *et al.*, 1993). A number of heterotrophic organisms have been investigated intensively for their involvement in processes such as BPR. These organisms include *Acinetobacter*, *Moraxella*, *Pseudomonas*, *Microlunatus*, *Achromobacter*, *Aeromonas*, *Vibrio*, *Citrobacter*, *Pasteurella*, *Enterobacter*,

Proteobacter, *Klebsiella*, *Bacillus* and coliforms like *Escherichia coli* and *Escherichia intermedium* (various authors as cited by Atkinson, 1999).

Many of these studies have implicated *Acinetobacter* as the principle agent responsible for BPR in activated sludge. Although Koch's principles for other nutrient removal processes, namely, N assimilation in biomass, nitrification and denitrification, as well as ammonia release from organic N, have been satisfied, the same cannot be said for EBPR processes (Jenkins and Tandoi, 1991). Due to *Acinetobacter*'s inability to fulfill the Koch-Henle postulates adapted to microbial ecology, the exact role of the organism in BPR has become dubious (Cloete *et al.*, 1985; Steyn and Cloete, 1989). These revised postulates state that (Steyn and Cloete, 1989):

- * the microbe in question must be associated with a certain phenomenon (BPR) under all circumstances;
- * the organisms must be isolated and studied in pure culture;
- * pure culture studies must render similar results to those found in the natural habitat;
- * the microbe must be present in sufficiently high numbers to warrant its association with the particular function.

The primary concern amongst wastewater biologists is that not all *Acinetobacter* strains exhibit P release concomitant with substrate uptake under anaerobic conditions, a prerequisite for classification as a PAO (Atkinson, 1999).

The effects of growth phase and initial cell concentration of *Acinetobacter* spp. on P release and uptake have been investigated by Momba and Cloete (1996a,b) and Rustrian *et al.* (1997). Momba and Cloete (1996b) found a linear relationship between initial biomass and phosphate uptake and that at low cell densities (10^2 - 10^5 per ml) there was a net release of P rather than uptake. The physiological state of the cells also determines the response of the biomass to P accumulation. Cloete and Bosch (1994) have observed that P is accumulated at the end of log and during stationary phase once active growth has ceased and concluded that a maximum number of cells in the stationary phase of growth should be accumulated in the aerobic zone to optimize biological P removal. Rustrian *et al.* (1997) found that *Acinetobacter* released more

P under anaerobic conditions when in the stationary phase of growth. Yet, P uptake was equally efficient with cells in the log and stationary phase. These results can have a direct implication on EBPR operations as growth conditions for the PAO community can be better optimized to improve and promote the mechanism (Atkinson, 1999).

2.12.2 Fungi

Activated sludge does not usually favour growth of fungi, although some fungal filaments are occasionally observed in activated sludge flocs. Fungi may grow abundantly under specific conditions of low pH, toxicity and nitrogen deficient wastes. The predominant genera found in activated sludge are *Geotrichum*, *Penicillium*, *Cephalosporium* and *Alternaria* (Bitton, 1994; Gray, 1989).

Gray (1989) found both endoparasitic and predatory nematophagous fungi from a number of activated sludge plants in Ireland, where they had a significant role both in floc formation and in the regulation of the nematode population density.

2.12.3 Protozoa

Protozoa are common components in activated sludge with population densities reaching up to 50 000 ml⁻¹ (Gray, 1989). Three classes are important in activated sludge: the *Rhizopodia*, *Mastigophora* and the *Ciliophora*. The ciliophora in activated sludge are mostly phagotrophic and are divided into free-swimming types (for example *Paramecium*), crawling types (for example *Aspidisca*) and the stalked types (for example *Vorticella*) (Buchan, 1984).